Position Paper on Anti-Microbial Resistance Diagnostics

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ABBREVIATIONS

ADA	Adenosine Deaminase
AIDS	Acquired Immune Deficiency Syndrome
AMK	Amoxicillin (brand name)
AMR	Antimicrobial Resistance
AUC	Area Under Curve
BCG	Bacillus Calmette-Guérin (TB vaccination)
BGGT	Blood Glutaraldehyde Gelification Time
CA-MRSA	Community Acquired - Methicillin-Resistant *Staphylococcus aureus*
CAP	Community Acquired Pneumonia
CDI	*Clostridium difficile* infection
CI	Confidence Interval
CNS	Coagulase-Negative *Staphylococci*
CoNS	Coagulase-Negative *Staphylococci*
CRI	Colorimetric Redox Indicator
CPT	Cycling Probe Technology
CSF	Cerebrospinal Spinal Fluid
CV	Coefficient of Variation
DDD	Defined Daily Dose
DFA	Direct Fluorescent Antibody
DNA	Deoxyribonucleic Acid
DOR	Diagnostic Odds Ratio
DPO-PCR	Dual Priming Oligonucleotide - Polymerase Chain Reaction
DST	Drug Susceptibility Testing
ED	Emergency Department
ELISA	Enzyme-Linked Immunosorbent Assay
FDA	Food and Drug Administration
FISH	Fluorescent In Situ Hybridization
FRET-PCR	Fluorescence Energy Transfer - Polymerase Chain Reaction
FVU	First Void Urine
GABHS	Group A beta-haemolytic *streptococci*
GAS	Group A *streptococci*
GDH	Glutamate Dehydrogenase
GP	General Practitioner
GPC	Gram-positive Cocci
GUM	Genitourinary Medicine
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
hMPV	Human Metapneumovirus
HRM	High Resolution Melting Curve
HTA	Health Technology Assessment
ICT	Immunochromatographic Test
ICU	Intensive Care Unit
IGRAS	Interferon Gamma Release Assays
IQR	Interquartile Range
INH	Isoniazid
KAN	Kanamycin
LAM	Lipoarabinomannan
LAMP	Loop-Mediated Isothermal Amplification
LE	Leucocyte Esterase
LFI	Lateral flow Immunochromatography
LRTI	Lower Respiratory Tract Infection
LMIC	Lower Middle Income Countries
MDR-TB	Multi-Drug-Resistant Tuberculosis
MIC	Minimum Inhibitory Concentration
MMLVA	Modified Multiple-Locus Variable-Number Tandem-Repeat Analysis
MODS	Microscopic Observation Drug Susceptibility

M-PCR	Multiplex- Polymerase Chain Reaction
MRCNS	Methicillin-Resistant Coagulase-Negative *Staphylococci*
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSSA	Methicillin-Susceptible *Staphylococcus aureus*
MLVA	Multiple-Locus Variable-Number Tandem-Repeat Analysis
NAAT	Nucleic Acid Amplification Test
NHS	National Health Service
NICE	National Institute of Health and Clinical Excellence
NIHR	National Institute of Health Research
NLCR	Neutrophil-Lymphocyte Count Ratio
NRA	Nitrate Reductase Assay
NPV	Negative Predictive Value
OR	Odds Ratio
PCB	Printed Circuit Board
PCR	Polymerase Chain Reaction
PCR - RFLP	Polymerase Chain Reaction - Restriction Fragment Length Polymorphism
PCT	Procalcitonin
PDMS	Permeable Polydimethylsiloxane
PFGE	Pulsed-Field Gel Electrophoresis
PNA-FISH	Peptide Nucleic Acid - Fluorescence In Situ Hybridization
POC	Point-of-Care
POCT	Point-of-Care Test
PPD	Purified Protein Derivative
PPV	Positive Predictive Value
ProADM	Proadrenomedullin
PZA	Pyrazinamide
QALY	Quality-Adjusted Life Year
RADT	Rapid Antigen Detection Tests
RCT	Randomised controlled trial
RNA	Ribonucleic Acid
rRNA	Ribosomal Ribonucleic Acid
RP	Respiratory Panel
RPLA	Reverse Passive Latex Agglutination
RR	Relative Risk
RSV	Respiratory Syncytial Virus
RTI	Respiratory Tract Infection
RT-PCR	Real Time-Polymerase Chain Reaction
SAT	Stool Antigen Test
SMCC	Short Multi-capillary Chromatography Column
SROC	Summary Receiver Operating Characteristic
ST	Sequence Type
sTREM-1	Soluble Triggering Receptor Expressed on Myeloid cells-1
suPAR	Soluble Urokinase-type Plasminogen Activator Receptor
TB	Tuberculosis
TRACE	Time Resolved Amplified Cryptate Emission
TST	Tuberculin Skin Test
UBT	Urea Breath Test
UTI	Urinary Tract Infection
VAP	Ventilator-Assisted Pneumonia
WBC	White Blood Cell
WHO	World Health Organisation

FOREWORD

There is no doubt that tackling antibiotic resistance is of major public health importance to global health systems. This report highlights the considerable number of new diagnostic technologies in development to underpin rational prescribing of antibiotics. What this report also shows is that innovative diagnostics will only provide part of the solution. It is therefore essential that ongoing strategies address barriers to change, as they are identified and when they arise, along with co-ordinated educational and informatics strategies that target unwarranted variations in practice and promote an evidence-based culture of change.

Approaches to antimicrobial resistance must continue to focus on both appropriateness as well as strategies to reduce antibiotic use. Importantly, interventions need to be sustained over the long term: the time taken to observe reductions in the incidence of antibiotic-resistant organisms is considerably longer than the time to reach high levels of resistance in the first place.

It has been consistently shown that in addressing changes in practice no single quality improvement strategy is more effective than another. Therefore a co-ordinated approach is essential: combining physician, patient and public education along with Informatics systems that are fit for purpose and highlight disparities in practice.

Based on current evidence single test assays will likely prove to be ineffective, therefore research and development should focus on multiplex systems reflecting clinical presentations that are often affected by multiple organisms. Part of the solution, though, lies with restructuring the development of the evidence base for many tests: the current system is burdensome and inefficient leading to a dearth of clinical trial data to aid application and uptake into clinical care.

What might a coordinated approach and therefore success look like? Rapid reporting of laboratory results, same-day results, infrastructure for research ready point-of-care testing sites, informatics systems and automatic alerts that promote appropriate prescribing, information systems that share best practice; along with a culture that develops and implements interventions aimed at specific healthcare settings, which constantly addresses barriers to change, will all have significant benefits for antibiotic stewardship in the future.

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EXECUTIVE SUMMARY

Antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections. It is a serious and escalating threat to global public health that requires concerted action across all government sectors and society.

The sheer volume of antibiotics prescribed is the central factor in increasing rates of AMR: currently half of all antibiotic consumption may be unnecessary. Hence, there is an urgent need to provide rapid evidence-based diagnostic tests to help clinicians better identify and treat micro-organisms causing infections. The norm should be for patients to be prescribed the correct antibiotic following the rapid identification of the causative micro-organism and its antibiotic susceptibility.

The aim of this report is therefore to provide an overview of the diagnostic technologies that improve the appropriate prescribing of antibiotics. Notably, three technologies have disrupted the testing pathway: genome sequencing, specific DNA probe detection (e.g. using PCR) and quantitation; proteomic mass spectrometry; and the development of point-of-care testing technologies.

It is based on a comprehensive search of the literature and is divided into applications relevant to primary and secondary care settings. Currently available laboratory-based and point-of-care tests and some technologies that are in development are included. The report provides a rapid overview of the current evidence, an assessment of the utility of these technologies and requirements for future research. Key recommendations are made based on our findings.

Overall 69 technologies were assessed in detail (see the Appendices for the individual technology summaries). The number of technologies aimed at improving diagnostic accuracy is substantial and many more are in development, with numerous patents filed each year. However, there is a striking lack of progress in developing robust evidence that will impact on practice. As a result many technologies are stuck at the laboratory phase or, having developed technical accuracy, have not progressed to clinical trials that provide cost-effectiveness and ultimately influence practice.

Key Recommendations

1. Policy should continue to coordinate and build on concerted efforts towards delivering multi-faceted interventions combining physician, patient and public education targeted at inappropriate antibiotic prescribing.
2. Informatics systems should be further utilised to improve prescriptions of antibiotic therapy and share best practice.
3. Commissioners should take into account regional and local plans for Quality, Innovation, Productivity and Prevention (QIPP) workstreams aimed at regular audit and feedback, with a focus on decreasing broad-spectrum antibiotic prescribing.
4. Ensure interventions prioritise antibiotic appropriateness along with antibiotic reduction strategies
5. Further evaluate rapid reporting of laboratory results, along with point-of-care testing, which suggest same-day result may have significant benefits for antibiotic stewardship.
6. Further evaluate point-of-care C-reactive protein (CRP) testing in primary care targeted towards reducing unnecessary prescribing.
7. Prioritise technologies which have the potential to both assess the pathogen as well as resistance that can inform and reduce inappropriate antibiotic prescriptions.
8. Tests measuring single pathogens are unlikely to prove effective in clinical practice and research should focus on multiplex systems reflecting clinical presentations that are often affected by multiple organisms.
9. Due to the sheer number of technologies in development, there is a need to support ongoing horizon scanning specific to antimicrobial resistance and emerging diagnostic technologies.
10. Streamline the development of the evidence base for many tests and fast-track clinical trial data to aid implementation into clinical care.
11. Further develop infrastructure for research ready point-of-care testing sites in primary and secondary care to act as rapid development centres in which technologies can be fast tracked through to implementation
12. Develop new smartphone applications, which are readily adopted by physicians and target barriers to adoption and facilitate improved knowledge on prescribing of antibiotics.

INTRODUCTION

The World Health Organisation recently stated antimicrobial resistance (AMR) threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi (WHO Antimicrobial resistance fact sheet). AMR is an increasingly serious threat to global public health that requires concerted action across all government sectors and society. Across the globe, a high proportion of common bacterial pathogens have multiple drug resistant strains, including bacteria that cause urinary tract infections, sexually transmitted infections, pneumonia and bloodstream infections. A high percentage of hospital-acquired infections are caused by highly resistant bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA) or multidrug-resistant Gram-negative bacteria.

Extensively drug-resistant tuberculosis has been identified in 84 countries and a total drug resistant form of the *Mycobacterium* has been recorded in Iran, India, Italy and South America. Treatment failures due to resistance to third-generation cephalosporins, the treatments of last resort for gonorrhoea, have been reported in 10 countries. Gonorrhoea is a prime example of the problems faced: it soon may become untreatable with no vaccines or new drug treatments in development. Moreover, it is recognised patients with infections caused by drug-resistant bacteria have worse clinical outcomes, increased mortality and consume more healthcare resources than patients infected with the same bacteria that are not resistant. The costs of AMR are also considerable: In the US, the annual hospital costs of AMR are estimated to be more than US$ 20 billion (Roberts et al).

Therefore we face a challenge resulting from two linked problems. Firstly, micro-organisms are becoming increasingly resistant to existing antibiotics, and in some cases resistant to all currently available antibiotics. Combined with the increasing virulence of some micro-organisms this can prove deadly: The European Centre for Disease Prevention and Control estimates AMR results in 25,000 deaths each year. Secondly, the development of new antibiotics in some areas is non-existent. As an example, almost no new antibiotics against multi-resistant Gram-negative bacteria are expected in the near future. The Chief Medical Officer for England highlighted this worrying trend in her 2011 Annual Report drawing attention to the increasing levels of antimicrobial resistance and the diminishing supply of new antibiotics (Davies).

Potential solutions to AMR include lifestyle changes, prevention strategies, screening and diagnostic strategies that address not only the whole population, but also focus on the at risk populations, e.g. the elderly and immunocompromised populations, as well as the discovery of new vaccines and treatment options. To combat this emerging threat a multiagency initiative - the UK Five Year Antimicrobial Resistance Strategy - was established in 2013 (DOH Five Year Antimicrobial Resistance Strategy). One of the seven key areas identified for future action is, *"timely diagnostics to guide health professionals to prescribe the right antibiotic for the right infection at the right time; a reduction in the use of antibiotics of 'last resort'*."

Historically diagnostic tests have primarily focussed on the identification of the infectious agent and its drug susceptibility. In some instances this was combined with tests reflecting the host's response to an infection, i.e. serology (antibodies) and inflammatory markers and/or cell surface antigens. The technology for the latter has been based on simple and rapid immunoassays together with techniques for determining the white blood cell count. The characterisation of the infectious agents, however, has involved lengthy culture techniques with the consequences of delays in result generation and decision making. These techniques have also underpinned important epidemiological work tracking infections rates, as well as drug resistance rates.

In the past two decades three technologies have changed the situation dramatically:

- genome sequencing, specific DNA probe detection (e.g. using PCR) and quantitation;
- proteomic mass spectrometry;
- the development of point-of-care testing (POCT) technologies.

Whole genome sequencing is likely to find its greatest application in the epidemiology of infections and antimicrobial resistance, as well as identifying new strains or organisms. The molecular probe techniques, incorporated into a robust POCT format, are likely to be the most appropriate approach for detection of an infectious agent, as well as drug susceptibility. Case studies in the Chief Medical Officer's report illustrate the importance of rapid infectious agent identification and clinical decision-making.

When assessing the evidence of the latest developments in the field of diagnostic testing, it is important to identify the specific clinical questions and decisions that the diagnostic results might address. These include:

- the setting in which the testing might take place;
- screening citizens and the at risk population, i.e. asymptomatic individuals;
- diagnostic use (rule in or rule out) in the patient suspected of having an infection;
- assessing the severity of that infection;
- guiding choice of treatment, i.e. susceptibility testing;
- monitoring response to treatment.

Currently, half of all antibiotic consumption may be unnecessary, which is contributing to the increasing problem of bacterial resistance (WHO Global Strategy for Containment of Antimicrobial Resistance). Antibiotics are often prescribed for self-limiting illnesses, sometimes caused by viruses, and diagnostic uncertainty is the key driver of this unnecessary use. In many clinical situations infections are not accurately diagnosed and, in the absence of an accurate diagnosis, clinicians prescribe antibiotics to err on the safe side. The sheer volume of antibiotics prescribed is the central factor in increasing rates of AMR.

Hence, there is an urgent need to provide rapid evidence-based diagnostic tests to help clinicians better identify and target organisms causing infections. The norm for patients should

be for treatment to commence, only when a bacterial infection is accurately identified. The correct antibiotic should be prescribed following rapid identification of the micro-organism alongside its antibiotic susceptibility.

Purpose of the report

The aim of this report is to provide an overview and a set of key recommendations for diagnostic technologies relevant to improving appropriate prescribing of antibiotics. This includes technologies relevant to decreasing the inappropriate use of antibiotics, such as pathogen detection, identification of antimicrobial resistance and markers of host response (i.e. distinguishing between viral and bacterial infection). The report assesses both currently available technologies (laboratory-based and point-of-care tests) and some that are in development. The report also provides a rapid overview of the current evidence for these technologies, as well as an assessment of their utility, requirements for further research and recommendations.

The report is based on the findings from a comprehensive search of the literature and is divided into applications relevant to primary and secondary care settings, rather than necessarily according to point-of-care and central laboratory testing sites. Increasingly important features of infectious disease testing and treatment management are process efficiency, a commitment to improve access to care and delivering more care closer to home. Consequently, a significant proportion of the new technology development is now focussed on point-of-care applications which can be used in both the primary and secondary care settings. Much of this new technology is summarised in the secondary care section of the report as adoption in the primary care setting is proving more challenging for both process and economic reasons.

Methods

We performed a comprehensive, broad search of the literature using the following databases up to September 2014: Cochrane Central Database of Controlled Trials, Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, Embase, Health Technology Assessment, NHS Economic Evaluation Database, Ovid MEDLINE(R) (including In-Process and Other Non-Indexed Citations) and Science Citation Index (Expanded and Conference Proceedings Citation Index). In addition we searched ClinicalTrials.gov and the WHO ICTRP clinical trials registries. We searched for news items using the NexisUK database and also performed a brief search for patents on Scopus (1986-present). It should be noted that the latter will not be comprehensive, as patents require a lengthy, specialised search of particular databases, however our search provides a sense of the kind of patents currently listed in this area of research.

We also contacted Industry via the British In Vitro Diagnostic Association (BIVDA) and AdvaMedDx (the US organisation with a similar role to BIVDA).

The database search results were reviewed first by title and then by abstract and categorised according to the topic area, such as pathogen detection, antimicrobial resistance and immunological markers/host response. The individual topics under these categories were then identified and an individual summary providing an overview of evidence for each topic was prepared, focussing on the most recent evidence and/or systematic reviews where possible (see Appendix Table 1).

For each identified technology summaries included:

- The level of evidence*;
- A summary of the implications for practice with regard to anti-microbial resistance;
- Definition;
- Summary of the evidence;
- Requirements for further research;
- Bottom line.

*Levels of evidence is a tool designed to help busy clinicians, researchers, or patients find the likely best quality evidence. For diagnostic tests, systematic reviews of cross sectional studies have been shown to provide the most reliable answers. Low levels of evidence should have less influence on practice.

Question	Step 1 (Level 1*)	Step 2 (Level 2*)	Step 3 (Level 3*)	Step 4 (Level 4*)	Step 5 (Level 5)
Is this diagnostic or monitoring test accurate? (Diagnosis)	Systematic review of cross sectional studies with consistently applied reference standard and blinding	Individual cross sectional studies with consistently applied reference standard and blinding	Non-consecutive studies, or studies without consistently applied reference standards**	Case-control studies, or "poor or non-independent reference standard**	Mechanism-based reasoning
What will happen if we do not add a therapy? (Prognosis)	Systematic review of inception cohort studies	Inception cohort studies	Cohort study or control arm of randomized trial*	Case-series or case-control studies, or poor quality prognostic cohort study**	n/a
Does this intervention help? (Treatment Benefits)	Systematic review of randomized trials or *n*-of-1 trials	Randomized trial or observational study with dramatic effect	Non-randomized controlled cohort/follow-up study**	Case-series, case-control studies, or historically controlled studies**	Mechanism-based reasoning

Results

Our database search strategy identified 8,972 results, the search of clinical trials databases provided 675 results, 654 news items were identified and 213 patents were found. From this search we assessed 69 technologies in detail (see appendices for individual technology summaries), providing some research evidence regarding the technology (recent studies or systematic reviews). However this should be not viewed as a comprehensive systematic review of all the evidence for the technology. We also summarised the evidence on interventions designed to improve antibiotic prescribing practices.

From the patent search it is evident that over the last almost 30 years a variety of new patents addressing microbial detection and antimicrobial resistance have been filed relating to either rapid pathogen detection or antimicrobial sensitivity. With our rapid search on Scopus already revealing at least seven new patents per year, the number is likely to be far in excess of this if a comprehensive patent search were to be conducted.

Several Industry representatives responded to our request via BIVDA and provided information on technologies relevant to antimicrobial resistance and infection management currently being developed by them. These included Roche, Siemens, Spectromics, MomentumBioScience, Randox Laboratories, Atlas Genetics, Abbott and Alere.

The US-based AdvaMedDx could not provide a complete response in the given time-frame, however they noted that they are currently forming an association work group on antibiotic resistance, and future outputs from this will be relevant to this topic.

OVERVIEW OF THE FINDINGS

A 2014 Nesta Longtitude survey, of over 1,000 UK GPs, reported 28% prescribed antibiotics several times a week even when they were unsure of the medical necessity; 90% felt pressure from patients to prescribe antibiotics; 70% prescribed antibiotics because they were unsure if the infection was viral or bacterial and 24% reported the reason was a lack of simple diagnostic tools. Both diagnostic tools and educational interventions are required to address antibiotic prescribing practices in primary care.

1. TECHNOLOGIES IN PRIMARY CARE

A 2005 systematic review of interventions to improve antibiotic prescribing in the outpatient setting (Arnold and Straus) assessed the effects of information or educational interventions for physicians. Topics addressed included the overuse of antibiotics for viral infections, choice of antibiotics for particular infection and the duration of prescription. Key findings of the review included:

- Using printed educational materials or audit and feedback alone resulted in no or only small changes in prescribing.
- Educational outreach visits and physician reminders produced mixed results.
- Interactive educational meetings appeared to be more effective than lectures.
- Overall a variety of multi-faceted interventions combining physician, patient and public education were the most successful in reducing inappropriate antibiotic prescribing.
- No single intervention could be applied to all settings, however a combination of methods including patient-based interventions and physician reminders show promise and require further investigation.

Regarding patient-based interventions, particularly the use of delayed prescriptions for infections, for which antibiotics were not immediately indicated, reductions in antibiotic use did not result in excess morbidity. Only one of four studies demonstrated a sustained reduction in the incidence of antibiotic-resistant bacteria associated with the intervention.

A subsequent review of physician-targeted interventions to improve antibiotic use for respiratory tract infections (Van der Velden et al) found that on average antibiotic prescription was reduced by 11.6%. Key findings included:

- Multiple interventions containing at least *'educational material for the physician'* were most often effective.
- No significant added value was found for interventions containing patient-directed elements.
- Communication skills training and near-patient testing providing the largest effects.

An interview-based study of GPs' views on interventions to promote judicious use of antibiotics was conducted in five European countries, including the UK. It found that overall

they preferred interventions that allowed discussion and comparison with local colleagues. This included the use of near-patient tests to reduce diagnostic uncertainty, as well as the involvement of other health professionals to increase their responsibility for prescribing (Tonkin-Crine et al). A recent international survey amongst GPs across five countries indicated that point-of-care tests for the diagnosis of infections were amongst the most commonly cited tests required in a needs assessment (Howick et al).

1.1. Technologies to identify antibiotic resistance

For the diagnosis of urinary tract infections (UTI) and simultaneous assessment of antimicrobial susceptibility, a urinary tract infection kit (Flexicult SSI - a petri dish with several compartments including five antimicrobials) appears promising and is currently being evaluated in an RCT based in primary care. The study aims to quantify the costs and effects of an optimised point-of-care test, guided diagnostic and treatment regime for symptoms of uncomplicated UTI. Results from this trial could influence how UTI is managed in primary care (Appendix 49).

A microfluidic biosensor using lytic bacteriophages and a penicillin-binding protein antibody to identify methicillin resistant *Staphylococcus aureus* is currently being developed. The test would provide results in approximately 10 minutes and has the potential to significantly impact on antibiotic treatment decisions, both in primary care and secondary care settings. However, the technology is in the early phases of development and requires substantial further research into its clinical application and cost-effectiveness (Appendix 12). A further point-of-care technology, which promises to be a rapid (around 10 minutes) system to assess microbial susceptibility, is currently being developed by Spectromics. However, this technology is still at the early development and patent application phase. It requires further development, evaluation and clinical assessment.

1.2. Technologies to improve pathogen detection/diagnosis

Point-of-care tests to detect pathogens

Gastrointestinal pathogens

Rapid tests to detect *Helicobacter pylori* stool antigens (e.g. Rapid Hp StAR) have been assessed in several studies and report sensitivities of 65-86% and specificities of 87-93%. A urine stick test to detect *H. pylori* antigens (Rapirun) has a reported sensitivity of 78-85% and specificity of 90-100%, and can be performed within 15 minutes. They are currently not sufficiently accurate to inform antibiotic prescription at the point-of-care however (Appendix 8).

Point-of-care tests for *Campylobacter* (e.g. ImmunoCard STAT CAMPY) have shown good accuracy, with sensitivity between 86% and 98%, depending on the reference standard, and specificity between 98% and 100%. However, the current evidence is based solely on laboratory studies and research addressing predictive value and utility in practice is currently lacking (Appendix 63).

Respiratory pathogens

There are a variety of rapid tests to aid the diagnosis of bacterial pharyngitis directly from throat swabs, which can be performed within five minutes. A comparative study of five tests showed all had 100% specificity, but varied in sensitivity (62-95%). The latter was influenced by the concentration of group A streptococci, clinical spectrum and physician characteristics. An RCT assessing the benefit of using a rapid test combined with a clinical score found no significant benefit for antibiotic use, and using a clinical score alone was more cost-effective. In addition, qualitative research has highlighted several concerns regarding the use of rapid tests in practice, including test validity. Implementation is unlikely until these concerns are addressed. Further research on validation of rapid tests for streptococcal infections is not warranted until the variability in sensitivity is reduced and tests are developed that can detect non-group A strains (Appendix 65).

For the diagnosis of influenza, there is a considerable array of point-of-care tests on the market and a meta-analysis has reported pooled sensitivity and specificity of 62% and 98%, respectively. Therefore these tests can potentially be used to rule in influenza, but are not good rule out tests. Some studies suggest that use of the test is associated with a reduction in antibiotic prescribing and may be particularly useful in children; however robust evidence on the utility for antibiotic prescribing is lacking and current evidence suggests that the tests may only be cost-effective when influenza probability is low (Appendix 14).

Genitourinary pathogens

Dipsticks are available that can estimate white blood cell counts and bacteria in urine. The test alone appears to be useful to exclude the presence of infection if the results of both nitrites and leucocyte–esterase are negative (Appendix 48). In some cases dipsticks have been used in conjunction with microscopy, and studies have shown that these rapid tests are negative in approximately 10% of children with UTI. Therefore research assessing the incremental accuracy of a rapid UTI test in children compared with clinical investigation alone is required (Appendix 47). The urinary tract infection kit with susceptibility testing (Flexicult SSI, mentioned above) will also be relevant for the diagnosis of UTI in primary care, and results from the ongoing trial should be evaluated to assess the utility of this test (Appendix 49).

Several rapid blood and serum tests for *Treponema pallidum* to diagnose syphilis are available, which can be performed in less than 20 minutes. Systematic reviews have shown that the tests have moderate to good accuracy in whole blood (sensitivity 74-86% and specificity 95-

99%). However, implementation research outcomes are generally poorly defined and evaluated. No specific evidence for the use of point-of-care syphilis tests in guiding antibiotic prescribing strategies has been identified (Appendix 61).

For *Neisseria gonorrhoeae*, rapid point-of-care tests (POCT) include dipsticks (sensitivity and specificity around 70%) and immunochromatographic tests (sensitivity 70%, specificity 96%), and these appear to be of value in high prevalence settings. However, the evidence suggests they do not provide any benefit compared to current standard practice in the UK (Appendix 51). All these test use immunoassays for antigen detection and therefore show lower analytical sensitivity than PCR-based tests.

A systematic review of the accuracy of *Chlamydia trachomatis* point-of-care tests has reported pooled sensitivity of 80% for vaginal swabs and 77% for first void urine, with specificity of 99% for both specimen types. However, economic analysis has indicated that antigen-based immunoassay point-of-care tests would be more costly and less effective than current laboratory-based PCR tests (Appendix 52). Point-of-care PCR-based assays (e.g. Cepheid), on the other hand, have been shown to have the potential to be more cost-effective than laboratory-based assays (Appendix 53).

Point-of-care tests to detect inflammation

There are several point-of-care tests on the market to measure various biomarkers of inflammation, such as C-reactive protein (CRP), procalcitonin and white blood cell counts. CRP testing is increasingly being used in primary care however the evidence for its use is mixed. Systematic reviews suggest that CRP point-of-care testing does reduce antibiotic prescribing at the index consultation. Overall, however, the evidence indicates that currently it should not be used alone or routinely (Appendix 37). It is interesting to note that CRP is used routinely in The Netherlands and Scandinavia and may warrant further investigation.

Systematic review of the accuracy of tests for procalcitonin to detect sepsis reported a mean sensitivity of 77% and specificity of 79%. Evidence from hospital settings (emergency care, ICU) suggests that using procalcitonin to inform initiation or termination of antibiotic use in respiratory infections may reduce total antibiotic exposure and duration of use. Its utility in primary care has not been established. A scoping document from the NICE Diagnostics Assessment Programme (June 2014) outlines an evaluation of the clinical utility and cost-effectiveness of laboratory-based procalcitonin testing to guide initiation and discontinuation of antibiotics in the hospital setting. Results from this assessment will also serve to inform further research requirements regarding utility in primary care (Appendix 13).

Various point-of-care white blood cell count devices are available, which in some cases can also measure red blood cells, haemoglobin and 3-part or 5-part differentials. Accuracy data for these devices is limited, but some studies have shown that the accuracy is adequate and that they may assist judicious antibiotic prescribing. However, other evidence has concluded

that white blood cell counts are less useful in ruling in serious infection and not useful for ruling it out. Therefore the role of these devices in the diagnosis of infection and antibiotic prescribing decisions may be in conjunction with other markers, but requires further research (Appendix 54).

SUMMARY OF KEY FINDINGS

- The effects of information or educational interventions for physicians show using printed educational materials or audit and feedback alone resulted in no or only small changes in prescribing; educational outreach visits and physician reminders produced mixed results. Overall a variety of multi-faceted interventions combining physician, patient and public education were the most successful in reducing inappropriate antibiotic prescribing.
- No single intervention could be applied to all settings, however a combination of methods including patient-based interventions and physician reminders show promise and require further investigation.
- Patient-based interventions, particularly the use of delayed prescriptions for infections, for which antibiotics were not immediately indicated, effectively reduced antibiotic use and did not result in excess morbidity. Only one of four studies demonstrated a sustained reduction in the incidence of antibiotic-resistant bacteria associated with the intervention.
- Multiple interventions containing at least 'educational material for the physician' were most often effective.
- A need exists for point-of-care technologies in primary care. Many of the technologies are protein antigen based and do not have the required analytical sensitivity. Nucleic acid laboratory based tests have the sensitivity but cause delay in the clinical pathway/decision making.
- Protein based assays are likely to be supplanted by nucleic acid based tests for infectious agents. They are able to perform resistant organism detection.
- CRP testing is increasingly being used in primary care however the evidence for the use of this point-of-care test is mixed. It is interesting to note that CRP is used routinely in The Netherlands and Scandinavia and based on the evidence warrants further investigation.
- Overall there is a lack of implementation research in primary care to assess the utility and cost-effectiveness of many of the technologies.

2. TECHNOLOGIES IN SECONDARY CARE

A 2013 systematic review examined interventions to improve antibiotic prescribing practices for hospital inpatients (Davey et al). Meta-analysis of 52 interrupted time series studies compared restrictive practices (physicians were given a limit on how they prescribed, e.g. they had to have approval from an infection specialist in order to prescribe an antibiotic) versus purely persuasive (physicians are advised how to prescribe or given feedback about how they prescribed) interventions. Key findings included:

- Restrictive interventions had significantly greater impact on prescribing outcomes at one month (32%, 95% CI: 2-61%) and on microbial outcomes at six months (53%, 95% CI: 31-75%), but there were no significant differences at 12 or 24 months.
- Interventions intended to decrease excessive prescribing were associated with reduction in antibiotic-resistant *Clostridium difficile, Staphylococcus aureus* and *Enterococcus faecalis* infections.
- Four interventions intended to increase effective prescribing for pneumonia were associated with significant reductions in mortality (risk ratio 0.89, 95% CI: 0.82-0.97).
- Nine interventions intended to decrease excessive prescribing were not associated with significant increase in mortality (risk ratio 0.92, 95% CI: 0.81-1.06).
- Structural interventions (e.g. measuring inflammatory markers or improved laboratory methods) had a median effect of 13.3% for the RCTs and 23.6% for the cluster-RCTs.
- Three studies assessing the effectiveness of rapid reporting of laboratory results suggested that same-day result reporting may have significant benefits for antibiotic stewardship.
- All studies assessing the effect of introducing tests for inflammatory markers showed that using these tests may significantly reduce antibiotic use for patients at low risk of infection; however the current evidence suggests these should not be used in isolation.

The review also concluded that rapid tests for microbes or antimicrobial resistance still require careful assessment using well-designed trials as the current evidence suggests that speed of reporting test results does not necessarily change prescribing behaviour. Regarding cost of implementation, the evidence was very limited with only 10 studies providing information on this. The review highlighted in particular the paucity of evidence about the cost-effectiveness of guideline implementation, stating that future studies should be directed to providing information about the resources required for development, dissemination and implementation of any interventions or recommendations.

Some studies have assessed the utility of automated alert systems in the hospital setting. For example, one US study of ICU patients receiving antibiotic treatment used an automated alert system. Upon the entry of the antibiotic order, the antibiotic and microbiologic history for each patient was electronically queried and patients were assigned to the alert group if they had exposure to the same antibiotic class being prescribed or had a positive culture isolating a Gram-negative organism with resistance to the prescribed antibiotic in the previous six months. Results from the study suggested that the automated alert could identify more than

40% of critically ill patients prescribed inappropriate antibiotic therapy for healthcare-associated infections and that using hospital informatics systems could improve prescription of antibiotic therapy (Micek et al).

2.1. Technologies to identify antibiotic resistance

PCR-based technologies

PCR-based technologies have been developed to rapidly detect genes conferring resistance to antibiotics. The technology involves amplifying resistance genes using specific probes, and tests have been developed for a range of bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), multi-drug resistant *Mycobacterium tuberculosis*, *Helicobacter pylori* and to detect carbapenemase-producing bacteria. The tests usually take around three hours to perform, with some taking less than two hours. A caveat of these tests is that they detect the presence of genes conferring resistance; however, this does not necessarily always correlate with phenotypic resistance. It also relies on characterised resistance mechanisms, and therefore the test is unable to detect novel resistance mechanisms.

Staphylococcus aureus

Systematic reviews comparing PCR to chromogenic agar-based methods for MRSA screening in hospitals suggest PCR methods have shorter turnaround times, but rapid screening methods are not associated with a significant decrease in MRSA acquisition rates. Some PCR technologies are being developed that can detect MRSA directly from clinical samples in less than two hours with high sensitivity, and can also detect vancomycin resistance (though with varying sensitivity and specificity). However, these tests are in the early phases of development and lack evidence from clinical trials in humans. Multiplex PCR targeting five genes for Panton-Valentine leucocidin-positive community-acquired MRSA is also at the early phases of development and is unlikely to impact on microbial resistance. Overall rapid testing for MRSA does not appear to offer advantages over conventional screening methods and there is currently insufficient evidence on the clinical effectiveness of these tests and their implementation (Appendix 16, Appendix 30, Appendix 31 and Appendix 34).

Mycobacterium tuberculosis

A systematic review of a rapid automated PCR test to detect rifampicin resistance suggests the test may be valuable as an add-on test following microscopy smear test, however the role of this test in the current diagnostic pathway is unclear. Also, we did not identify any cost-effectiveness studies and given the expense of the test, this lack of data prevents uptake in the NHS (Appendix 11). Several additional mutations associated with *M. tuberculosis* resistance to

amikacin, kanamycin and capreomycin have been identified; the sensitivity and specificity of future diagnostics could be improved by their inclusion (Appendix 26).

Helicobacter pylori

PCR-based tests for clarithromycin-resistant *H. pylori* have been assessed in several diagnostic accuracy studies, using biopsy or gastric wash samples, showing good sensitivity (98-100%) and moderate specificity (81-83%). One particular test, the string test, does not require biopsy and may warrant further investigation as a non-invasive method to obtain gastric fluid. However, additional research is required as one study using this method reported rather low sensitivity (67%) but good specificity (97%) (Appendix 9).

Carbapenemase resistance

Carbapenems are broad spectrum β-lactam antibiotics and several bacteria produce carbapenemases, conferring resistance to these antibiotics. Currently an indicator carbapenem is used as a first screen for resistance. Several commercially available multiplex PCR assays are available, reporting good sensitivities and specificities (above 95%) from a variety of samples. For example, one test for carbapenemase-producing Gram negative bacteria from rectal swabs is due for commercial release in the USA in 2015. However, these tests require clinical validation and cost-effectiveness research to establish their utility in clinical care (Appendix 2).

Other genotyping technologies

Rapid sequencing of the whole genome of a bacterium, combined with bioinformatics tools, can provide data on determinants of antibiotic resistance. Technology involving the sequencing of *M. tuberculosis* katG mutations, which are associated with high-level isoniazid resistance, is also in the early phases of development. However, these technologies currently appear to be impractical in day-to-day clinical practice, and may be more suited to tracking outbreaks of infections (Appendix 20, Appendix 25). For *M. tuberculosis* several genotypic tests have been developed to detect multidrug resistant TB and they have shown high sensitivity and specificity (above 98%); however there is a need to assess their cost-effectiveness and utility in a low prevalence setting (Appendix 10).

Another genotyping technology, high resolution melting curve analysis, can rapidly detect resistance genes, such as those for rifampicin and pyrazinamide resistance in *M. tuberculosis*. It may be a good alternative to conventional drug susceptibility tests however there is a lack of clinical trial evidence on the utility and cost-effectiveness to inform implementation (Appendix 28, Appendix 29).

Several microarrays for antimicrobial susceptibility have been developed, sometimes in combination with real-time PCR. They can simultaneously detect large numbers of antibiotic resistance genes (e.g. carbapenemases), but their utility in practice is hampered by the lack of clinical studies on their accuracy (Appendix 2, Appendix 18).

Fluorescent in situ hybridisation (FISH) is based on fluorescently labelled DNA probes, which can recognise specific sequences related to antimicrobial resistance; for example, *H. pylori* in biopsy samples. These methods have shown good sensitivity and specificity (84-97% and 91-94%, respectively), however their utility requires further investigation (Appendix 9).

Microfluidic systems ("lab on a chip") are currently in development and may provide rapid systems (results available in 30 minutes to four hours) using extremely small volumes of reagents for the detection of antibiotic susceptibility. These may be of use in hospital settings or at the point-of-care in primary and other ambulatory care settings. They are a new innovation that could potentially aid point-of-care testing of antimicrobial susceptibility but are in the early phases of development and require further assessment (Appendix 15).

Mass spectrometry

Mass spectrometry (matrix-assisted laser desorption/ionisation time-of flight; MALDI-TOF MS) aims to differentiate spectra from resistant and susceptible bacterial isolates using whole cells or crude extracts. This technology has been validated in *Enterobacteriaceae* and *Pseudomonas aeruginosa* but is not commercially available. It is still in the early phases of development regarding clinical utility and it is as yet unclear if this approach offers sufficient sensitivity and specificity (Appendix 2, Appendix 17).

Phenotypic tests

A rapid phenotypic test for carbapenemase resistance using a colour indicator (RAPIDEC CARBA NP) has shown good sensitivity and specificity compared to molecular techniques. It is currently under evaluation and due to be released in the UK in late 2014. Evaluation of the clinical and cost-effectiveness needs to be performed before implementation in a clinical setting can be considered (Appendix 2). Phenotypic tests to detect multidrug resistant TB have shown high sensitivity and specificity (above 98%), however their utility in low-prevalence settings remains to be assessed (Appendix 10).

Tests using strips with antimicrobial agents (e.g. the E-test, AB Biodisk) are well known and relatively simple to use. They can be used to test antimicrobial susceptibility either on isolated bacteria or directly on clinical samples in the laboratory. A study has shown that the use of this test was associated with fewer fever days and fewer days of antibiotics in ventilator-associated pneumonia. The test may have utility in practice; however a systematic assessment of the current evidence regarding clinical utility and cost-effectiveness is required (Appendix 7).

Cell lysis assays based on detecting bacterial cell lysis after incubation with an antibiotic have been validated for the detection of quinolone and ampicillin resistance in *Escherichia coli* and carbapenem resistance in *Acinetobacter baumannii*. This approach has not been tested using clinical samples however (Appendix 19).

New antimicrobial susceptibility technologies currently being developed may have utility in either the hospital or low-resource settings. These include a colorimetric microwell array assay using a cell phone-based microphotometric system. This has been tested using urine samples with varying concentrations of bacteria; however the test is in the development phase and requires further assessment (Appendix 46).

Biosensor platforms ranging from lateral flow test strips and microfluidic devices to cell-based sensors have been developed over the last few years. They can provide results within an hour and clinical validation studies on urine samples in urinary tract infection studies have shown high sensitivity and specificity (89-100% and 97-98%, respectively). These tests may have utility in antibiotic treatment decisions in UTI. Further research into microfluidic technology and automation may make these useful diagnostic aids at the point-of-care (Appendix 45). Microfluidics with susceptibility testing for the diagnosis of UTI has been shown to be more rapid than PCR-based tests and could aid diagnosis as well as antimicrobial treatment. However, current evidence is laboratory-based and randomised controlled trials are required to determine patient benefit (Appendix 50).

Rapid diagnostic tests with immunoassays, in some cases utilising lateral flow immunochromatography, have been developed to detect MRSA. They have shown high sensitivity and specificity (above 94%) however, in clinical practice their benefits over conventional methods remain to be assessed (Appendix 32). Chemiluminescence-based methods to detect *S. aureus* strains with reduced sensitivity to vancomycin have also shown some promise with sensitivity and specificity greater than 95%; however these require validation and clinical trials to assess their accuracy (Appendix 33).

Rapid immunoblot-based assays (e.g. Nanologix QuickTest) for the detection of group B *Streptococcus*, which can also determine antimicrobial susceptibility, have been developed (turnaround time of around six hours). A study assessing the accuracy of this test in pregnant women reported a sensitivity and specificity of 97% and 88%, respectively. Rapid testing of group B *Streptococcus* could reduce the unnecessary antibiotic use in women going into labour who have not had prior screening, or those going into pre-term labour. However, these tests require further validation of their accuracy in the intended population as well as assessment of their clinical utility and cost-effectiveness (Appendix 67).

2.2. Technologies to improve pathogen detection/diagnosis

Gastrointestinal pathogens

Several rapid tests for the detection of gastrointestinal pathogens have been developed, including multiplex PCR and immunological assays. Tests are able to detect a variety of gastrointestinal pathogens, including *Salmonella*, pathogenic *Escherichia coli*, *Campylobacter* and *Clostridium difficile*. A 2014 HTA assessment of two multiplex PCR assays (Luminex xTag and MassCode), which can be used to directly test stool samples for common important enteropathogens (including *C. difficile*, *Salmonella* spp, *Campylobacter* spp and norovirus), found that although the ability of the tests to detect multiple pathogens from one stool sample was promising (sensitivity and specificity of over 90% for some pathogens), the sensitivity for the key enteric pathogen *Salmonella enterica* was low (around 40%). Overall the report concluded that these multiplex systems require further research and are not currently ready for deployment (Appendix 1). A systematic review of other rapid diagnostic tests (PCR-based or immunological) for individual gastrointestinal pathogens found that several of the tests appeared to have high sensitivity and specificity, especially PCR tests for *Salmonella, Campylobacter* and *E. coli* O157; however, the cost-effectiveness of implementing these tests is unclear and requires further research (Appendix 62).

For the diagnosis of *C. difficile*, a systematic review of studies assessing PCR techniques reported high sensitivity and specificity (87-92% and 94-97%, respectively). Sensitivity and specificity was dependent on the prevalence of disease. PCR may be a useful method but the test is expensive (Appendix 41). Several other tests for *C. difficile,* including new selective culture media and fluorescent in situ hybridisation (FISH), allow for species identification and toxin detection within the same day and would have high clinical relevance (Appendix 40). Immunochromatography tests, which can be done in less than 30 minutes, could also be a reliable first-line method for detecting *C. difficile* (Appendix 38). These tests are promising however they require clinical trial evidence for their accuracy, utility and cost-effectiveness. New technologies in the development phase include a multi-capillary gas chromatography column along with artificial neural network software (Appendix 42) and a modified multiple-locus variable-number tandem-repeat analysis for the identification of *C. difficile* during institutional outbreaks (Appendix 39). These tests are in the early phases of development and the latter may require substantial technical expertise.

For the diagnosis of *Helicobacter pylori*, several non-invasive tests are available. In the hospital setting, these include the carbon-13 urea breath test and the stool antigen test, both of which are recommended in the current NICE guidance on dyspepsia and gastro-oesophageal reflux disease (CG184) (Appendix 8).

Respiratory pathogens

Rapid technologies for the diagnosis of *Mycoplasma pneumonia* include PCR, microfluidics, loop-mediated isothermal amplification (LAMP) and DNA microarray assays. PCR and real-time PCR may aid the diagnosis of *Mycoplasma* community acquired pneumonia however there is a lack of clinical trial evidence and it is expensive. A systematic review comparing PCR with serology testing showed a great deal of heterogeneity in the results and suggested using both test types (Appendix 3, Appendix 4, Appendix 57). Microfluidics tests have been developed for the detection of *Mycoplasma pneumonia* and these are more rapid than PCR (60 minutes vs 165 minutes) with the potential for high throughput and affordability (Appendix 58). LAMP is a simple technology suitable for low-resource settings. DNA microarray assays are easy to use, affordable and with potential high throughput. However regarding all these tests, it is unlikely that single test assays will be useful in clinical practice (Appendix 55, Appendix 56). Rapid culture methods that have been developed seem to provide little benefit as they are expensive and still take one to three days (Appendix 59).

An automated colorimetric microbial detection system (Bac T/Alert 3D) has been evaluated in the diagnosis of community acquired pneumonia (CAP). It appears to be a rapid, reliable and cost-effective method compared to conventional culture. Randomised comparative studies are required to confirm initial findings (Appendix 6). For CAP, an immunochromatographic test to detect the presence of *S. pneumoniae* C-polysaccharide in urine has been developed, which gives results in 15 minutes (BinaxNOW-SP). The test has a sensitivity and specificity of 74% and 97%, respectively, and may be a useful addition to the current diagnostic work-up of CAP. However, adequately powered randomised clinical trials and economic evaluation are required to inform clinical application (Appendix 5).

For *Mycobacterium tuberculosis*, several tests have been developed for use in resource-limited settings. These include tests to detect circulating mycobacterial antigens (e.g. lipoarabinomannan) in serum, sputum, urine, pleural fluid and cerebrospinal fluid. A systematic review of these tests has shown that the range of sensitivities and specificities is extremely broad. Overall they do not appear to be sufficiently accurate and the quality of the evidence to support their use limits their utility (Appendix 21). Other rapid diagnostic tests for tuberculosis infection include nucleic acid amplification tests, molecular probes and biochemical assays. Studies from low-prevalence countries strongly suggest that assays based on the RD1 specific antigens are more accurate than the tuberculin skin test or purified protein derivative assays for the detection of latent tuberculosis (Appendix 27). Commercial rapid serological tests described as bedside tests in the hospital or clinic setting continue to produce inaccurate estimates of sensitivity and specificity. The current WHO policy statement recommends against their use (Appendix 22). Glutaraldehyde tests, which use blood glutaraldehyde gelification time for the diagnosis of pulmonary TB, appear to be rapid, easy and cost-effective in low resource settings, and research should address their role as an add-on test (Appendix 24). A recent systematic review of the utility of interferon-gamma release assays in *M. tuberculosis* diagnosis in children suggests this is a promising approach, but

interpretation of results may be difficult, resulting in diagnostic uncertainty, both regarding ruling in and ruling out (Appendix 23).

Multiplex PCR molecular panels for the simultaneous detection of both bacterial and viral respiratory pathogens (e.g. Biofire FilmArray RP) have a short turnaround time (approx. one hour) and they may be efficient in the hospital setting. A number of accuracy studies have reported sensitivity between 84-100% and specificity between 98-100%, however these were mostly retrospective studies, a lower level of evidence. An evaluation into the implementation of the FilmArray RP on outcomes for children admitted to hospital with acute respiratory tract illness reported significantly reduced times to test result, but there was no significant impact on antibiotic prescribing and use (Appendix 60, Appendix 64). Other respiratory panel tests for the diagnosis of respiratory viruses (e.g. mariPOC, Prodesse ProFLU, Verigene) have shown high specificities (99-100%), but very variable sensitivities for the different viruses (25-89%). A 2014 systematic review on the use of rapid viral detection in children presenting with acute respiratory infection to the emergency department concluded that although there appeared to be a trend towards decreased antibiotic use, this was not statistically significant. Overall there is currently insufficient evidence regarding the utility of these tests in reducing antibiotic prescribing and an adequately powered trial with antibiotic use as an outcome is needed. A 2014 UK randomised controlled trial assessing the impact and cost-effectiveness of rapid diagnostic testing for influenza, respiratory syncytial virus and *S. pneumoniae* on the management of the elderly and high-risk adults found no evidence that rapid testing influenced antibiotic prescribing or clinical outcomes (Appendix 60).

For group B *Streptococcus* infection, a multiplex PCR assay (Cepheid Xpert GBS) allows for the rapid detection of these bacteria (turnaround time 35-52 minutes). An accuracy study in pregnant women of this test compared to culture results has shown sensitivity between 87% - 92% and specificity between 85%-96%, depending on the sample used. However, there is currently no evidence for the utility of this test and its role in the clinical pathway (i.e. as replacement or add-on test to current culture methods) (Appendix 67).

Sepsis

Real-time PCR tests are commercially available to detect the presence of several pathogens simultaneously (including bacteria, fungi, and, in some cases, resistance genes) in patients with suspected sepsis (e.g. Magiplex Sepsis, SepsiTest, SeptiFast). Accuracy studies have shown sensitivity and specificity of 75% and 92%, respectively for SeptiFast, while SepsiTest and Magiplex Sepsis had significantly lower sensitivity and several limitations. Of note, a NIHR HTA systematic review assessing the diagnostic accuracy of multi-pathogen real-time PCR in the management of patients with suspected sepsis is currently under way and publication is expected in April 2015. Further research should await the results of this study (Appendix 44). A new platform based on PCR and mass spectrometry, which can potentially rapidly (within 8 hours) identify hundreds of bacteria, viruses and fungi simultaneously, has been developed by Abbott and is due to be launched within 12 months (www.abbott.co.uk). However, this will

require further research regarding clinical utility and cost-effectiveness to inform implementation.

Automated culture systems containing molecules that bind compounds that may inhibit growth of micro-organisms have been developed (e.g. Bac T/Alert). Together with simple lysis-based methods and mass spectrometry this allows for more rapid identification of micro-organisms in the diagnosis of sepsis and could impact on the clinical management of antibiotic treatment. Randomised trials are required to confirm the findings of comparative studies (Appendix 66). A new technology currently in development (planned launch early 2015), Enzymatic Template Generation and Amplification (ETGA), promises to provide an even faster method (one day) to detect bloodstream pathogens along with antimicrobial susceptibility testing. This will require further assessment prior to implementation (www.momentumbio.co.uk).

Combinations of biomarkers, such as C-reactive protein (CRP), procalcitonin (PCT), interleukin-6, neutrophil-lymphocyte count ratio (NLCR) and soluble urokinase-type plasminogen activator receptor (suPAR) have been assessed for the diagnosis of sepsis. Combining CRP, PCT and temperature may be an approach to improve detection of nosocomial infection in the ICU, and NLCR shows promise as a cheap and rapid marker to differentiate bacterial bloodstream infection patients; however, there is a lack of clinical studies assessing the utility of this strategy. Several new biomarkers are being assessed for their relevance in the diagnosis of infection, as well as the utility of combining markers, however these are in the early stages of assessment and require further clinical validation (Appendix 43).

For the diagnosis of *Staphylococcus aureus* and coagulase-negative staphylococcus, rapid diagnostic tests with fluorescence in situ hybridisation (FISH) have been shown to be highly accurate (sensitivity and specificity 99% and 90%, respectively). They could improve diagnostic accuracy and shorten time to commencement of antibiotic therapy. However, this technology also lacks clinical trial data and cost-effectiveness assessment (Appendix 36). An immunochromatographic test, the BinaxNow *Staphylococcus aureus*, appears to be accurate for the detection and differentiation of *S. aureus* from coagulase-negative staphylococci directly from blood culture bottles (sensitivity 96%, specificity 99%) (Appendix 35). Yet, as for the above technologies, there is a lack of clinical and cost-effectiveness studies to inform uptake.

Genitourinary pathogens

Real-time nucleic acid amplification tests (NAAT) for both *Chlamydia trachomatis* and *Neisseria gonorrhoea,* using urine or cervical/vaginal swabs, can provide results in 30 to 90 minutes and may be of use in genitourinary medicine clinics. An accuracy study reported sensitivities ranging from 98-100% (depending on the sample and pathogen to be detected) and specificity of 99% (Appendix 53). Multiplex PCR-based arrays for the simultaneous detection of multiple sexually transmitted pathogens are also available (e.g. Atlas Genetics iO

system, Randox STI Array), however assessment of the research evidence regarding accuracy and clinical utility is required. An early stage development of a PCR-based rapid test for *Trichomonas vaginalis* has also been described (Appendix 68). A study in four UK GUM clinics showed that rapid NAAT test results shortened the patient pathway and were less expensive, potentially leading to more appropriate and rapid patient care. Utility and implementation studies are currently lacking.

Other technologies

Breath analysis

Breath has been considered for a number of years as an underutilised resource for diagnosis and monitoring of disease. It is now being researched in relation to the diagnosis and management of infectious diseases (including sepsis) (Appendix 69). This technology is in the early stages of development and there are no reported clinical studies with the exception of the use of the urea breath test.

With further development and/or research, many of the new technologies mentioned above could have potential use as point-of-care tests, with shorter time to result. It is also clear that, although the current state of the evidence is poor, the trend is moving toward molecular testing, offering greater analytical sensitivity as well as analytical specificity. Point-of-care testing will significantly reduce time to decision making, although large multiplexing may be more difficult with point-of-care tests.

SUMMARY OF KEY FINDINGS

- With further developments many of the new technology developments mentioned could have potential use as point-of-care tests, with shorter time to result, as well as use in the clinic setting.
- Many technologies lack clinical trial data, validation and cost-effectiveness assessments.
- There is a current need to streamline the development of the evidence base for many tests and fast-track clinical trial data to aid implementation into clinical care.
- Automated alert can identify critically ill patients prescribed inappropriate antibiotic therapy for healthcare-associated infections.
- Better utilization of hospital informatics systems could improve prescription of antibiotic therapy.
- Interventions in hospital-based settings intended to decrease excessive prescribing can be associated with reductions in antibiotic-resistant bacteria.
- Interventions aimed at increasing effective prescribing for pneumonia are associated with significant reductions in mortality.
- Studies assessing the effectiveness of rapid reporting of laboratory results suggest that same-day result reporting may have significant benefits for antibiotic stewardship.
- Introducing tests for inflammatory markers may significantly reduce antibiotic use for patients at low risk of infection.

3. OTHER INTERVENTIONS

3.1 Educational interventions

A cluster-RCT in general practice in Norway, where the intervention group had two visits by academic peers providing education on the guidelines and research evidence for acute respiratory tract infection, as well as feedback on antibiotic prescribing practices. They reported a reduction in odds (0.72) in prescribing of antibiotics for acute respiratory tract infections compared to the control group (Gjelstad et al).

A US cluster randomized trial in a paediatric primary care network reported that clinician education (one hour session) combined with audit and feedback (one year) resulted in decreased broad-spectrum antibiotic prescribing by 12% (Gerber et al).

Practical measures such as providing an interactive booklet on respiratory tract infections in children in UK primary care have also been shown to significantly reduce antibiotic prescription (absolute risk reduction 21%) (Francis et al).

Some technology-based interventions have also been assessed. A recent multinational cluster RCT (including UK) assessed the effect of internet-based training on targeting testing and negotiating with patients on prescribing practices for respiratory tract infections. The study reported that internet training achieved notable reductions in prescribing rates (risk rates between 0.38 and 0.53, depending on the intervention), was easily accessible and could be used across national language and cultural boundaries (Little et al 2013).

A UK study on the development and implementation of a smartphone application for the delivery of antimicrobial prescribing policy reported that clinicians rapidly adopted the mobile phone application. 71% reported that using the application improved their antibiotic knowledge and helped them adhere to policy. Several barriers were identified, such as IT infrastructure and clinician awareness to ensure applications are up-to-date, concerns regarding the mobile phones as potential reservoirs of infection and the effect of the patient perception of their use on the doctor-patient interaction (Charani et al). The study did not measure the specific effect of using the application on the prescribing practices.

A 2008 systematic review (Ranji et al 2008) that included 34 studies reported a 10% median reduction in antibiotics use. Overall, no single quality improvement strategy appeared to be more effective. However, active clinician education strategies were associated with non-significant decreases in prescribing compared to passive education strategies (13% vs. 7%, p=0.096). The findings in this review were further supported by a 2006 AHRQ report (Ranji, et al 2006), which also reported no single quality improvement strategy was seemingly more effective than another, highlighting active clinician education seemed to be more effective and the review concluded that reductions in overall prescribing might be better achieved through targeting all acute respiratory infections rather than single conditions. A Cochrane review on interventions to improve antibiotic prescribing practices in ambulatory care that included 39 studies concluded the effectiveness of interventions targeting prescribing depends largely on the behaviour and the barriers to change within a particular community setting. Whilst no

single intervention was recommended multi-faceted interventions, which include educational components at many levels, might be effective after barriers to change have been addressed (Arnold et al 2005).

Interventions aimed at increasing the prescribing of certain recommended first-line antibiotics for specific infections are more likely to produce substantial changes in prescribing than those interventions targeting overall inappropriate antibiotic use.

A further systematic review of antibiotic prescribing in long-term care facilities included four low quality studies and reported interventions involving local consensus procedures, educational strategies, and locally developed guidelines may improve the quality of prescribing, but due to the low quality evidence did not draw any firm conclusions (Fleming et al 2013).

A systematic review of strategies to reduce antibiotic prescribing for children with respiratory tract infections in primary care included 17 studies and reported that interventions combining parent education with clinician behaviour change decreased prescribing rates by up to 21% (Vodicka et al 2013). Computerised prescribing prompts were shown to increase the appropriateness of prescribing. Yet, prescribing rates of antibiotics for children have declined over time but remain high and often unnecessary. Automatic computer prompts and promotion of leadership or involving clinicians in the design of guidelines and education strategies was reported to be more effective in the review, particularly when compared to passive strategies, such as leaflets or posters targeting only parent, which do not appear to affect prescribing. Providing information on self-care and specific actionable advice on when to re consult was more effective than providing generic information to parents on the appropriateness of antibiotics. A further review on the effectiveness of physician-targeted interventions to improve **antibiotic** use for respiratory tract infections in adults and children included 58 studies, describing 87 interventions, reported that multiple interventions, which contained at least educational materials targeted towards clinicians, were more effective than patient-directed elements. Communication skills training and near-patient testing reported the largest effects (Van der Velden et al 2012).

SUMMARY OF KEY FINDINGS

- No single quality improvement strategy was more effective than another
- Academic peers providing education on the guidelines and research evidence for acute respiratory tract infection had significant effects on prescribing.
- Clinician education combined with audit and feedback resulted in decreased broad-spectrum antibiotic prescribing.
- Interactive booklets on respiratory tract infections in children in UK primary care have been shown to significantly reduce antibiotic prescriptions.
- Internet training might achieve notable reductions in prescribing rates.
- Smartphone applications are readily adopted by physicians and can improve knowledge on prescribing of antibiotics.
- Active clinician education seems to be more effective than passive education strategies.
- The effectiveness of interventions targeting prescribing depends largely on the behaviour and the barriers to change within a particular community setting.
- Interventions combining parent education with clinician behaviour can affect prescribing rates.
- Automatic computer prompts and promotion of leadership or involving clinicians in the design of guidelines and education strategies is more effective.

3.2 Delayed antibiotic prescribing

A 2013 systematic review on delayed antibiotic prescribing reported there were no differences between immediate, delayed and no antibiotic prescription on most clinical outcomes. In patients with respiratory infections that clinicians feel it is safe not to prescribe antibiotics immediately, withholding antibiotics (with the advice to return if symptoms did not improve) is likely to result in the least antibiotic use, while maintaining similar patient satisfaction and clinical outcomes to delayed antibiotics (Spurling et al).

An RCT set in UK primary care randomised children (aged 3 years old or above) with acute pharyngitis to three management strategies: delayed prescription, clinical score designed to identify streptococcal infection (FeverPAIN) or rapid antigen test used according to clinical score. The study found that routinely providing a delayed antibiotic prescription was less effective than targeted use of antibiotics using a clinical score (with or without rapid antigen testing). The latter reduced antibiotic use (in the delayed antibiotics group 46% used antibiotics, compared to 29% and 27% in the other two groups) (Little et al, 2013).

Trial data suggests that antibiotic use reduces the risk of complications of sore throat. However a recent large prospective cohort study of over 12,000 adults in the UK concluded that risks of suppurative complications or reconsultation in adults were reduced by antibiotics, but by not as much as the trial evidence suggests (Little et al, 2014). It is also interesting to note that a 2014 systematic review of the effectiveness and safety of antibiotics for preventing complications from undifferentiated acute respiratory tract infections in children (under 5 years) found only four trials which were all of low quality. It concluded that the quality of evidence currently available does not provide strong support for antibiotic use as a means of reducing the risk of otitis or pneumonia in children up to five years of age with undifferentiated respiratory tract infections (Alves Galvao et al).

SUMMARY OF KEY FINDINGS

- Delayed prescription strategies were likely to provide similar benefits to immediate antibiotic prescription.
- Not prescribing any antibiotics (with the advice to return if symptoms did not improve) is likely to result in the least antibiotic use, while maintaining similar patient satisfaction and clinical outcomes to delayed antibiotics.
- Delayed antibiotic prescription was less effective than targeted use of antibiotics using a clinical score.

3.3. Combining diagnostic tests with other clinical information/Diagnostic algorithms

The use of biomarkers and rapid microbiological diagnosis is discussed elsewhere across this report. Using procalcitonin to inform antibiotic prescription has been shown to safely reduce frequency and duration of antibiotics in lower respiratory tract infection (e.g. Albrich et al) and a meta-analysis of procalcitonin combined with clinical assessment had a sensitivity and specificity of 77% and 79%, respectively, in the diagnosis of sepsis (Wacker et al). However the clinical utility and cost-effectiveness of this test remains to be addressed.

A 2013 systematic review of point-of-care CRP testing and antibiotic prescribing in primary care showed that CRP testing was associated with a significant reduction in antibiotic prescribing at the index consultation (RR 0.75, 95% CI 0.67 to 0.83) (Huang et al). However, the evidence to date suggests testing should not be used alone or routinely in primary care. It is interesting to note that CRP testing is used in some European countries, and not others, which suggests this variation and the reasons that might explain such variation in testing and also in treatment rates warrants further investigation. As highlighted across the report, rapid laboratory microbiological diagnosis (e.g. PCR, MALDI-TOF, microarrays, etc.) has the potential to both assess the pathogen as well as any antibiotic resistance and could be used to inform antibiotic prescription. However substantial further research is required regarding utility and cost-effectiveness before these can be implemented in routine diagnostic decisions.

Some tests may be best used in conjunction with other diagnostic indicators. An RCT in UK general practice assessed the effectiveness of a clinical score with or without rapid streptococcal antigen detection tests compared to delayed antibiotic prescribing. It found that while targeted use of antibiotics for acute sore throat with a clinical score reduces antibiotic use, antigen tests used according to a clinical score provided similar benefits but with no clear advantages over a clinical score alone (Little et al, 2013). The accompanying qualitative study of the views of healthcare professionals and patients on the use of rapid streptococcal antigen detection tests highlighted several concerns regarding implementation of these tests, such as validity, time and resource use, as well as effect on the clinician-patient interaction. These would require addressing before implementation of such tests (Leydon et al).

Several relevant studies are also currently ongoing: For example, a large German trial assessing the effect of communication training or the combination of communication training with a point-of-care test on the prescribing of antibiotics for respiratory tract infections. This study is expected to complete in January 2015 (Altiner et al). A Belgian trial has also been registered on clinicaltrials.gov, titled "Optimising Diagnosis and Antibiotic Prescribing for Acutely Ill Children in Primary Care (ERNIE2)". The estimated trial completion date is January 2015 (http://clinicaltrials.gov/show/NCT02024282).

SUMMARY OF KEY FINDINGS

- CRP testing is associated with a significant reduction in antibiotic prescribing at the index consultation; however the test should not be used in isolation.
  Rapid microbiological diagnosis has the potential to both assess the pathogen as well as any antibiotic resistance and could be used to inform and reduce antibiotic prescriptions.
- Variation in testing and treatment rates across practices and across different countries and the reasons that might explain such variation warrants further investigation.

4. ONGOING RESEARCH

As highlighted across the report, antimicrobial resistance and improved pathogen detection are key health research areas and we have identified several relevant projects, which are currently under way in the UK:

For improved diagnosis of pathogens:
Tuberculosis: Diagnosis via nanosensor arrays (Applied Nanodetectors Ltd); identification of multi-drug resistant TB (Enigma Diagnostics); multifunctional integrated point-of-care systems for TB diagnosis (Epigem Ltd); point-of-care TB test (Microsens, ProteinLogic, The Ideas Studio Ltd); next generation platforms for interferon gamma release assays (National Physics Laboratory); biomarker signatures as diagnostic for TB (ProteinLogic); point-of-care TB diagnostic with drug susceptibility testing (QuantmDx Ltd, Smiths Detection); feasibility of using amplification assays for point-of-care TB diagnosis (TwistDx Ltd).
Community acquired pneumonia: Exhaled breath test (Applied Nanodetectors Ltd), feasibility of using amplification assays for point-of-care *Mycoplasma pneumoniae* diagnosis (TwistDx Ltd).
Chlamydia and gonorrhoea: Ultra-rapid point-of-care diagnostic system (Altas Genetics); point-of-care duplex tests for chlamydia and gonorrhoea (Diagnostics for the real world), highly sensitive point-of-care platform for chlamydia and gonorrhoea detection (Mast Group Ltd); wireless biosensors for chlamydia detection and monitoring (OJ-Bio Ltd); multiplex point-of-care detection of multiple STI pathogens (Randox).
Methicillin-resistant Staphylococcus aureus (MRSA): point-of-care biosensors (Elisha systems Ltd, Sterilin Ltd), single-use point-of-care diagnostics (Geneform Technologies Ltd).
Gastrointestinal infections: rapid point-of-care test for urinary tract infection (Mologic), rapid diagnostic platform for *C. difficile* diagnosis (Sarum Biosciences).

Infection and sepsis:
Biomarker-based systems and flow cytometric systems (Beckton Dickinson); rapid multiplex systems (Biogene); laser optics to detect infectious agents (Cascade Technologies); disposable biosensors (highland Biosciences); point-of-care molecular tests (Health Protection Agency); protein markers for early onset sepsis (Inanovate); bioluminogenic pathogen detection (Hygiena International Ltd); rapid and microfluidic point-of-care tests for bacterial sepsis (L3 technology Ltd, Mast Group Ltd, Microlab Devices); portable immunosensors (Magna Parva), biomarkers for sepsis (Mologic); multiplex point-of-care biochip arrays for sepsis (Randox), intervention modelling in sepsis (X-labs).

New technologies:
Broad spectrum point-of-care diagnostics using high speed molecule detection systems including bioinformatics (Base4Innovations); point-of-care lateral flow devices (Foresite Diagnostics Ltd); single-step nucleic acid extraction methods for point-of-care tests (Lumora Ltd); novel methodology to detect Extended Spectrum Beta Lactamase-producing bacteria (Smiths Detection).

Clinical impact and cost-effectiveness studies:
Clinical value of point-of-care *Clostridium difficile* diagnosis and impact of point-of-care MRSA testing in emergency admissions (Cepheid); development of tools to assess cost-effectiveness and impact of point-of-care *Chlamydia* testing (Diagnostics for the real world); tools for the assessment of the impact of point-of-care tests for sexually transmitted infections (Health Protection Agency); quantification of the economic impact of point-of-care sepsis testing (Integrated Medicines); tool for the socio-economic assessment of the impact of point-of-care STI testing (Matrix Insight Ltd).

DISSEMINATION AND IMPLEMENTATION

Barriers to Adoption and Dissemination of Diagnostic Tests

In a systematic review of organisational factors influencing technology adoption and assimilation in the NHS, it was highlighted by Sir Derek Wanless, in his submission to the Health Select Committee, that '*the UK has been slow to adopt and diffuse new technologies*' (Robert et al). The Kings Fund also noted the adoption of new technology in the NHS is slow and disparate (Liddell et al). More recently the Department of Health's Innovation, Health and Wealth report emphasized a number of barriers to adoption and diffusion of new technologies. These included a general absence of an innovation culture, together with an absence of evidence to support innovation, as well as a financial framework that currently does not encourage innovation and change (Department of Health: Innovation Health and Wealth).

In relation to *in vitro* diagnostic tests there appear to be fundamental problems that are slowing down innovation and adoption: a slow rate of adoption and dissemination, and poor evidence of impact on outcomes, or return on investment. The NHS Atlas of variation has highlighted the slow pace of adoption and dissemination. For example, four years after the introduction of calprotectin, there was an almost 450-fold variation in it utilisation across primary care trusts. In the case of brain natriuretic peptide and its use in heart failure, launched in the UK 12 years ago there is an almost 300-fold variation in utilisation (NHS Atlas of Variation in Diagnostic Services).

The rate of adoption and dissemination of POCT is currently slower than that of laboratory based tests. A recent review of diagnostic tests effects on changes in patient outcome that included 140 trials found that outcomes were only significantly improved in 18% of these studies (Siontis et al); concluding that few tests currently have documented benefits on patient outcomes. Furthermore, compliance with guidelines for the use of established tests is variable and national guidance is seemingly an ineffective strategy to change testing behaviour (Driskell et al).

A 2009 report on the organisational and behavioural barriers to medical technology adoption (York Health Economics Consortium) reported key findings that resonate with the experiences of using *in vitro* diagnostics and represents an accurate picture of the barriers facing the adoption of new tests (Price). A more recent report by Deloittes on improving access to diagnostics makes similar points (Deloitte Centre for Health Solutions).

Key barriers to adoption are:

a) Lack of an innovation culture
b) Absence of priority setting
c) Limited Evidence-Base
d) Inadequate reimbursement schemes

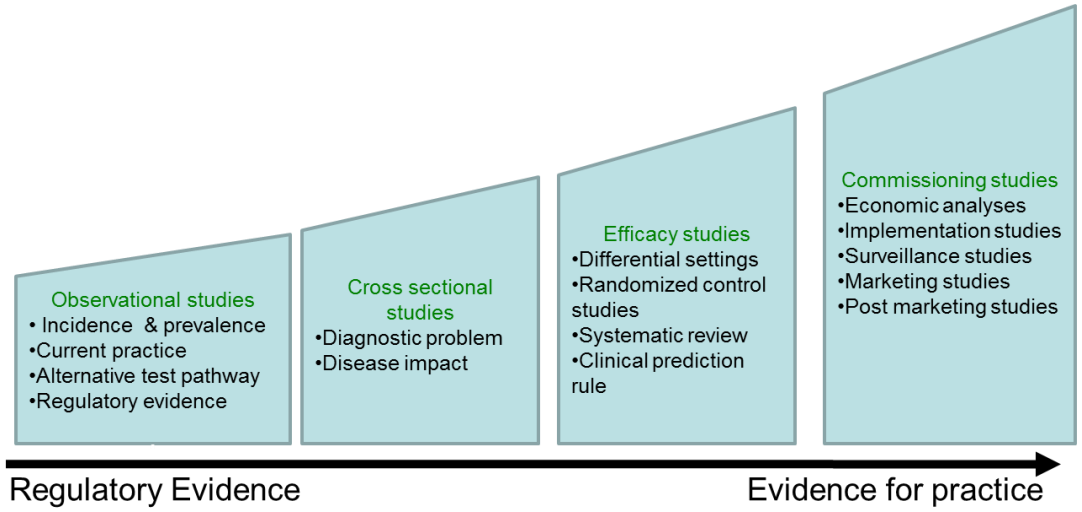
e) Budget Silos
f) Absence of decommissioning strategies
g) Insufficient modelling pathways to inform adoption
h) Developing a communication and implementation plan
i) Identifying new and emerging diagnostic technologies

***a) Lack of an innovation culture**:*
Managers, as well as staff in general, are risk averse and resistant to change. There is anecdotal experience in the field of *in vitro* diagnostics that there can be resistance to change within an organisation if the innovation is likely to lead to a reduction in the organisation's income stream. The organisation and management of diagnostic services into silos can further exacerbate this problem.

b) Absence of priority setting:
Defined by the World Health Organisation as the *"identification of unmet needs"*, priority setting is considered to be lacking in many organisations. This is particularly evident in the case of *in vitro* diagnostics where the business model is focussed on activity rather than outcomes. By being seen as a commoditised service, there is little incentive to demonstrate the impact on patient outcomes. Regulatory approval is currently focussed on analytical performance and test complexity, rather than on clinical effectiveness, although this is likely to change in the coming years.

***c) Limited Evidence Base*:**
The fundamental evidence requirements for any innovation in healthcare are seen as clinical and cost-effectiveness, together with a clear understanding of the safety and risks to patients associated with any innovation. It is also important to recognise that innovation can benefit a number of stakeholders including patients, clinicians, provider organisations, commissioners and governments. The adoption phase of any new technology also requires a clear pathway for translating this evidence into practice. In the case of the majority of *in vitro* diagnostic tests the main body of evidence is generally limited to that required to obtain regulatory approval. The evidence requirements for *in vitro* diagnostic tests however are very demanding. A test may be of value in a number of ways, e.g. screening, diagnosis, treatment optimisation and monitoring, as well as in a range of settings, e.g. self-, primary, secondary and tertiary care.

Several studies, including the horizon scanning work undertaken in Oxford (Monitoring and Diagnosis Oxford), have shown that the quality of the evidence to meet the requirements outlined here is generally quite limited. The number of technologies aimed at improving diagnostic accuracy is substantial and there are many more in development, with numerous patents filed each year. However, what is striking is the lack of progress in developing the evidence base for many technologies. As a consequence many are stuck at the laboratory phase or, having developed technical accuracy, have not progressed, or are not quite ready to progress to clinical trials that can provide evidence for practice and ultimately change clinical care.



d) Inadequate Reimbursement schemes:

In countries where formal reimbursement schemes exist, the foundation of such schemes is based on the complexity of the test (i.e. the time taken and equipment required to perform the test) and the cost of the consumables. Similar less formal tariffs operate in most other countries. The two major consequences of this are (i) a business model (i.e. cost-per-test) that rewards the number of tests performed – as against the benefits to using those tests (i.e. cost-per-outcome), and (ii) a disincentive to develop new tests.

e) Budget silos:

Budget silos are reported to be a major challenge for managers. Firstly, when a new clinical service or treatment is introduced invariably the role that the laboratory might play in the innovation is not considered. Secondly, and by far the more challenging, is the benefit of using any test that has been demonstrated to be clinically effective, does not accrue to the laboratory where the cost of the test was incurred. This may have important consequences for the introduction of tests related to antimicrobial resistance, as clinicians and clinical managers rarely understand the financial consequences of using diagnostic tests; e.g. in primary care the diagnostic test budget is often part of a block contract with secondary care.

f) Absence of decommissioning strategies:

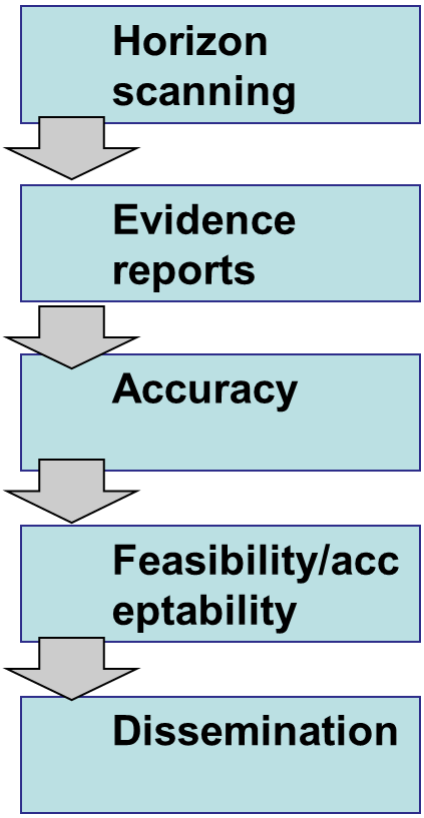
It is generally expected that adoption of new technology should replace the use of previous technology and/or process; the evidence indicates that decommissioning is not rigorously pursued. Thus while it might, in theory, be straightforward to reduce use of drugs (e.g. antibiotics) and blood products – as they are both considered as commodities, it is more difficult to decommission other resource categories, e.g. from reducing length of hospital stay, or clinic visits. As suggested earlier it may also be difficult because of the adverse effects on organisational income; a current example would be the transition of care from the secondary to the primary care sector, supported by the use of POCT.

g) Insufficient modelling pathways to inform adoption:

Two of the barriers outlined above, resource utilisation and process change, have begun to be addressed through the use of economic modelling. The former is based on simulation modelling approaches whilst the latter is primarily based on the use of 'Lean Thinking', now an integral part of quality improvement (Silvester et al). The Technology Strategy Board (now Innovate UK) following its investment of point-of-care technology development for infectious disease markers ran a competition for the development of modelling packages that could be employed in point-of-care testing scenarios. Sherrard-Smith et al have recently presented the first output from one of these developments enabling commissioners to model the epidemiological impact of POCT for Chlamydia in local settings; the same model can provide a health economic assessment of the use of POCT.

h) Developing a communication and implementation plan:
The need for communication involving all stakeholders underpins the overcoming of the barriers outlined above, from the cognition of the unmet needs through to implementation of new tests and devices. From the perspective of the diagnostic services professionals, this would be best achieved by refocussing of roles and working as part of the clinical team (Ham et al).

Communication is likely to play a major part in willingness on the part of clinicians to adopt new tests. It is interesting therefore to note, in a recent study of attitudes of primary care physicians to the use of POCT, the basic concerns were about the accuracy of tests, as well as the costs (including equipment maintenance etc.). There were also concerns about the over-reliance on the use of tests, and the risk of undermining clinical skills (Jones et al).



i) Identifying new and emerging diagnostic technologies:
New technologies are emerging at an increasing pace with numerous new patents filed. By the time of dissemination of this current report, the evidence base for many of the technologies outlined in the report will have moved on. Therefore, it would seem logical to have an ongoing horizon scanning service solely dedicated to antimicrobial resistance and emerging diagnostic technologies.

The aims of a horizon scanning are to:

- Identify new diagnostic technologies with most potential in primary and secondary care. Prioritise those with greatest health impact;
- Rapid evidence reports;
- Field studies of new point-of-care tests;
- Disseminate results back to HTA, NICE, Commissioners etc.

Increasingly variations in practice show the need to develop and implement interventions aimed at specific healthcare settings, which address the barriers to change as they are identified and when they arise. Without ongoing multifaceted strategies to address barriers and new technology is likely to fail. Addressing clinician barriers to change remains an important issue to address. At the clinical interface reasons for overuse of antibiotics are complex and multifactorial: three key elements are pressure from patients, diagnostic uncertainty and constraints on time. In addition clinicians may consider prescribing guidelines a limitation to their clinical freedom. Clinicians also want to prescribe what they think are the optimal medications for any given patients, which often can mean the use of a broad-spectrum agent, which protects against possible resistant organisms, at the expense of concerns over long term resistance.

Furthermore, to affect antibiotic resistance rates, and barriers to change, interventions need to be sustained over the long term: results from modelling studies suggest the time it takes to observe reductions in the incidence of antibiotic-resistant organisms is considerably longer than the time to reach high levels of resistance in the first place (Stewart et al 1998).

To understand, identify and overcome barriers to change NICE recommends (NICE, How to change practice) that any organisation needs to:

- Understand the types of barriers faced in healthcare.
- Identification of the barriers and the gap between recommended and current practice
- Understand that no one method will overcome the different barriers faced, and often interventions might prove effective for different people in different contexts.

A Health Foundation report on the literature on healthcare professional views on quality improvement initiatives concluded that engaging clinicians in improvement activities is likely to remain difficult and such non-engagement is a long-standing problem that is multifactorial and not limited to the UK (Health Foundation Report).

LIMITATIONS OF THE REPORT

In this report, we have sign-posted some of the key diagnostic technologies related to antimicrobial resistance and prescribing, however this should not be viewed as a definitive list of all the devices and tests on the market, but rather the types of technologies that are available or in development. We have focussed on the published literature as this allowed for a brief assessment of the current evidence base for the various technologies, since implementation of any technology should be informed by robust evidence regarding not only accuracy, but also clinical utility and cost-effectiveness. Although we have highlighted some evidence related to each technology (identifying systematic reviews where possible), the time-frame did not allow for a comprehensive review of the body of evidence, therefore this would need to be performed when considering a particular technology for further assessment or implementation.

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Appendix

TABLE 1: Summary table of diagnostic technologies

Technology	Conditions / Use	Summary	Level of Evidence	App-endix
PRIMARY CARE				
Rapid point-of-care blood and serum tests	Diagnosis of influenza	Point-of-care influenza tests could reduce antibiotic prescribing, particularly in children, but a lack of evidence and cost-effectiveness prevents widespread uptake in the NHS.	1	14
	Diagnosis of *Campylobacter*	Isolated point-of-care tests, which test for only one pathogen, are unlikely to prove effective given the range of intestinal pathogens and the current evidence of their effectiveness is lacking.	5	63
	Diagnosis of syphilis	Point-of-care syphilis tests appear to show adequate accuracy; however their utility and cost-effectiveness in informing antibiotic prescribing strategies in the UK setting requires assessment.	1	61
	Measurement of C-reactive protein	Evidence for the use of Point-Of-Care CRP tests to guide antibiotic treatment is mixed. It can be a useful tool to aid clinical assessment but the evidence suggests it should not be used alone or routinely.	1	37
	Measurement of procalcitonin	Point-of-care tests for procalcitonin to guide antibiotic treatment, are not ready for deployment in the NHS and require further research to develop the evidence base.	1	13
	Measurement of white blood cell counts	The role of point-of-care white blood cell count devices in diagnosis of serious infection and use to inform antibiotic prescribing (perhaps in conjunction with other markers) requires further research.	1	54
Rapid point-of-care urine stick tests	Diagnosis of *H. pylori*	Current point-of care urine tests for *H. pylori* are not accurate enough to warrant their widespread use in the NHS.	1	8
	Diagnosis of urinary tract infection (UTI)	Urine dipstick test alone seems to be useful in all populations to exclude the presence of infection if the results of both nitrites and leukocyte-esterase are negative.	1	48
	Diagnosis of UTI in children	Studies assessing the incremental accuracy of a rapid test for UTI in children compared with a clinical investigation alone are required.	1	47
Urinary culture and antibiotic susceptibility kit	Diagnosis of UTI	The use of a UTI diagnosis kit with susceptibility testing could aid diagnosis and, more importantly, antimicrobial treatment. Results of a recently completed RCT could influence how we manage UTI.	4	49
Rapid point-of-care urine or swab tests	Diagnosis of *C. trachomatis*	Current evidence suggests that the laboratory-based PCR tests are still the most accurate and cost-effective for diagnosing chlamydia and there is currently no robust evidence to support the use of POC chlamydia tests.	1	52
	Diagnosis of *N. gonorrhoeae*	The utility of these point-of-care tests for gonorrhoea in the UK setting is currently limited and based on current research, it is unclear if such tests would provide any benefit compared to current standard practice	1	51
Microfluidic biosensor	Detection of antibiotic resistance	Point–of-care tests for antibiotic resistance could have a significant impact on antibiotic treatment regimes in a wide range of diseases across both primary and secondary care. They are in the early stages of development and require a coordinated research strategy to facilitate their implementation into NHS care.	5	12
SECONDAY CARE				
Multiplex PCR	Diagnosis of gastrointestinal pathogens	Multiplex PCR systems, used for diagnosis of common gastrointestinal pathogens, are not ready for deployment in the NHS and require further research to develop the evidence base.	1	1
	Diagnosis of viral and bacterial respiratory pathogens	Implementation of a biofilm array panel reduces the time to test result and increase the proportion of results received in ED before admission, but currently has no significant impact on antibiotic prescription and use.	3	64
	Detection of PVA positive MRSA	Rapid tests with multiplex PCR and pulsed-field electrophoresis for the detection of PVA-positive MRSA are in the early stage of development and are unlikely to impact on microbial resistance.	5	34
	Detection of carbapenemase-producing bacteria	The clinical and cost-effectiveness of rapid laboratory-based tests, which detect carbapenemase-producing bacteria, needs to be established before implementation in the NHS can be considered.	3-4	2
PCR and other rapid tests	Diagnosis of gastrointestinal	Rapid diagnostic assays, especially PCR, for *Salmonella, Campylobacter* and *E. coli* O157 are highly accurate; however evidence regarding the	3	62

	pathogens	relative cost of implementing these tests in practice is currently sparse and unclear.		
PCR	Diagnosis of *M. pneumoniae* community acquired pneumonia	PCR has the potential to aid the diagnosis of *M. pneumoniae* prior to prescribing. However, the lack of clinical trials and cost-effectiveness information limit its application in the NHS.	1	3
	Diagnosis of methicillin resistant *S. aureus* (MRSA)	The evidence regarding the use of rapid diagnostic tests with PCR for the detection of MRSA in hospitalised patients is insufficient for implementation. It does not appear to offer advantages over conventional methods.	1	30; 31
	Detection of antimicrobial susceptibility	PCR-based techniques for susceptibility testing are in the early phases of development and currently lack evidence from clinical trials in humans.	3	16
	Detection of rifampicin resistance in *M. tuberculosis*	XpertMTB/RIF may be valuable as an add-on test following smear microscopy however it is not clear how the test would fit into the current diagnostic pathway. The test is expensive and lack of cost-effectiveness data prevents widespread uptake in the NHS.	1	11
	Evaluation of genetic mutations associated with *M. tuberculosis* resistance	Additional mutations appear to be associated with antibiotic resistance and could improve sensitivity and specificity of future diagnostics.	1	26
	Detection of *H. pylori* antibiotic resistance	PCR-based tests to detect genes conferring resistance to clarithromycin and can inform antibiotic prescribing practices. Further research on the utility and cost-effectiveness is required.	2	9
	Detection of group B *Streptococcus*	The role of rapid tests for the detection of Group B streptococcus is unclear. Cost effectiveness analyses are required to understand whether such tests offer any value over current screening methods.	2-3	67
	Detection of *Trichomonas vaginalis*	The rapid PCR-based test for *T. vaginalis* is at the early stages of development and requires further accuracy studies, as well as evidence regarding utility and cost-effectiveness.	5	68
PCR and String test	Detection of *H. pylori* antibiotic resistance	The string test does not require biopsy and may warrant further investigation as a less invasive method to obtain gastric fluid for testing.	2	9
PCR and high resolution melting curve analysis	Detection of pyrazinamide resistance in *M. tuberculosis*	Rapid detection of pyrazinamide resistant *M. tuberculosis* is in the early phase of assessment.	5	28
	Detection of rifampicin resistance in *M. tuberculosis*	HRMC might be a good alternative to conventional drug susceptibility tests in clinical practice; however its utility is limited by lack of clinical trial evidence.	1	29
Real-time PCR	Diagnosis of *M. pneumoniae* community acquired pneumonia	Real time PCR could promote targeted treatment for community acquired pneumonia but currently not hospitalised patients. The lack of clinical trials and cost-effectiveness information limit its application in the NHS.	2	4
	Diagnosis of sepsis	A systematic review of the accuracy of real-time PCR tests for multiple pathogens in the diagnosis of sepsis is currently in process and further research should await the results of this review.	1	44
	Diagnosis of chlamydia and gonorrhoea	Real-time PCR testing in clinics could reduce cost and clinician time and may lead to more appropriate and rapid care of patients, reducing number of patients lost to follow-up; however, utility and implementation studies are currently lacking.	5	53
PCR and LAMP (potential point-of-care test)	Diagnosis of *C. difficile*	PCR is a rapid useful test for diagnosing, with high sensitivity and specificity, but is expensive.	1	41
Loop-mediated isothermal amplification (LAMP)	Diagnosis of *M. pneumoniae*	Loop mediated isothermal amplification has high specificity, is easy to use and can rapidly detect *Mycoplasma*; however single test assays are unlikely to prove useful in clinical practice.	3	56
Modified multiple-locus variable number tandem repeat analysis	Diagnosis of *C. difficile*	Modified MLVA has high set-up costs, requires technical expertise and currently lacks clinical trial evidence.	5	39
DNA Microarray	Diagnosis of *M.*	ArrayStrip microarray is easy to use with potential high throughput,	5	55

	pneumoniae	high information content, and affordability. However, single test assays are unlikely to prove useful in clinical practice.		
	Detection of carbapenemase-producing bacteria	The clinical and cost-effectiveness of rapid laboratory-based tests, which detect carbapenemase-producing bacteria, needs to be established before implementation in the NHS can be considered.	3-4	2
	Detection of antimicrobial susceptibility	Microarray utility is limited by a lack of clinical studies including information on sensitivity and specificity.	5	18
Multiplex molecular platform (potential point-of-care tests)	Respiratory viruses and bacteria	Rapid detection of respiratory viruses may aid diagnosis of respiratory infections and inform decisions on antibiotic prescribing thus avoiding overuse of antibiotics. However, an adequately powered trial with antibiotic use as an outcome is currently needed.	1	60
Sequencing	Detection of mutations associated with high-level isoniazid resistance in *M. tuberculosis*	Identification of katG mutations associated with high-level isoniazid resistance in *Mycobacterium tuberculosis* is in the early phase of development.	5	25
Fluorescence in situ hybridisation (FISH)	Diagnosis of *S. aureus*	FISH could improve diagnostic accuracy and shorten time to commencement of targeted antimicrobial therapy. However there is a lack of clinical trials and cost-effectiveness for targeted, rapid strategies for delivery of antimicrobial therapy.	5	36
	Detection of *H. pylori* antibiotic resistance	Fluorescent in situ hybridization (FISH) can detect genes conferring resistance to clarithromycin and can inform antibiotic prescribing practices. Further research on the utility and cost-effectiveness is required.	2	9
Selective culture media and FISH	Diagnosis of *C. difficile*	Allows *C. difficile* strain typing and resistance testing, which allows for species identification and toxin detection within the same day. However the technology currently lacks clinical trial data and cost-effectiveness studies.	5	40
Rapid culture media	Diagnosis of *M. pneumoniae*	Rapid culture is expensive and seems to offer little benefit given it still takes one to three days to detect mycoplasma	3	59
Colorimetric culture systems	Diagnosis of community acquired pneumonia	The Bac T/Alert 3D test is reliable, time-saving, cost-effective and might reduce mortality. Randomised comparisons are required to confirm the important findings of the comparative study.	2	6
	Bloodstream infections	The Bac T/Alert 3D test is reliable, time-saving, cost-effective and might reduce mortality. Randomised comparisons are required to confirm the important findings of the comparative studies.	2	66
Colorimetric indicator tests	Carbapenemase-producing bacteria	The clinical and cost-effectiveness of rapid laboratory-based tests, which detect carbapenemase-producing bacteria, needs to be established before implementation in the NHS can be considered.	3-4	2
E-test antibiotic strips	Antimicrobial susceptibility in ventilator-associated pneumonia	Rapid E-test was associated with fewer days of fever, fewer days of antibiotic administration until resolution of the episode of ventilator-associated pneumonia. Implementation in the NHS is limited by insufficient evidence; therefore a further trial with clinical and cost-effectiveness is warranted.	2	17
Short multi-capillary gas chromatography column	Diagnosis of *C. difficile*	Short multi-capillary chromatography column is in the early phase of development. The accuracy of the technology requires improvement.	5	42
Microfluidics ("lab on a chip") (potential point-of-care tests)	Diagnosis of *M. pneumoniae*	Microfluidics tests are easy to use with potential high throughput and affordability; however single test assays are unlikely to prove useful in clinical practice.	3	58
	Diagnosis of urinary tract infection	The use of microfluidics with susceptibility testing could aid diagnosis and, more importantly, antimicrobial treatment in UTI. Randomised controlled trials are required to determine patient benefit.	4	50
	Antimicrobial susceptibility testing	Microfluidic systems are a new innovation that could potentially aid point-of-care testing for antimicrobial susceptibility however they are in the early phase of development.	5	15
Biosensor platforms (potential point-of-care tests)	Antimicrobial susceptibility testing	Biosensor systems could have a role in improving the rapid detection of UTI. Research aimed at integrating biosensors with microfluidic technology and automation of sample processing for point of care application is required.	4	45
Mass	Detection of	The clinical and cost-effectiveness of rapid laboratory-based tests,	3-4	2

spectrometry (MALDI-TOF)	carbapenemase-producing bacteria	which detect carbapenemase-producing bacteria, needs to be established before implementation in the NHS can be considered.		
	Detection of antimicrobial susceptibility	MALDI-TOF MS is in the early phase of development and it remains to be seen if this approach offers sufficient sensitivity and specificity to influence clinical practice.	5	17
Immuno-chromatography (point-of-care tests)	Diagnosis of *C. difficile*	Immunochromotography tests of stool specimens for *C. difficile* infection could prove to be a reliable first-line method. Further clinical studies are warranted.	4	38
	Diagnosis of *S. aureus*	A rapid test that is still in the early stage of development but does have high accuracy. It could shorten the time between the test and commencement of antimicrobial therapy.	5	35
	Diagnosis of community acquired pneumonia	Immunochromatographic test could be a useful addition to the current diagnostic workup for community acquired pneumonia. However, a lack of clinical trials and cost-effectiveness information limit its application in the NHS.	1	5
	Diagnosis of streptococcal infections	Additional research on RADTs is not required until the variability in sensitivity is reduced and the detection of non-group A strains that commonly cause Streptococcal throat are developed. Further validation of the symptom-based clinical score (FeverPAIN) is required.	4	65
	Diagnosis of MRSA	Rapid diagnostic tests with immunoassay have high sensitivity and specificity for detecting MRSA, however their use in clinical practice and the benefits over conventional detection methods remains to be elucidated.	3	32
Antigen detection tests	Diagnosis of tuberculosis	Antigen detection test for TB are unlikely to impact on antibiotic resistance. The poor quality of the available evidence limits their utility. The quality of the evidence remains very low. The WHO policy statement recommends against serological tests.	1	21, 22
	Diagnosis of *H. pylori*	NICE guidance currently recommends the use of *H.pylori* stool antigen test for the assessment of patients with dyspepsia.	1	8
	Diagnosis of Group B *Streptococcus*	The role of rapid tests for the detection of Group B strep is unclear. Cost effectiveness analyses are required to understand whether such tests offer any value over current screening methods.	2-3	67
Interferon gamma release assays	Diagnosis of tuberculosis	IGRAs show promise for improving TB diagnosis in immunocompetent children aged over 5 in high income settings. However neither of the available tests can rule out nor confirm the certainty of diagnosis, and interpretation of results may be difficult.	1	23
Glutaraldehyde test	Diagnosis of tuberculosis	Along with conventional diagnostic tests, the glutaraldehyde test could be a rapid, easy, cost-effective and reliable test for the diagnosis TB, particularly in low resource settings.	3	24
Rapid TB tests:	Diagnosis of tuberculosis	Studies from low-prevalence countries strongly suggest that the RD1 antigen-based assays are more accurate than TST- and PPD-based assays for diagnosis of latent TB infection.	1	27
Genotypic and phenotypic tests	Identification of multi-drug resistant *M. tuberculosis*	These tests could help to diagnose MTBDR faster compared to conventional culture in areas with high TB prevalence. However, in areas of low prevalence, the possibility of false negatives exists and a lack of cost-effectiveness data negates impact on practice.	1	10
Combination of markers	Diagnosis of sepsis	Combining biomarkers might be a useful strategy for improving diagnosis, however there is a lack of clinical studies and cost effectiveness, which is preventing uptake.	3	43
Cell phone-based micro-photometric system	Antimicrobial susceptibility testing	Gas-permeable microwell arrays are simple to use and likely to be a cost effective for antimicrobial susceptibility testing and require further clinical studies to assess their role in clinical care.	5	46
Cell lysis assays	Antimicrobial susceptibility testing	Cell lysis techniques show good correlation with microdilution and E-test data, and have the ability to provide approximate MIC values. However, these techniques are yet to be tested using clinical samples.	5	19
Chemi-luminescence drug susceptibility assays	Detection of *S. aureus* with reduced sensitivity to vancomycin	A rapid test that may supersede classical methods for testing susceptibility, however validation studies and clinical trials are needed to test and confirm diagnostic accuracy.	5	33
Breath tests	Carbon-13 urea breath test for the diagnosis of *H. pylori*	NICE guidance currently recommends the use of *H. pylori* Carbon-13 urea breath test for the assessment of patients with dyspepsia.	1	8
	Breath test for infectious disease diagnosis/sepsis	Breath tests for the diagnosis of infectious agents are at the proof-of-concept stage and require substantial further validation, assessment of accuracy and clinical utility	5	69

TABLE 2: Summary table of diagnostic technologies by pathogen and use

Disease/Pathogen	Use	Technology	Summary	Level of Evidence	Appendix
Multiple gastrointestinal pathogens	Pathogen detection	Multiplex PCR	Multiplex PCR systems, used for diagnosis of common gastrointestinal pathogens, are not ready for deployment in the NHS and require further research to develop the evidence base.	1	1
	Pathogen detection	Multiplex PCR	Implementation of a biofilm array panel reduces the time to test result and increase the proportion of results received in ED before admission, but currently has no significant impact on antibiotic prescription and use.	3	64
	Pathogen detection	PCR	Rapid diagnostic assays, especially PCR, for *Salmonella, Campylobacter* and *E. coli* O157 are highly accurate; however evidence regarding the relative cost of implementing these tests in practice is currently sparse and unclear.	3	62
Campylobacter	Pathogen detection	Point-of-care blood/serum tests	Isolated point-of-care tests, which test for only one pathogen, are unlikely to prove effective given the range of intestinal pathogens and the current evidence of their effectiveness is lacking.	5	63
Clostridium difficile	Pathogen detection	PCR and LAMP (potential point-of-care test)	Real-time PCR testing in clinics could reduce cost and clinician time and may lead to more appropriate and rapid care of patients, reducing number of patients lost to follow-up; however, utility and implementation studies are currently lacking.	1	41
	Pathogen detection	Modified multiple-locus variable number tandem repeat analysis	Modified MLVA has high set-up costs, requires technical expertise and currently lacks clinical trial evidence.	5	39
	Pathogen detection and antimicrobial resistance	Selective culture media and FISH	Allows *C. difficile* strain typing and resistance testing, which allows for species identification and toxin detection within the same day. However the technology currently lacks clinical trial data and cost-effectiveness studies.	5	40
	Pathogen detection	Short multi-capillary gas chromatography column	Short multi-capillary chromatography column is in the early phase of development. The accuracy of the technology requires improvement.	5	42
	Pathogen detection	Immuno-chromatography (potential point-of-care test)	Immunochromotography tests of stool specimens for *C. difficile* infection could prove to be a reliable first-line method. Further clinical studies are warranted.	4	38
Helicobacter pylori	Pathogen detection	Point-of-care urine test	Current point-of care urine tests for *H. pylori* are not accurate enough to warrant their widespread use in the NHS.	1	8
	Pathogen detection	Antigen detection tests	NICE guidance currently recommends the use of *H.pylori* stool antigen test for the assessment of patients with dyspepsia.	1	8
	Pathogen detection	Carbon-13 urea breath test	NICE guidance currently recommends the use of *H. pylori* Carbon-13 urea breath test for the assessment of patients with dyspepsia.	1	8
	Pathogen detection and antimicrobial resistance	PCR and string test	The string test does not require biopsy and may warrant further investigation as a less invasive method to obtain gastric fluid for testing.	2	9
	Detection of antimicrobial resistance	PCR	PCR-based tests to detect genes conferring resistance to clarithromycin and can inform antibiotic prescribing practices. Further research on the utility and cost-effectiveness is required.	2	9
	Detection of antimicrobial resistance	Fluorescence in situ hybridisation (FISH)	Fluorescent in situ hybridization (FISH) can detect genes conferring resistance to clarithromycin and can inform antibiotic prescribing practices. Further research on the utility and cost-effectiveness is required.	2	9
Chlamydia trachomatis	Pathogen detection	Point-of-care urine or swab test	Current evidence suggests that the laboratory-based PCR tests are still the most accurate and cost-effective for diagnosing chlamydia and there is currently no	1	52

			robust evidence to support the use of POC chlamydia tests.		
Neisseria gonorrhoea	Pathogen detection	Point-of-care urine or swab test	The utility of these point-of-care tests for gonorrhoea in the UK setting is currently limited and based on current research, it is unclear if such tests would provide any benefit compared to current standard practice	1	51
Chlamydia and N. gonorrhoea	Pathogen detection	Real-time PCR	Real-time PCR testing in clinics could reduce cost and clinician time and may lead to more appropriate and rapid care of patients, reducing number of patients lost to follow-up; however, utility and implementation studies are currently lacking.	5	53
Syphilis	Pathogen detection	Point-of-care blood/serum tests	Point-of-care syphilis tests appear to show adequate accuracy; however their utility and cost-effectiveness in informing antibiotic prescribing strategies in the UK setting requires assessment.	1	61
Urinary tract infection	Detection of infection	Point-of-care urine test	Urine dipstick test alone seems to be useful in all populations to exclude the presence of infection if the results of both nitrites and leukocyte-esterase are negative.	1	48
	Detection of infection	Point-of-care urine test	Studies assessing the incremental accuracy of a rapid test for UTI in children compared with a clinical investigation alone are required.	1	47
	Detection of infection and susceptibility to antimicrobials	Point-of-care urine culture test with antibiotic susceptibility detection	The use of a UTI diagnosis kit with susceptibility testing could aid diagnosis and, more importantly, antimicrobial treatment. Results of a recently completed RCT could influence how we manage UTI.	4	49
	Pathogen detection and antimicrobial resistance	Microfluidics ("lab on a chip") (potential point-of-care test)	The use of microfluidics with susceptibility testing could aid diagnosis and, more importantly, antimicrobial treatment in UTI. Randomised controlled trials are required to determine patient benefit.	4	50
Mycoplasma pneumoniae	Pathogen detection	PCR	PCR has the potential to aid the diagnosis of *M. pneumoniae* prior to prescribing. However, the lack of clinical trials and cost-effectiveness information limit its application in the NHS.	1	3; 57
	Pathogen detection	Real-time PCR	Real time PCR could promote targeted treatment for community acquired pneumonia but currently not hospitalised patients. The lack of clinical trials and cost-effectiveness information limit its application in the NHS.	2	4
	Pathogen detection	Loop-mediated isothermal amplification (LAMP)	Loop mediated isothermal amplification has high specificity, is easy to use and can rapidly detect *Mycoplasma*; however single test assays are unlikely to prove useful in clinical practice.	3	56
	Pathogen detection	DNA Microarray	ArrayStrip microarray is easy to use with potential high throughput, high information content, and affordability. However, single test assays are unlikely to prove useful in clinical practice.	5	55
	Pathogen detection	Rapid culture media	Rapid culture is expensive and seems to offer little benefit given it still takes one to three days to detect mycoplasma	3	59
	Pathogen detection	Microfluidics ("lab on a chip") (potential point-of-care test)	Microfluidics tests are easy to use with potential high throughput and affordability; however single test assays are unlikely to prove useful in clinical practice.	3	58
Community acquired pneumonia	Pathogen detection	Colorimetric culture systems	The Bac T/Alert 3D test is reliable, time-saving, cost-effective and might reduce mortality. Randomised comparisons are required to confirm the important findings of the comparative study.	2	6
	Pathogen detection	Immuno-chromatography (point-of-care)	Immunochromatographic test could be a useful addition to the current diagnostic workup for community acquired pneumonia. However, a lack of clinical trials and cost-effectiveness information limit its application in the NHS.	1	5
Multiple respiratory viruses and bacteria	Pathogen detection	Multiplex molecular platform (potential point-of-care test)	Rapid detection of respiratory viruses may aid diagnosis of respiratory infections and inform decisions on antibiotic prescribing thus avoiding overuse of antibiotics. However, an adequately powered trial with	1	60

			antibiotic use as an outcome is currently needed.		
Staphylococcus aureus	Pathogen detection	Fluorescence in situ hybridisation	FISH could improve diagnostic accuracy and shorten time to commencement of targeted antimicrobial therapy. However there is a lack of clinical trials and cost-effectiveness for targeted, rapid strategies for delivery of antimicrobial therapy.	5	36
	Pathogen detection	Immuno-chromatography (point-of-care)	A rapid test that is still in the early stage of development but does have high accuracy. It could shorten the time between the test and commencement of antimicrobial therapy.	5	35
	Pathogen detection and antimicrobial sensitivity.	Chemi-luminescence drug susceptibility assays	Detection of S. aureus with reduced sensitivity to vancomycin A rapid test that may supersede classical methods for testing susceptibility, however validation studies and clinical trials are needed to test and confirm diagnostic accuracy.	5	33
Methicillin resistant Staphylococcus aureus	Detection of pathogen and antimicrobial resistance	Multiplex PCR	Rapid tests with multiplex PCR and pulsed-field electrophoresis for the detection of PVA-positive MRSA are in the early stage of development and are unlikely to impact on microbial resistance.	5	34
	Detection of pathogen and antimicrobial resistance	PCR	The evidence regarding the use of rapid diagnostic tests with PCR for the detection of MRSA in hospitalised patients is insufficient for implementation. It does not appear to offer advantages over conventional methods.	1	30; 31
	Pathogen detection	Immuno-chromatography (point-of-care)	Rapid diagnostic tests with immunoassay have high sensitivity and specificity for detecting MRSA, however their use in clinical practice and the benefits over conventional detection methods remains to be elucidated.	3	32
Mycobacterium tuberculosis	Detection of antimicrobial susceptibility/rifampicin resistance	PCR	XpertMTB/RIF may be valuable as an add-on test following smear microscopy however it is not clear how the test would fit into the current diagnostic pathway. The test is expensive and lack of cost-effectiveness data prevents widespread uptake in the NHS.	1	11
	Detection of rifampicin resistance	PCR	Additional mutations appear to be associated with antibiotic resistance and could improve sensitivity and specificity of future diagnostics.	1	26
	Detection of rifampicin resistance	PCR and high resolution melting curve analysis	HRMC might be a good alternative to conventional drug susceptibility tests in clinical practice; however its utility is limited by lack of clinical trial evidence.	1	29
	Detection of pyrazinamide resistance	PCR and high resolution melting curve analysis	Rapid detection of pyrazinamide resistant *M. tuberculosis* is in the early phase of assessment.	5	28
	Detection of isoniazid resistance	Sequencing	Identification of katG mutations associated with high-level isoniazid resistance in *Mycobacterium tuberculosis* is in the early phase of development.	5	25
	Pathogen detection	Antigen detection tests	Antigen detection test for TB are unlikely to impact on antibiotic resistance. The poor quality of the available evidence limits their utility. The quality of the evidence remains very low. The WHO policy statement recommends against serological tests.	1	21, 22
	Pathogen detection	Interferon gamma release assays	IGRAs show promise for improving TB diagnosis in immunocompetent children aged over 5 in high income settings. However neither of the available tests can rule out nor confirm the certainty of diagnosis, and interpretation of results may be difficult.	1	23
	Pathogen detection	Glutaraldehyde test	Along with conventional diagnostic tests, the glutaraldehyde test could be a rapid, easy, cost-effective and reliable test for the diagnosis TB, particularly in low resource settings.	3	24
	Pathogen detection	Rapid TB tests:	Studies from low-prevalence countries strongly suggest that the RD1 antigen-based assays are more accurate than TST- and PPD-based assays for diagnosis of latent TB infection.	1	27
	Pathogen detection: Identification of multi-drug resistant *M.*	Genotypic and phenotypic tests	These tests could help to diagnose MTBDR faster compared to conventional culture in areas with high TB prevalence. However, in areas of low prevalence, the possibility of false negatives exists and a lack of cost-effectiveness data negates impact on practice.	1	10

	tuberculosis				
Group B Streptococcus	Pathogen detection	PCR	The role of rapid tests for the detection of Group B streptococcus is unclear. Cost effectiveness analyses are required to understand whether such tests offer any value over current screening methods.	2-3	67
	Pathogen detection	Immuno-chromatography (point-of-care)	Additional research on RADTs is not required until the variability in sensitivity is reduced and the detection of non-group A strains that commonly cause Streptococcal throat are developed. Further validation of the symptom-based clinical score (FeverPAIN) is required.	4	65
	Pathogen detection	Antigen detection tests	The role of rapid tests for the detection of Group B strep is unclear. Cost effectiveness analyses are required to understand whether such tests offer any value over current screening methods.	2-3	67
Sepsis	Pathogen detection	Colorimetric culture systems	The Bac T/Alert 3D test is reliable, time-saving, cost-effective and might reduce mortality. Randomised comparisons are required to confirm the important findings of the comparative studies.	2	66
	Pathogen detection	Breath tests	Breath tests for the diagnosis of infectious agents are at the proof-of-concept stage and require substantial further validation, assessment of accuracy and clinical utility	5	69
	Diagnosis of sepsis	Real-time PCR	A systematic review of the accuracy of real-time PCR tests for multiple pathogens in the diagnosis of sepsis is currently in process and further research should await the results of this review.	1	44
Trichomonas vaginalis	Pathogen detection	PCR	The rapid PCR-based test for *T. vaginalis* is at the early stages of development and requires further accuracy studies, as well as evidence regarding utility and cost-effectiveness.	5	68
Influenza	Pathogen detection	Rapid point-of-care tests to diagnose influenza	Point-of-care influenza tests could reduce antibiotic prescribing, particularly in children, but a lack of evidence and cost-effectiveness prevents widespread uptake in the NHS.	1	14
HOST RESPONSE					
C-reactive protein	Assessment of inflammation	Point-of-care blood/serum tests	Evidence for the use of Point-Of-Care CRP tests to guide antibiotic treatment is mixed. It can be a useful tool to aid clinical assessment but the evidence suggests it should not be used alone or routinely.	1	37
Procalcitonin	Assessment of inflammation	Point-of-care blood/serum tests	Point-of-care tests for procalcitonin to guide antibiotic treatment, are not ready for deployment in the NHS and require further research to develop the evidence base.	1	13
White blood cell counts	Assessment of inflammation	Point-of-care blood/serum tests	The role of point-of-care white blood cell count devices in diagnosis of serious infection and use to inform antibiotic prescribing (perhaps in conjunction with other markers) requires further research.	1	54
Combinations of markers	Diagnosis of sepsis	Various tests	Combining biomarkers might be a useful strategy for improving diagnosis, however there is a lack of clinical studies and cost effectiveness, which is preventing uptake.	3	43
ANTIBIOTIC RESISTANCE (multiple pathogens)					
Multiple pathogens	Detection of antimicrobial resistance	Point-of-care tests	Point–of-care tests for antibiotic resistance could have a significant impact on antibiotic treatment regimes in a wide range of diseases across both primary and secondary care. They are in the early stages of development and require a coordinated research strategy to facilitate their implementation into NHS care.	5	12
	Detection of antimicrobial resistance	PCR	PCR-based techniques for susceptibility testing are in the early phases of development and currently lack evidence from clinical trials in humans.	3	16
	Detection of antimicrobial resistance	DNA Microarray	Microarray utility is limited by a lack of clinical studies including information on sensitivity and specificity.	5	18
	Detection of antimicrobial resistance	E-test antibiotic strips	Rapid E-test was associated with fewer days of fever, fewer days of antibiotic administration until resolution of the episode of ventilator-associated pneumonia. Implementation in the NHS is limited by insufficient	2	7

			evidence; therefore a further trial with clinical and cost-effectiveness is warranted.		
	Detection of antimicrobial susceptibility	Microfluidics ("lab on a chip") (potential point-of-care test)	Microfluidic systems are a new innovation that could potentially aid point-of-care testing for antimicrobial susceptibility however they are in the early phase of development.	5	15
	Detection of antimicrobial susceptibility	Biosensor platforms (potential point-of-care tests)	Biosensor systems could have a role in improving the rapid detection of UTI. Research aimed at integrating biosensors with microfluidic technology and automation of sample processing for point of care application is required.	4	45
	Detection of antimicrobial susceptibility	Mass spectrometry (MALDI-TOF)	MALDI-TOF MS is in the early phase of development and it remains to be seen if this approach offers sufficient sensitivity and specificity to influence clinical practice.	5	17
	Detection of antimicrobial susceptibility	Cell phone-based micro-photometric system	Gas-permeable microwell arrays are simple to use and likely to be a cost effective for antimicrobial susceptibility testing and require further clinical studies to assess their role in clinical care.	5	46
	Detection of antimicrobial susceptibility	Cell lysis assays	Cell lysis techniques show good correlation with microdilution and E-test data, and have the ability to provide approximate MIC values. However, these techniques are yet to be tested using clinical samples.	5	19
	Detection of carbapene-mase producing bacteria	Multiplex PCR	The clinical and cost-effectiveness of rapid laboratory-based tests, which detect carbapenemase-producing bacteria, needs to be established before implementation in the NHS can be considered.	3-4	2
	Detection of carbapene-mase producing bacteria	DNA Microarray	The clinical and cost-effectiveness of rapid laboratory-based tests, which detect carbapenemase-producing bacteria, needs to be established before implementation in the NHS can be considered.	3-4	2
	Detection of carbapene-mase producing bacteria	Colorimetric indicator tests	The clinical and cost-effectiveness of rapid laboratory-based tests, which detect carbapenemase-producing bacteria, needs to be established before implementation in the NHS can be considered.	3-4	2
	Detection of carbapene-mase producing bacteria	Mass spectrometry (MALDI-TOF)	The clinical and cost-effectiveness of rapid laboratory-based tests, which detect carbapenemase-producing bacteria, needs to be established before implementation in the NHS can be considered.	3-4	2

TABLE 3: Summary table of diagnostic technologies including companies/research groups, level of readiness and ongoing UK-funded research

Technology	Conditions / Use	Company or research group	Level of Readiness	App-endix
PRIMARY CARE				
Rapid point-of-care blood and serum tests	Diagnosis of influenza	Alere, Becton Dickinson, Quidel, Sekisui, Meridian Bioscience, SA Sceintific, (all USA), All Diag (France), Medix Biochemica (Finland) , CorisBioconcept (Belgium), Denka Seiken, Fujirebio (both Japan)	On the market	14
	Diagnosis of *Campylobacter*	Immunocard Stat! CAMPY, Meridian Bioscience, USA	On the market	63
	Diagnosis of syphilis	Determine, Abbott (USA), SD Bioloines (S Korea),Syphicheck, Qualpro (India), VisiTect, Omega (UK),	On the market	61
	Measurement of C-reactive protein	Alere, Orion Diagnostica, Axis Shield, Eurolyser Smart	On the market with all of these companies	37
	Measurement of procalcitonin	Brahms	On the market	13
	Measurement of white blood cell counts	Philips Hemocue Sysmex	All on the market	54
	Home monitoring of white cell count	Philips Healthcare. Funded by Innovate UK	Commercialisation 2015	*
	Sepsis markers	Mast Group, PHE, University of Manchester. Funded by Innovate UK	Commercialisation date not known	*
Rapid point-of-care urine stick tests	Diagnosis of *H. pylori*	RAPIRUN, Otsuka Pharmaceutical Co., Japan	On the market	8
	Diagnosis of urinary tract infection (UTI)	Menarini, Siemens and Roche are main suppliers	All on the market	48
	ESBL urinary tract infections	Mologic, University of Swansea. Funded by Innovate UK	Commercialisation date not known	*
	Diagnosis of UTI in children	Menarini, Siemens and Roche are main suppliers	All on the market	47
Urinary culture and antibiotic susceptibility kit	Diagnosis of UTI	Test kit made by Statens Serum Institut., Copenhagen	On the market	49
Rapid point-of-care urine or swab tests	Diagnosis of *C. trachomatis*	Alere	On the market	52
	Diagnosis of *N. gonorrhoeae*	PATH GC Check, Seattle Biostar, City of Industry, California NOW gonorrhoea test, Alere	All three on the market	51
	Diagnosis of *C trachomatis and N. gonorrhoeae*	Atlas Genetics and PHE. Funded by Innovate UK	CE marking mid 2015	*
	Diagnosis of *C trachomatis and N. gonorrhoeae*	Mast Group Ltd, PHE, Liverpool University, John Moores University. Funded by Innovate UK	CE marking date not known	*
	Diagnosis of *C trachomatis and N. gonorrhoeae*	Diagnostics for the Real World, University of Cambridge. Funded by Innovate UK	CE marking date not known	*
	MRSA in the community	Geneform technologies Ltd. Funded by Innovate UK	Commercialisation date not known	*
Microfluidic biosensor	Detection of antibiotic resistance	University researchers USA	Not on the market and readiness not known	12
SECONDAY CARE				
Rapid point-of-care testing platforms	Bacterial sepsis	L3 Technologies Ltd, Sepsis Ltd, University of Liverpool. C	Commercialisation date not known	*
	Infectious disease	Optigene, University of Southampton, LGC Ltd. Funded by	Commercialisation	*

	detection	Innovate UK	date not known	
	Influenza A/B	Iquum, recently purchased by Roche	Likely within the next 12 months	60
	Influenza A/B	Oxford Nanopore, PHE, Science and Technology Facilities Council. Funded by Department of Trade and industry	Commercialisation date not known	*
Automated laboratory platforms	Infectious disease detection and antibiotic resistance	Abbott IRIDICA: in development	Commercialisation date not known	
	Infectious disease detection and antibiotic resistance	Cepheid GeneXpert; Roche Cobas, LightCycler, and SeptiFast; Becton Dickinson GeneOhm; BioFire FilmArray recently acquired by Biomerieux	Range of markers on the market	
Single molecule, short DNA probes and bioinformatics	POCT platform	Base4Innovation, University of Cambridge, Wellcome Trust Sanger institute. Funded by Innovate UK	Commercialisation date not known	*
Phage-lysin actuated biosensor	Detection of MRSA	Sterilin, FERA. Funded by Innovate UK	Commercialisation date not known	*
Multiplex PCR	Diagnosis of gastrointestinal pathogens	Masscode Luminex xTag	Both on market	1
	Diagnosis of viral and bacterial respiratory pathogens	BioFire Diagnostics, now part of Biomerieux	On the market	64
	Detection of PVA positive MRSA	Research application (Japan); commercialisation not known	Commercialisation not known	34
	Detection of carbapenemase-producing bacteria	Cepheid. USA Check-Point. Netherlands	Available in 2015	2
	Biochip array for ten STI agents	Randox and University of Hull. Funded by Innovate UK	Commercialisation date not known	*
	Biochip array for sepsis pathogens	Randox and Queens University Belfast. Funded by Innovate UK	Commercialisation date not known	*
PCR and other rapid tests	Diagnosis of gastrointestinal pathogens	Unable to identify commercial assays evaluated	Commercial assays available	62
PCR	Diagnosis of *M. pneumoniae* community acquired pneumonia	Cepheid Mologic Roche LightCycler	All on the market	3
	Use of recombinase polymerase in the rapid diagnosis of *M. pneumoniae* community acquired pneumonia	TwistDX and Kingston University and Vet Labs Agency. Funded by Innovate UK	Commercialisation date not known	*
	Diagnosis of methicillin resistant *S. aureus* (MRSA)	XPert, Cepheid, USA GeneOhm, Becton Dickinson, USA MRSA ID, Biomerieux, France Cobas, Roche	All on the market	30; 31
	Detection of antimicrobial susceptibility	GeneXpert, Cepheid, USA	On the market	16
	Detection of rifampicin resistance in *M. tuberculosis*	GeneXpert Cepheid platform, USA	On the market	11
	Multidrug resistant tuberculosis	Enigma Diagnostics Ltd, health Research Ltd, Imperial College London. Funded by Innovate UK	Commercialisation date not known	*
	Evaluation of genetic mutations	Research application, commercialisation not undertaken	Not commercially available	26

	associated with *M. tuberculosis* resistance			
	Detection of *H. pylori* antibiotic resistance	Seeplex H-pylori, Seegene, S Korea	On the market	9
	Detection of group B *Streptococcus*	GeneXpert, Cepheid, USA	On the market	67
	Detection of *Trichomonas vaginalis*	Rapid PCR-based test for *T. vaginalis* is at the early stages of development Atlas Genetics. Platform funded by Innovate UK	Commercialisation date not known	*68
	ESBL bacteria detection by POCT	Smith Detection Ltd and University of Edinburgh. Funded by Innovate UK	Commercialisation date not known	*
	Detection of C difficile	Roche Cobas 4800	On the market	41
Whole genome sequencing	Antimicrobial susceptibility	Research groups	Not known	20
PCR and String test	Detection of *H. pylori* antibiotic resistance	HDC Corporation, CA, USA	On the market	9
PCR and high resolution melting curve analysis	Detection of pyrazinamide resistance in *M. tuberculosis*	Research application only	Not commercially available	28
	Detection of rifampicin resistance in *M. tuberculosis*	Systematic review of research data using a specific molecular technique	Not commercially available	29
Real-time PCR	Diagnosis of *M. pneumoniae* community acquired pneumonia	HIRATAN; research technique in Japan	Not commercially available	4
	Diagnosis of sepsis	Sepsitest: Molzym Molecular Diagnostics Magicplex Sepsis test: Seegene, Korea SeptiFast LightCycler: Roche	On the market	44
	Diagnosis of chlamydia and gonorrhoea	Cepheid GeneXPert	On the market	53
PCR and LAMP (potential point-of-care test)	Diagnosis of *C. difficile*	PCR – Becton Dickinson GeneOhm, Cepheid XPert , Roche Light Cycler, GenProbe Progastro GenProbe LAMP research only	All on the market	41
Loop-mediated isothermal amplification (LAMP)	Diagnosis of *M. pneumoniae*	Research application in Japan; commercialisation not known	Not commercialised	56
Modified multiple-locus variable number tandem repeat analysis	Diagnosis of *C. difficile*	Research application; commercialisation not known	Not commercially available	39
DNA Microarray	Diagnosis of *M. pneumoniae*	ArrayStrip microarray is a research application platform; commercialisation not known	Not commercially available	55
	Detection of carbapenemase-producing bacteria	Check-Point, Netherlands	On the market	2
	Detection of antimicrobial susceptibility	Check-Points, Netherlands	On the market	18
Multiplex molecular platform (potential point-of-care tests)	Respiratory viruses and bacteria	Mari-POCT, Spain Nanosphere Verigene Respiratory Virus Plus, USA Prodesses, GenProbe, USA FilmArray, BioFire (now part of Biomerieux) Respiratory Multiplex Array, Randox, UK (not POCT assay	All on the market	60
	Sepsis markers	PHE, Atlas Genetics, Randox, Cardiff University of	Commercialisation	*

		Nottingham Trent University. Funded by Innovate UK	date not known	
Multifunctional Integrated Microsystem	Sepsis markers	Epigem Ltd, MV Diagnostics Ltd, SAW Dx Ltd, Universities of Edinburgh, Glasgow and Southampton and University College London. Funded by Innovate UK	Commercialisation date not known	*
Sequencing	Detection of mutations associated with high-level isoniazid resistance in *M. tuberculosis*	Research application in early stage of development	Not commercially available	25
Fluorescence in situ hybridisation (FISH)	Diagnosis of *S. aureus*	A recently launched approach by Sepsis Diagnostics, UK	Recently launched	36
	Detection of *H. pylori* antibiotic resistance	seaFAST *H. pylori* Combi-Kit; SeaPro Theranostics International, Lelystad, The Netherlands	On the market	9
Selective culture media and FISH	Diagnosis of *C. difficile*	Research application; commercialisation not known	Not commercially available	40
Rapid culture media	Diagnosis of *M. pneumoniae*	Research application; commercialisation not known	Not commercially available	59
Cellular analysis	Sepsis diagnostic and monitoring	Becton Dickinson, Edinburgh, St Thomas' and Newcastle Hospitals. Funded by Innovate UK	Commercialisation date not known	*
Colorimetric culture systems	Diagnosis of community acquired pneumonia	Bac T/Alert 3D, large analyser, Biomerieux	On the market	6
	Bloodstream infections	The Bac T/Alert 3D, Biomerieux	On the market	66
Colorimetric indicator tests	Carbapenemase-producing bacteria	Biomerieux	On the market	2
E-test antibiotic strips	Antimicrobial susceptibility in ventilator-associated pneumonia	AB, Biodisk www.genetixbiotech.com Biomerieux	On the market	7
Short multi-capillary gas chromatography column	Diagnosis of *C. difficile*	Research application; commercialisation not known	Not commercialised	42
Microfluidics ("lab on a chip") (potential point-of-care tests)	Diagnosis of *M. pneumoniae*	Research application, Duke university Medical centre, USA: commercialisation not known	Not on the market	58
	Diagnosis of urinary tract infection	Research application, University of Arizona, USA: commercialisation not known	Not on the market	50
	Antimicrobial susceptibility testing	Early stage development, first papers published 2013	Early development	15
	Detection of sepsis markers	Biogene Ltd, University of Hull. Funded by Innovate UK	Commercialisation date not known	*
	Detection of sepsis markers	Microlab devices, Forsite Diagnostics, University of Liverpool. Funded by Innovate UK	Commercialisation date not known	*
Biosensor platforms (potential point-of-care tests)	Antimicrobial susceptibility testing	Research application, Stanford University, School of Medicine: commercialisation not known	Not commercially available	45
	Detection of sepsis	Highland Biosciences. Funded by Innovate UK	Commercialisation date not known	*
	Wireless biosensors for detection of chlamydia	OJ Bio Ltd. Funded by Innovate UK	Commercialisation date not known	*
Mass spectrometry (MALDI-TOF)	Detection of carbapenemase-producing bacteria	MALDI-TOF laboratory instrumentation application	Some applications published	2
	Detection of antimicrobial susceptibility	MALDI-TOF MS is a laboratory analyser.	Some applications published	17

Immuno-chromatography (point-of-care tests)	Diagnosis of *C. difficile*	Alere Meridian Bioscience	On the market	38
	Diagnosis of *S. aureus*	Alere	On the market	35
	Diagnosis of community acquired pneumonia	Alere	On the market	5
	Diagnosis of streptococcal infections	OSOM Ultra Strep A, Alere Quickview Dipstick Strep A, TK Diagnostic, Oxford Sterptatest, DECTRA PHARM Clearview, Alere IMI Strep A, Alere	All on the market	65
	Diagnosis of MRSA	Alere	On the market	32
	Next generation lateral flow devices	Forsite Diagnostics Ltd. Funded by Innovate UK.	Commercialisation date not known	*
Multiplexed immunoassay platform	Sepsis markers inc CRP, IL6 and sICAM-1	Mologic, I Innovations, University of Aston. Funded by Innovate UK.	Commercialisation date not known	*
	TB biomarker signature for diagnosis	Proteinlogic Ltd, Microtest Matrices Ltd, University of Sheffield, London School of Hygiene and Tropical Medicine. Funded by Innovate UK.	Commercialisation date not known	*
	PROCID STI protein markers	Amies Innovation Ltd, Intrinsiq Materials Ltd, P1 Technology, Ryedale Group Ltd, The Needham group, University of Leeds. Funded by Collaborative Research and Development	Commercialisation date not known	*
Endo-lysin bound magnetometer platform	Detection of *C Difficile*	Sarum Biosciences and University of the West of England. Funded by Innovate UK.	Commercialisation date not known	*
Antigen detection tests	Diagnosis of tuberculosis	Antigen detection test for TB are unlikely to impact on antibiotic resistance. The poor quality of the available evidence limits their utility. The quality of the evidence remains very low. The WHO policy statement recommends against serological tests.	Not relevant for future	21, 22
	Diagnosis of *H. pylori*	stool antigen test -several	On the market	8
	Diagnosis of Group B *Streptococcus*	Quick Test, Nanologix GeneXpert, Cepheid, USA	On the market	67
	Sepsis pathogens	Magna Parva Ltd, Applied Enzyme technology Ltd, Chelsea Technologies Group Ltd, University of Cardiff. Funded by Innovate UK.	Commercialisation date not known	*
Interferon gamma release assays	Diagnosis of tuberculosis	Quest diagnostics, USA	On the market	23
Glutaraldehyde test	Diagnosis of tuberculosis	Along with conventional diagnostic tests, the glutaraldehyde test could be a rapid, easy, cost-effective and reliable test for the diagnosis TB, particularly in low resource settings.	Not relevant to UK	24
Rapid TB tests:	Diagnosis of tuberculosis	Systematic review which concluded that molecular tests are best – see other relevant technologies for this analyte	Tests available	27
	Detection of MDR TB	Smith Detection Ltd and Queen Mary University of London. Funded by Innovate UK	Commercialisation date not known	*
	Recombinase polymerase of the rapid detection of TB	TwistDx Ltd and the London School of Hygiene and Tropical Medicine	Commercialisation date not known	*
Genotypic and phenotypic tests	Identification of multi-drug resistant *M. tuberculosis*	INNO-Lipa, Fujirebio, Japan Genotype MTBDR and Genotype Plus, Hain Lifescience, Nehren, Germany	Both on the market	10
Inflammatory markers CRP	Immunoassay	Most large laboratory analysers have CRP on menu, Roche, Siemens, Abbott, Beckman Coulter Several POCT devices: Orion Diagnostic, Finland, Nycocard, Axis Shield, Norway, Cholestec LDX, Affinion, Alere, UK, Eurolyser Smart	On the market, both lab and POCT systems	
Inflammatory	Immunoassay	Brahms, Siemens and Roche, as well as possibly other main	All on the market	13

markers procalcitonin		lab analysers		
Combination of markers	Diagnosis of sepsis	Several CRP,PCT IL-6, and TNFalpha assays available separately as well as digital thermometers; no specific package. Some additional markers in research phase only	Components available commercially	43
Cell phone-based micro-photometric system	Antimicrobial susceptibility testing	Research application: commercialisation not known	Not commercially available	46
Cell lysis assays	Antimicrobial susceptibility testing	Research groups in the main	Not known	19
Chemi-luminescence drug susceptibility assays	Detection of *S. aureus* with reduced sensitivity to vancomycin	Research application only (Japan); commercialisation not known	Not known	33
Charge transfer trace chemical analysis		The Ideas Studio Ltd, Ancon technologies Ltd. Funded by Innovate UK	Commercialisation date not known	*
Flow cytometry	Multi-pathogen detection	Becton Dickinson, Kings College London, University of Newcastle. Funded by Innovate UK	Commercialisation date not known	*
Spectroscopic phenotypic detection	Antimicrobial resistance platform	Spetromic, Manchester. In early research phase	Commercialisation date not known	*
Automated blood culture	Rapid rule out of blood stream infections	Momentum Bioscience	On the market	
Breath tests	Carbon-13 urea breath test for the diagnosis of *H. pylori*	Carbon-13 urea breath test – several available	On the market	8
	Breath test for infectious disease diagnosis/sepsis	Research and development phase: no commercialisation known	No commercialisation to date	69
	VOC biomarkers for TB	Applied Nanodetectors Ltd and London School of Hygiene and Tropical Medicine. Funded by Innovate UK	Commercialisation date not known	*

*Ongoing research, funded in the UK over the last four years.

APPENDIX 1
TECHNOLOGY: MULTIPLEX POLYMERASE CHAIN REACTION (PCR) FOR THE DIAGNOSIS OF GASTROINTESTINAL PATHOGENS

Bottom line: *Multiplex polymerase chain reaction systems, used for diagnosis of common gastrointestinal pathogens, are not ready for deployment in the NHS and require further research to develop the evidence base.*

Level of evidence	
A HTA assessment of two multiplex assays for gastrointestinal pathogens concluded that neither is currently ready for deployment in the NHS.	**Level 1: Validating cohort study**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
This technology is a rapid test to identify infectious from non-infectious diarrhoea by detecting several common gastrointestinal pathogens simultaneously from one sample, informing initiation of appropriate antibiotics or stopping treatment where it is not appropriate.	**High**

1. Definition

In the hospital setting, approximately 90% of diarrhoea cases have non-infectious causes. Multiplex polymerase chain reaction (PCR) systems are used for simultaneous diagnosis of common important enteropathogens directly from stool samples, including *Clostridium difficile*, *Campylobacter* spp., *Salmonella* spp. and norovirus. Identification of the pathogen can inform antibiotic treatment (either to initiate a new antibiotic or to stop antibiotics, e.g. in the case of norovirus).

2. Summary of the Evidence

A recent Health Technology Assessment report (Pankhurst 2014) evaluated the MassCode and Luminex xTag multiplex PCR systems for rapid integrated PCR-based diagnostics for gastrointestinal pathogens to improve routine hospital infection control practice. In a UK hospital setting, MassCode assay sensitivities for each organism compared with standard microbiological testing ranged from 43% to 94% and specificities from 95% to 98%, with particularly poor performance for *Salmonella enterica*.

The Luminex xTag gastrointestinal assay showed high sensitivities (> 92%) and specificities (> 96%) for *Campylobacter* spp., *C. difficile* and norovirus, but for *S. enterica*, sensitivity was 46% with specificity 99%. Overall, the Luminex xTag gastrointestinal panel showed similar or superior sensitivity and specificity to the MassCode assay. However, the low sensitivity to detect a key enteric pathogen - *S. enterica* - makes it an unrealistic option for most microbiology laboratories.

3. *Requirements for further research*

To improve workflows in microbiology laboratories, reduce workload for infection control practitioners, and improve outcomes for NHS patients, further research on deoxyribonucleic acid-based multiplex gastrointestinal diagnostics is required. The report highlighted the substantial burden of, and difficulties in, dealing with infective diarrhoea, and the need for improved molecular/genetic diagnostics for better and faster diagnostics of gastrointestinal pathogens.

4. References

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APPENDIX 2
TECHNOLOGY: RAPID LABORATORY-BASED TESTS FOR DETECTION OF CARBAPENEMASE-PRODUCING BACTERIA

Bottom line: *The clinical and cost-effectiveness of rapid laboratory-based tests, which detect carbapenemase-producing bacteria, needs to be established before implementation in the NHS can be considered.*

Level of evidence	
Several molecular assays exist, including microarray and PCR, to detect carbapenemase- and β-lactamase-encoding genes; some of which are commercially available whilst others are still under development. The RAPIDEC CARBA NP test, that detects the presence of β-lactam antibiotic-resistant bacteria, is currently under evaluation and due to be released in the UK in late 2014. The MALDI-TOF mass spectrometry assay is becoming increasingly available to laboratories, but still requires validation as a diagnostic method.	**Level 3-4: mostly cohort studies and case-control studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
The RAPIDEC CARBA NP test could rapidly detect the presence of β-lactam antibiotic-resistant bacteria, informing antibiotic prescription.	**High**
Molecular assays (microarray and PCR) can detect a broad range of carbapenemase-encoding genes from clinical isolates, and could be used to inform antibiotic prescription.	**High**
MALDI-TOF is a rapid method to rapidly detect antibiotic resistance across a range of organisms and may decrease the time-frame of resistance detection. The assay is not currently commercially available and requires validation.	**High**

1. Definition

Carbapenems are a class of β-lactam antibiotics with broad antibacterial activity. Some bacteria, e.g. *Enterobacteriaceae* and *Pseudomonas aeruginosa,* produce enzymes called carbapenemases, which render them resistant to these antibiotics. Currently an indicator carbapenem is used as a first screen for resistance (NHS Public Health England). There are several supplementary laboratory tests available to distinguish carbapenemase producers:

a. The RAPIDEC CARBA NP test (bioMérieux) is a rapid phenotypic test (~2hrs) which detects carbapenemase using a colour indicator.
b. Molecular tests, including DNA microarray-based assays and multiplex real-time PCR assays, simultaneously detect genes encoding clinically important β-lactamases, including carbapenemases, directly from culture material within a few hours (~7-24hrs).
c. Matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry is an assay that generates profiles of antibiotic resistance by detecting mass changes due to hydrolysis of the carbapenem molecule. The test requires pre-incubation of the test organism with carbapenem, but is rapid (~2hrs). The assay is not currently commercially available and requires validation.

2. Summary of the evidence

RAPIDEC CARBA NP test:

Two evaluations indicated it was 100% sensitive and 100% specific compared with molecular-based techniques (Nordmann et al; Vasoo et al). One study (Tijet et al) reported a sensitivity and specificity of 73% and 100%, respectively; with positive and negative predictive values of 100% and 69%, respectively. Differences in accuracy may be attributable to differences in methodology or culture conditions. The latter study concluded that this test could present a rapid, inexpensive method for laboratories to identify potential carrier isolates which could then be further confirmed by molecular methods such as PCR. A 2014 prospective survey evaluating an efficient and cost-effective strategy to detect and characterize carbapenemase-producing *Enterobacteriaceae* showed that using the CARBA

NP test as a first screening, followed by the use of molecular techniques (PCRs or DNA microarray), and was an efficient strategy (Dortet et al).

MOLECULAR TESTS:
Evaluations of commercially available DNA microarrays for the detection of β-lactamase-encoding genes (Check-MDR CT101, Check-MDR CT102; Check-Points B.V., The Netherlands) from clinical isolates of *Enterobacteriaceae* showed a sensitivity of 97-100% and specificity of 98-100% compared to standard molecular and phenotypic methods (Bogaerts 2011, Cuzon 2012, Stuart 2012). An oligonucleotide microarray, currently in development, correctly identified 98% of the covered carbapenemase genes and several β-lactamases (Braun 2014). This microarray is currently not commercially available, but in development and may be extended and adapted to an automated point-of-care device in the future. Results indicate these assays may provide an accurate tool to discriminate most clinically relevant carbapenemases and β-lactamases, and are less time consuming and labour intensive than current laboratory methods, such as phenotypic and PCR testing.
There are several commercially available real-time multiplex PCR assays available detecting genes encoding for carbapenemases (Avlami 2010, Kaase 2012) with reported sensitivities and specificities of 96-98% and 98-100%, respectively. PCR tests are also being developed and improved, e.g. Expert Carba-R, Cepheid, USA is a rapid test to detect carbapenemase-producing gram negative bacteria from rectal swabs and is due for commercial release in USA in 2015. Overall, molecular tests are the only reliable method to detect production of multiple carbapenemases simultaneously.

MALDI-TOF:
MALDI-TOF offers a potential method to rapidly detect carbapenemase production by *Enterobacteriaceae* more rapidly than most other tests (2hrs or less) (Sparbier 2012, Burckhardt 2011) and some studies report sensitivity and specificity of 100% (Kempf 2012).

3. Requirements for further research
Further studies to validate the accuracy, validity and clinical utility of the above tests are required, particularly in the setting in which they would be implemented, along with cost-effectiveness analyses and an assessment of the most efficient strategy or combination of tests.

4. References

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APPENDIX 3
TECHNOLOGY: PCR FOR COMMUNITY-ACQUIRED PNEUMONIA CAUSED BY MYCOPLASMA PNEUMONIAE

Bottom line: *PCR has the potential to aid the diagnosis of M. pneumoniae prior to prescribing. However, the lack of clinical trials and cost-effectiveness information limit its application in the NHS.*

Level of evidence	
Compared with serology, commercial PCR tests showed superiority in diagnosis of *M. pneumonia*, but cannot replace serology alone. PCR plus serology may be effective screening tools for reliable and accurate diagnosis.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
PCR can result in earlier detection of MP infection compared with conventional culture techniques, and also show higher specificity. PCR inhibitors in collected samples can cause false-negative results; and sample contamination can cause false-positives.	**Medium**

1. Definition

PCR methods target the adherence protein P1 or the 16S RNA gene, thereby enabling direct detection of *M. pneumonia* from respiratory secretions (Nilsson et al, 2008). This is thought to be superior to serology which relies on antibodies to *M. pneumonia* (antibodies may not appear until 14 days after symptom onset). PCR tests can be conducted in hospital or clinic laboratories.

2. Summary of the evidence

Fourteen studies were included in the systematic review; all had a case-control design, and none reported blinded interpretation of test results (Zhang et al, 2011). Meta-analysis showed a summary estimate of sensitivity 62% (95% CI, 45% to 76%), and specificity 96% (95% CI, 93% to 98%). Heterogeneity between results was substantial ($p < 0.001$).

3. Requirements for further research

Although *M. pneumoniae* can be detected in patients without respiratory diseases by PCR, only 60% of infection is detected by targeting the P1 gene detects. Future research should be focused on targeting other gene sites on MP which will increase the diagnostic yield.

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APPENDIX 4
TECHNOLOGY: REAL-TIME PCR FOR COMMUNITY-ACQUIRED PNEUMONIA CAUSED BY MYCOPLASMA PNEUMONIAE

Bottom line: *Real time PCR could promote targeted treatment for Community Acquired Pneumonia but currently not hospitalized patients. The lack of clinical trials and cost-effectiveness information limit its application in the NHS.*

Level of evidence	
The HIRA-TAN technique yielded reproducible results and provided useful information to plan the course of treatment of pneumonia. Results are comparable with multiple PCR technique.	**Level 2: Multi-centre observational study**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
HIRA-TAN is able to discriminate therapeutic targets from commensal organisms, and can also detect foreign organisms in the sputum. It does not require pathogen-by-pathogen identification methods. However, in long-term hospitalised patients, HIRA-TAN is unable to confirm diagnosis by *S. aureus* or anaerobes.	**Medium**

1. Definition

This is an extension of conventional PCR methods which optimizes newly developed primers and cut-off values. HIRA-TAN is able to discriminate between commensal and infective organisms in sputum specimen. HIRA-TAN can be conducted in hospital/clinic laboratories.

2. Summary of the evidence

One large multi-centre, observational study in Japan including 568 patients was identified (Hirama et al, 2014). The prospective study used HIRA-TAN technique for the rapid identification of causative agents in pneumonia. 23 different gene sites were targeted. HIRA-TAN was able to identify the causative pathogens of pneumonia in 60% of the cases (97% for *H. influenzae*; 93% for *P. aeruginosa*; 81% for *Klebsiella pneumoniae*; 91% for *M. catarrhalis*; 88% for *E. coli*; 78% for MRSA and 92% for *S. pneumoniae*); and was able to determine when the pneumonia-causing organism was a commensal organism or a foreign organism in a single assay.

3. Requirements for further research

Development of cutoff values at which HIRA-TAN can discriminate between commensalism and infectivity for *S. aureus* and anaerobes.

4. References

- Hirama T, Minezaki S, Yamaguchi T, Kishi E, Kodama K, Egashira H, et al. HIRA-TAN: a real-time PCR-based system for the rapid identification of causative agents in pneumonia. Respir Med. 2014 Feb;108(2):395-404

APPENDIX 5
TECHNOLOGY: IMMUNOCHROMATOGRAPHIC TEST FOR COMMUNITY-ACQUIRED PNEUMONIA CAUSED BY STREPCOCOCCAL PNEUMONIA

Bottom line: *Immunochromatographic test could be a useful addition to the current diagnostic workup for community acquired pneumonia. However, a lack of clinical trials and cost-effectiveness information limit its application in the NHS.*

Level of evidence	
The immunochromatographic test could be a useful addition to the current diagnostic workup for CAP. However, evidence from the meta-analysis does not address whether rapid diagnosis with BinaxNOW-SP would impact the initial management of CAP patients.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
BinaxNow assay appears to have higher pooled sensitivity compared to culture, and it also has a high specificity. There is a need to determine its accuracy and effect on subsequent prescribing patterns in primary care alongside its cost effectiveness.	**Medium**

1. Definition

The BinaxNOW *Streptococcus pneumoniae* test is an immunochromatographic test (ICT) for the presence of the pneumococcal C-polysaccharide coat protein in urine. It is a rapid diagnostic test for *S. pneumonia* infected patients producing a result within 15 minutes of a urine sample being obtained. ICT tests can be conducted in hospital/clinic laboratories; samples can be collected on admission or within 48 hours after admission.

2. Summary of the evidence

Twenty seven studies were included (Sinclair et al, 2013). Participants in most studies were predominantly middle-aged or elderly, except for the studies which included HIV-positive or AIDS patients. No study had an overall low risk of bias, and none met the requirement for a perfect reference standard. After adjusting for the imperfect and variable nature of the reference standard, meta-analysis revealed a higher sensitivity of 74.0% (Credible interval (CrI), 66.6% to 82·3%) and specificity of 97.2% (CrI, 92.7% to 99.8%). There was substantial heterogeneity across studies, and this did not decrease with adjustment for covariates. The results were consistent with a previous meta-analysis of 24 studies (16 of which were included in the new report) (Boulware et al, 2007).

3. Requirements for further research

The meta-analysis does not address whether rapid diagnosis with BinaxNOW-SP would impact the initial management of CAP patients or changes to the initial management of CAP patients. Adequately powered randomized clinical trials are required to allow for a more robust assessment of the diagnostic accuracy and reliability of ICT.

4. References

- Sinclair A, Xie X, Teltscher M, Dendukuri N. Systematic review and meta-analysis of a urine-based pneumococcal antigen test for diagnosis of community-acquired pneumonia caused by *Streptococcus pneumoniae*. J Clin Microbiol. 2013 Jul;51(7):2303-10.
- Boulware DR, Daley CL, Merrifield C, Hopewell PC, Janoff EN. Rapid diagnosis of pneumococcal pneumonia among HIV-infected adults with urine antigen detection. J. Infect. 2007; 55:300–309

APPENDIX 6
TECHNOLOGY: BAC T/ALERT 3D SYSTEM FOR COMMUNITY-ACQUIRED PNEUMONIA

Bottom line: *The Bac T/Alert 3D test is reliable, time-saving, cost-effective and might reduce mortality. Randomized comparisons are required to confirm the important findings of the comparative study.*

Level of evidence	
Compared with conventional culture, automation was superior in terms of recovery and time to detect pathogens.	**Level 2: Comparative study**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
The Bac T/Alert system is reliable, time-saving and cost-effective and might reduce mortality.	**High**

1. Definition

The Bac T/ Alert 3D is a fully automated colorimetric, blood culture system, which incubates and agitates cultures, and has membrane sensors for detecting microbial growth based on changes in pH and carbon dioxide. The Alert system can be used in hospital or clinic settings. Blood, sputum or pleural fluid samples for testing are collected before initiation of antibiotic therapy.

2. Summary of the evidence

One comparative study compared Bac T/Alert 3D with conventional culture. 124 cases of CAP were included in the study. The average time for detection of pathogens was 16.6 hours (range 11.6 – 40 hrs) by the Bac T/Alert 3D system compared to 48 hours (range 36 – 72 hrs) by culture method. The rate of isolation with automation was four-times higher compared to the conventional blood culture method. False positives were also lower when compared with conventional culture.

3. Requirements for further research

Randomized comparisons are required to confirm the findings of the comparative study.

4. References

- Capoor MR, Nair D, Aggarwal P, Gupta B. Rapid diagnosis of community-acquired pneumonia using the BacT/Alert 3D system. Braz J Infect Dis. 2006 Oct;10(5):352-6.

APPENDIX 7
TECHNOLOGY: E-TEST ANTIBIOTIC STRIPS (AB BIODISK) FOR VENTILATOR-ASSISTED PNEUMONIA (VAP)

Bottom line: *Rapid E-test was associated with fewer days of fever, fewer days of antibiotic administration until resolution of the episode of ventilator-associated pneumonia. Implementation in the NHS is limited by insufficient evidence; therefore a further trial with clinical and cost-effectiveness is warranted.*

Level of evidence	
Rapid E-test was associated with fewer days of fever, fewer days of antibiotic administration until resolution of the episode of ventilator-associated pneumonia, decreased antibiotic consumption, less *C. difficile*-associated diarrhoea, lower costs of antimicrobial agents, and fewer days receiving mechanical ventilation.	**Level 2: RCT**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
A Rapid E-test improves antimicrobial use and lowers costs of antibiotics in cases of ventilator-associated pneumonia. Performance of the E-test is very simple and is within the reach of any microbiology laboratory. However, interpretation of the results in polymicrobial cases is more difficult and requires a certain degree of expertise.	**High**

1. Definition

The E-test (AB Biodisk) is a well-known antimicrobial susceptibility test that uses antimicrobial agent-inoculated strips and is inoculum size-independent. The test strips can be used on isolated bacteria or applied directly to clinical samples. The test can be utilized in hospital settings.

2. Summary of the evidence

One RCT with 1,220 patients was identified. Patients from the E-test group had fewer days of fever per episode (4.6 vs. 7.8 days), required fewer days of antibiotic administration to resolve the VAP episode (15.7 vs. 18.9 days), consumed fewer antibiotics (i.e., received fewer defined daily dosages; 31 vs. 43 doses) and experienced less *C. difficile*-associated diarrhoea (1.8% vs. 9.6%). In addition, the early availability of microbiological information led to an improvement in the adequacy of antibiotic therapy. Overall, the E-test group and the control group differed in the percentage of days of adequate therapy (95% vs. 76%) and in the percentage of adequate DDDs prescribed (91% vs. 68%). The cost of the antimicrobial agents prescribed per episode in the E-test group and control group were €666 and €984, respectively. There was a decrease in time receiving mechanical ventilation from the diagnosis of VAP in the E-test group versus the control group (8 days vs. 12 days; $P < .05$). Trends towards a shorter total ICU stay (23 days vs. 27 days), fewer overall days receiving mechanical ventilation (17 days vs. 19 days), and a shorter stay in the ICU after diagnosis of VAP (13 days vs. 17 days) were observed, but did not reach statistical significance.

3. Requirements for further research

Studies evaluating the diagnostic value of a test for detecting the less-commonly observed organisms implicated in the pathogenesis of VAP are required.

4. References

- Bouza E, Torres MV, Radice C, Cercenado E, de Diego R, Sánchez-Carrillo C, Muñoz P. Direct E-test (AB Biodisk) of respiratory samples improves antimicrobial use in ventilator-associated pneumonia. Clin Infect Dis. 2007 Feb 1;44(3):382-7.

APPENDIX 8
TECHNOLOGY: NON-INVASIVE TESTS FOR THE DIAGNOSIS OF HELICOBACTER PYLORI

Bottom line: *NICE guidance currently recommends the use of Helicobacter pylori stool antigen test (SAT) and Carbon-13 urea breath test for the assessment of patients with dyspepsia. Current point-of care tests, however, are not accurate enough to warrant their widespread use in the NHS.*

Level of Evidence	
Treatment of dyspepsia and gastro-oesophageal reflux disease with antibiotics is only recommended based on a positive *H. pylori* test. Several non-invasive laboratory tests can accurately detect *H. pylori* and point-of-care tests are available, however there accuracy prevents widespread implementation.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
The *Helicobacter pylori* stool antigen test (SAT) can detect the presence of *H. pylori* in stool samples and is currently recommended by NICE guidance to inform antibiotic prescription.	**High**
The Carbon-13 urea breath test can detect the presence of *H. pylori* by measuring labelled CO_2 in exhaled breath, which results from the bacteria's ability to convert ingested ^{13}C-labelled urea to ammonia and CO_2. The test is currently recommended by NICE guidance to inform antibiotic prescription.	**High**
The Rapirun Stick is a point-of-care test that can measure anti- *H. pylori* antibodies form urine samples. If the accuracy of these point-of-care tests can be improved these may assist antibiotic prescribing decisions.	**High**

1. Definition

a. The *Helicobacter pylori* stool antigen test (SAT) is a laboratory-based, non-invasive test using antibodies to detect *H. pylori antigens* in stool samples.
b. Carbon-13 urea breath test (UBT) is a non-invasive test to detect *H. pylori* by its ability to convert urea to ammonia and carbon dioxide.
c. Rapid immunochromatographic tests for *H. pylori* are point-of-care stool antigen tests that can be performed by clinicians, giving a colour indicator result in ~5 minutes.
d. The Rapirun Stick test is an *H. pylori* antibody stick-type tests that measures anti-*H. pylori* antibodies in urine, with results available in ~15 minutes.

2. Summary of the evidence

H. pylori infection has been shown to be uncommon in dyspeptic patients at endoscopy. In a 2012 UK study, *H. pylori* was cultured in only 6.4% of 2,063 patients attending hospitals (McNulty et al.). A 2006 systematic review of 22 studies including 2,499 patients showed the monoclonal stool antigen tests had sensitivity 94% (95% CI 93–95%), and specificity 97% (CI 96–98%), and were more sensitive than the polyclonal tests (Gisbert et al). A subsequent 2014 systematic review of 45 studies (5,931 patients) of the *H. pylori* SAT test compared to either endoscopy or UBT showed pooled sensitivity and specificity of 92% and 94%.

A 2009 systematic review of 30 studies (3,415 patients) of the efficacy and cost-effectiveness of ^{13}C-UBT compared to other non-invasive tests and to biopsy showed sensitivity and specificity higher than 90% in 84% of the studies for the ^{13}C UBT; overall it had higher sensitivity and specificity than the SAT and IgG serology tests (Nocon et al).

The above two tests for H. pylori are currently recommended by NICE guidance [CG184].

Several studies have assessed the accuracy of rapid immunochromatographic (ICT) *H. pylori* tests. For example, a study of 109 children and adolescents with abdominal symptoms assessing the accuracy of the Rapid Hp StAR test compared to histology reported sensitivity of 65% and specificity of 93%.

(Koluglu et al). A 2013 study assessed the accuracy of three rapid stool antigen ICT tests reporting sensitivities ranging from 68% to 86%, and specificities between 87% and 92% (Korkmaz et al). Current NICE guidelines do not recommend these point-of-care serological tests due to inadequate performance.

One 2014 study of 200 patients undergoing upper gastrointestinal endoscopy assessed the accuracy of urine Rapirun *Helicobacter pylori* Antibody Stick test compared to biopsy, and reported a sensitivity and specificity of 85% and 90%, respectively (Quach et al). Another study assessing the accuracy of urine-based *H. pylori* tests in 101 children with upper abdominal symptoms reported 78% sensitivity and 100% specificity for the Rapirun stick test compared to UBT and SAT (Okuda et al). However, the current evidence for these tests is limited and requires validation.

3. Requirements for further research

The SAT and UBT tests are non-invasive laboratory tests currently recommended in the NICE guideline on dyspepsia and gastro-oesophageal reflux disease for the detection of *H. pylori* infection, along with laboratory serology where validated. Investigation into the optimal testing procedure and pathway may be warranted. *H. pylori* point-of-care tests are currently not recommended due to inadequate performance; however, further research may be warranted to improve and validate these tests as they may aid more rapid decisions around antibiotic prescribing.

4. References

- Gisbert JP, de la Morena F, Abraira V. Accuracy of monoclonal stool antigen test for the diagnosis of H. pylori infection: A systematic review and meta-analysis. Am J Gastroenterol 2006;101:1921-30.
- Korkmaz H1, Kesli R, Karabagli P, Terzi Y. Comparison of the diagnostic accuracy of five different stool antigen tests for the diagnosis of Helicobacter pylori infection. Helicobacter. 2013 Oct;18(5):384-91.
- McNulty CA, Lasseter G, Shaw I, Nichols T, D'Arcy S, Lawson AJ, Glocker E. Is Helicobacter pylori antibiotic resistance surveillance needed and how can it be delivered? Aliment Pharmacol Ther. 2012 May;35(10):1221-30
- NICE Guideline CG184 Dyspepsia and gastro‐oesophageal reflux disease: Investigation and management of dyspepsia, symptoms suggestive of gastro‐oesophageal reflux disease, or both. September 2014.
- Nocon M, Kuhlmann A, Leodolter A, Roll S, Vauth C, Willich SN, Greiner W. Efficacy and cost-effectiveness of the 13C-urea breath test as the primary diagnostic investigation for the detection of Helicobacter pylori infection compared to invasive and non-invasive diagnostic tests. GMS Health Technol Assess. 2009 Oct 21;5:Doc14.
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- Quach DT, Hiyama T, Shimamoto F, Le QD, Ho LX, Vu NH, Yoshihara M, Uemura N. Value of a new stick-type rapid urine test for the diagnosis of Helicobacter pylori infection in the Vietnamese population. World J Gastroenterol. 2014 May 7;20(17):5087-91.
- Zhou X, Su J, Xu G, Zhang G. Accuracy of stool antigen test for the diagnosis of Helicobacter pylori infection in children: A meta-analysis. Clin Res Hepatol Gastroenterol. 2014 Mar 10. pii: S2210-7401(14)00045-X. [Epub ahead of print].

APPENDIX 9
TECHNOLOGY: DETECTION OF ANTIBIOTIC RESISTANCE IN HELICOBACTER PYLORI

Bottom line: *Several rapid molecular tests are available to detect clarithromycin-resistant H. pylori in gastric samples. One identified test, the string test, does not require biopsy and may warrant further investigation as a less invasive method to obtain gastric fluid for testing.*

Level of evidence	
Molecular testing (e.g. PCR, microarrays, fluorescent in situ hybridization) for *H. pylori* genes conferring resistance to clarithromycin is a more rapid alternative to culture methods, and has been tested across a number of diagnostic accuracy studies.	**Level 2: Cohort studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
PCR-based tests to detect genes conferring resistance to clarithromycin and can inform antibiotic prescribing practices	**Medium**
Fluorescent in situ hybridization (FISH) can detect genes conferring resistance to clarithromycin and can inform antibiotic prescribing practices	**Medium**
String test and PCR-restriction fragment length polymorphism (RFLP) can detect genes conferring resistance to clarithromycin, can inform antibiotic prescribing practices and may be less invasive than biopsy	**Medium**

1. Definition

a. PCR-based tests of gastric biopsy specimens and gastric washes to detect *H. pylori* genes conferring resistance to clarithromycin.
b. Fluorescent in situ hybridization (FISH) is based on fluorescently labelled DNA or peptide nucleic acid probes that hybridize with specific rRNA sequences of *H. pylori* from biopsy samples.
c. String test and PCR-restriction fragment length polymorphism (RFLP) to detect *H. pylori* clarithromycin resistance.

2. Summary of the evidence

Molecular testing (e.g. PCR, microarrays, fluorescent in situ hybridization) for *H. pylori* genes conferring resistance to clarithromycin is a more rapid alternative to culture methods. Several PCR-based methods are available. One study assessed the accuracy of two PCR-based methods, (dual priming oligonucleotide [DPO]-PCR and fluorescence energy transfer [FRET] PCR), compared to bacterial culture, in detecting clarithromycin resistant *H. pylori*. It reported sensitivity of DPO-PCR and real-time FRET-PCR of 98% and 100%, while specificity was 83% and 81%, respectively (Lehours et al). A study assessing a commercially available rapid fluorescent in situ hybridization (FISH) test, (seaFAST *H. pylori* Combi-Kit; SeaPro Theranostics International, The Netherlands) compared to culture, reported a sensitivity and specificity of FISH for the detection of *H. pylori* in biopsy specimens of 97% and 94% respectively (Morris et al). A retrospective and prospective evaluation of a peptide nucleic acid-fluorescence in situ hybridization (PNA-FISH) method compared to culture followed by an agar dilution test (Etest) reported a sensitivity and specificity of 84% and 91% respectively.

Although molecular testing frequently requires frozen storage of biopsy samples, some evidence suggest PCR could also be performed on samples stored at room temperature (Li et al). Studies have also indicated that gastric washes can be used as an alternative to biopsy samples in PCR testing (Baba et al).

The string test (Entero-Test *H. pylori*, HDC Corporation, CA, United States) is an alternative sampling method to biopsy. A 90-cm nylon string coiled inside a gelatin capsule is used. A free-end looped string protrudes through a hole in the other end of the capsule, which is secured to the patient's cheek with tape before the capsule is swallowed. One hour after swallowing, the string is retrieved (in a swift motion to prevent gag reflex). The withdrawn string yields 1-2ml gastric juice, which is then tested using

PCR-RFLP. One study assessing the accuracy of this method reported sensitivity and specificity of the string test to detect genotypic clarithromycin resistance of 67% and 97%, respectively (Wu et al).

3. Requirements for further research

Several rapid molecular tests are available to detect clarithromycin-resistant *H. pylori* in gastric samples and these can be performed in conjunction with the analysis of biopsy samples for the presence of *H. pylori*. One test identified, the string test, does not require biopsy and may warrant further investigation as a less invasive method to obtain gastric fluid for testing. Further research should address the most efficient and cost-effective method to detect antibiotic resistance in these samples.

4. References

- Baba S1, Oishi Y, Watanabe Y, Oikawa R, Morita R, Yoshida Y, Hiraishi T, Maehata T, Nagase Y, Fukuda Y, Nakazawa M, Ishigouoka S, Hattori N, Suzuki H, Toyota M, Niwa H, Suzuki M, Itoh F. Gastric wash-based molecular testing for antibiotic resistance in *Helicobacter pylori*. Digestion. 2011;84(4):299-305.
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- Wu JY, Wang SS, Lee YC, Yamaoka Y, Graham DY, Jan CM, Wang WM, Wu DC. Detection of genotypic clarithromycin-resistant *Helicobacter pylori* by string tests. World J Gastroenterol. 2014 Mar 28;20(12):3343-9.

APPENDIX 10
TECHNOLOGY: RAPID DIAGNOSTIC TESTING FOR MULTIDRUG RESISTANCE IN TUBERCULOSIS (MTB-DR)

Bottom line: *These tests could help to diagnose MTBDR faster compared to conventional culture in areas with high TB prevalence. However, in areas of low prevalence, the possibility of false negatives exists and a lack of cost-effectiveness data negates impact on practice.*

Level of evidence	
When used alone, these tests cannot accurately predict MDR-TB in areas with low TB prevalence.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
These test are accurate and may prove valuable in areas with high prevalence of TB but a lack of cost-effectiveness data negates implementation.	**Low**

1. Definition

a. Genotypic tests: Based on detection of mutations conferring resistance to anti-TB drugs;
INNO-LiPA Rif. TB; Genotype® MTBDR assay; Genotype® MTBDRplus assay.

b. Phenotypic tests: Susceptibility tests performed in solid or liquid media;
Microscopic observation drug susceptibility assay (MODS); Colorimetric Redox Indicator (CRI) assay; Nitrate Reductase Assay (NRA).
These tests can be used in hospital/clinic settings.

2. Summary of the evidence

The most recent systematic review with 60 publications, including the 6 different tests was identified. It was unclear whether patient selection (8% high risk; 42% unknown risk) or index test performance (27% low risk) could have introduced bias.

Test	***Nos. of studies***	***Nos. of participants***	***Sensitivity***	***Specificity***
INNO-LiPA Rif. TB	4	947	94%	99%
MTBDR assay	Not included in meta-analysis as only 3 studies identified			
MTBDRplus assay	12	3,337	98%	99%
MODS	10	1,395	98%	99%
CRI assay	14	1,629	98%	99%
NRA assay	19	2,289	98%	99.8%

For all the tests, there was a high NPV when the prevalence of rifampicin resistance was ≤ 30%. PPV values were considerably decreased for INNO-LiPA Rif. TB assay, the MTBDRplus assay and MODS when the prevalence of rifampicin resistance was < 5%.

3. Requirements for further research

There is a need to asses cost-effectiveness and develop tests with high PPV in low prevalence circumstances.

4. References

- Arentz M, Sorensen B, Horne DJ, Walson JL. Systematic Review of the Performance of Rapid Rifampicin Resistance Testing for Drug- Resistant Tuberculosis. PLoS ONE 2013; 8(10): e76533. doi:10.1371/journal.pone.0076533

APPENDIX 11
TECHNOLOGY: XPERT® MTB/RIF ASSAY FOR PULMONARY TUBERCULOSIS AND RIFAMPICIN RESISTANCE IN ADULTS

Bottom line: *XpertMTB/RIF may be valuable as an add-on test following smear microscopy however it is not clear how the test would fit into the current diagnostic pathway. The test is expensive and lack of cost-effectiveness data prevents widespread uptake in the NHS.*

Level of Evidence	
The XpertMTB/RIF is sensitive and specific in adults with suspected TB, with or without HIV infection.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
XpertMTB/RIF may be valuable as an add-on test following smear microscopy in patients previously found to be smear-negative for TB.	**Medium**

1. Definition
XpertMTB/RIF is an automated PCR test which utilizes the GeneXpert® platform as an initial test for rifampicin resistance thus replacing the conventional initial test - phenotypic drug susceptibility testing (DST). The tests can be conducted in intermediate level laboratories in hospital/clinic settings.

2. Summary of the evidence
The most recent systematic review with 27 studies and 9,557 participants was identified: 59% of trials were performed in low-to-middle-income countries (LMICs) and most of the studies had low risk of bias. As an initial test replacing smear microscopy, Xpert® MTB/RIF had a pooled sensitivity of 89% and specificity 99%. As an add-on test following a negative smear microscopy result, the pooled sensitivity and specificity of Xpert®MTB/RIF was 67% and 99%, respectively. For smear-positive, culture-positive TB, Xpert® MTB/RIF's pooled sensitivity was 98%.

In comparison with smear microscopy, Xpert® MTB/RIF increased TB detection among culture-confirmed cases by 23%. If pooled sensitivity estimates for Xpert® MTB/RIF and smear microscopy are applied to a hypothetical cohort of 1000 patients where 10% of those with symptoms have TB, Xpert® MTB/RIF will diagnose 88 cases and miss 12 cases, whereas sputum microscopy will diagnose 65 cases and miss 35 cases. For rifampicin resistance detection, Xpert® MTB/RIF pooled sensitivity was 95% and specificity was 98%.

Balance of benefit and harm
Xpert® MTB/RIF has higher sensitivity for TB detection in smear-positive than smear-negative patients. It is also helpful for rapid initiation of MDR-TB treatment, pending results from conventional testing. However, it does not eliminate the need for culture and phenotyping which are required for monitoring.

3. Requirements for further research
Cost-effectiveness studies are required to determine the wider impact.

4. References
Steingart KR, Schiller I, Horne DJ, Pai M, Boehme CC, Dendukuri N. Xpert® MTB/RIF assay for pulmonary tuberculosis and rifampicin resistance in adults. Cochrane Database Syst Rev. 2014 Jan 21;1:CD009593

APPENDIX 12
TECHNOLOGY: BACTERIOPHAGE BIOSENSORS FOR ANTIBIOTIC-RESISTANT BACTERIA

Bottom line: *Point–of-care tests for antibiotic resistance could have a significant impact on antibiotic treatment regimes in a wide range of diseases across both primary and secondary care. They are in the early stages of development and require a coordinated research strategy to facilitate their implementation into NHS care.*

Level of evidence	
Biosensors using lytic bacteriophages have the potential to rapidly detect antibiotic resistance, but are in the early stages of development.	**Level 5: Laboratory Proof of concept study**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Point-of-care tests for antibiotic resistance could have a significant impact on the choice of treatment, both in primary and secondary care.	**Medium**

1. Definition

This microfluidic biosensor technology uses transformed lytic bacteriophages and penicillin-binding protein antibody conjugated to latex beads to discriminate between methicillin resistant and methicillin sensitive *Staphylococcus aureus.* This laboratory-based method can detect bacterial antibiotic resistance in 10-12 minutes.

2. Summary of the evidence

This technology provides the potential for a rapid and cost-effective method to identify antibiotic-resistant bacteria, however it is in the early stages of development (proof of concept) and thus far no evidence has been identified as to its clinical application.

3. Requirements for further research

There is currently no evidence on the accuracy or utility of this technology. Substantial further research is required regarding accuracy, feasibility and utility.

4. References

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APPENDIX 13
TECHNOLOGY: POINT-OF-CARE PROCALCITONIN-GUIDED ANTIBIOTIC TREATMENT

Bottom line: *Point-of-care tests for procalcitonin (PCT), to guide antibiotic treatment, are not ready for deployment in the NHS and require further research to develop the evidence base.*

Level of Evidence	
The point-of-care PCT test could be a useful addition to guide antibiotic treatment. Only one such test is currently available. Its use in emergency departments (ED) yields similar sensitivities and specificities as other inflammatory markers, but its use in primary care has yet to be established. Existing evidence is based on one high quality RCT and cross sectional studies.	**Level 1: RCT**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
PCT-Q - The technology is a semi-quantitative rapid immunochromatographic test that uses lateral-flow immunochromatography to measure PCT, informing initiation of appropriate antibiotics or stopping treatment when it is not appropriate.	**High**

1. Definition

The blood marker procalcitonin (PCT) increases rapidly during bacterial infections but remains low in viral infections and other inflammatory processes. Therefore, PCT may be used to support clinical decision making for the initiation and discontinuation of antibiotic therapy in patients with a clinical suspicion of infection. The standard laboratory method of measuring PCT uses a luminometric immunoassay or an ultra-sensitive immunoassay using TRACE (Time Resolved Amplified Cryptate Emission) technology. Only one point-of-care PCT test is currently available, the PCT-Q® semi-quantitatively measures PCT values in the serum of patients. A new diagnostic test for sepsis using whole blood is in development (Wacker et al).

2. Summary of the evidence

A 2013 systematic review assessed the evidence of PCT as a marker for sepsis included studies (3,244 patients). Bivariate analysis yielded a mean sensitivity of 77% and specificity of 79%. A 2012 Cochrane review assessed the evidence for using PCT to initiate or terminate antibiotic use in respiratory infections and included individual patient data from 14 RCTs (4,211 patients). Total antibiotic exposure was significantly reduced overall (median from 8 (IQR: 5 to 12) to 4 (IQR: 0 to 8) days. A further 2013 review including 18 RCTs assessed the evidence for PCT guidance compared to using clinical criteria alone to manage antibiotic therapy in patients with infections. In adult ICU patients, PCT-guided discontinuation of antibiotics reduced antibiotic duration by 2.1 days without increasing morbidity or mortality. In adult patients with respiratory tract infections, PCT guidance significantly reduced antibiotic duration by 2.4 days, antibiotic prescription rate by 22%, and total antibiotic exposure without affecting morbidity or mortality.

A 2011 Horizon Scanning Report identified five studies that evaluated the point-of-care PCT test compared to quantitative PCT testing on the diagnosis of serious bacterial infection (SBI) in the ED. The sensitivity ranged from 57% to 100% and the specificity ranged from 64% to 89%. Overall, it appears that point-of-care PCT tests in the ED have similar sensitivities and specificities to other inflammatory markers. No studies have directly assessed the diagnostic value of PCT in diagnosing SBI in primary care.

A June 2014 scoping document from the NICE Diagnostics Assessment Programme outlines an evaluation on the clinical utility and cost-effectiveness of laboratory-based procalcitonin testing to guide initiation and discontinuation of antibiotics in the ED and ICU, and results from this assessment will further inform practice.

3. Requirements for further research

Further high quality studies are required to compare the diagnostic value of PCT compared to other inflammatory markers either alone or in combination at different time points and to confirm the safety of PCT guided antibiotic treatment. Further studies of the accuracy and utility of point-of-care PCT tests and comparisons of point-of-care PCT tests with point-of-care devices for other inflammatory markers for patients presenting with acute infections are also required. Future studies should also establish cost-effectiveness by considering country-specific costs of PCT measurement and potential savings in consumption of antibiotics and other healthcare resources, as well as secondary cost savings due to lower risk of side effects and reduced antimicrobial resistance.

4. References

- NICE Diagnostics Assessment Programme: Diagnosis and monitoring of sepsis: procalcitonin testing (ADVIA Centaur BRAHMS PCT assay, BRAHMS PCT Sensitive Kryptor assay, Elecsys BRAHMS PCT assay, LIAISON BRAHMS PCT assay and VIDAS BRAHMS PCT assay). Final scope. June 2014
- NIHR SPCR Horizon Scan Report 0021: Diagnostic Technology: Point-of-care test for procalcitonin to improve the early diagnosis of serious bacterial infections in patients presenting in primary care.13 February 2012
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- Wacker C, Prkno A, Brunkhorst FM, Schlattmann P. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. Lancet Infect Dis 2013; 13: 426–35

APPENDIX 14
TECHNOLOGY: POINT-OF-CARE INFLUENZA TESTS

Bottom line: *Point-of-care influenza tests could reduce antibiotic prescribing, particularly in children, but a lack of evidence and cost-effectiveness prevents widespread uptake in the NHS.*

Level of evidence	
Point-of-care influenza tests might decrease antibiotic prescribing, however robust evidence of the utility and cost-effectiveness data is lacking.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Influenza can be ruled in but not ruled out using rapid tests. There is some evidence to suggest testing reduces antibiotic prescriptions more so in children than adults.	**Medium**

1. Definition

Point-of-care tests for influenza are mostly chromatographic immunoassays using antibodies against highly conserved viral nucleoproteins to detect influenza A and/or B. They are rapid - most providing results within 15min - and can be performed at the bedside or in general practice. There are a large number of tests commercially available (>20).

2. Summary of the evidence

A 2012 meta-analysis of the accuracy of rapid influenza tests in adults and children assessed 159 studies evaluating 26 different tests and reported a pooled sensitivity and specificity of 62% and 98%, respectively (Chartrand et al). The positive and negative likelihood ratios were 34.5 and 0.38, respectively. Overall the sensitivity was lower in adults (54%) than in children (66%) and sensitivity for influenza A was higher than for influenza B (65% vs 52%). The review concluded that influenza can be ruled in, but not ruled out using rapid tests. Some studies have assessed the influence of rapid influenza testing on antibiotic prescribing. One study in Thailand (Bhavani et al) reported a significant decrease in antibiotic prescription using a rapid influenza test, as the likelihood of antibiotic prescription for influenza positive patients was 0.41 times the likelihood for influenza negative patients. However, robust evidence on the utility of rapid influenza testing to inform antibiotic prescribing is currently lacking. Some studies indicate that a rapid influenza test affects management of febrile children as the confirmation of influenza virus infection decreases additional diagnostic tests ordered (such as complete blood counts, lumbar punctures and urinalysis) (Hojat et al). Studies indicate that rapid testing is only cost-effective when influenza probability is low (e.g. early in the influenza season or when it is uncommon). An Italian Health Technology Assessment report on the potential benefits of rapid influenza testing by GPs in managing influenza appropriately concluded that "*given the poor returns and high costs associated with community use of rapid influenza testing"* the recommendation was that rapid influenza tests should not be provided and *"no further studies on the topic should be conducted with public funding*".

3. Requirements for further research

A substantial body of literature on the accuracy of point-of-care influenza tests exists, along with some implementation studies, however the utility and cost-effectiveness in the context of antibiotic prescribing strategies remains to be assessed. A community-based trial of the effect on antibiotic prescribing for children is warranted. Point-of-care test need to show they are pragmatic and feasible in primary care setting to facilitate implementation.

4. References

- Age.na.s. HTA report: Rapid (bed-side) tests for influenza. Rome, September 2008. http://www.salute.gov.it/imgs/C_17_pagineAree_1202_listaFile_itemName_0_file.pdf

- Bhavnani D1, Phatinawin L, Chantra S, Olsen SJ, Simmerman JM. The influence of rapid influenza diagnostic testing on antibiotic prescribing patterns in rural Thailand. Int J Infect Dis. 2007 Jul;11(4):355-9.
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- Hojat K, Duppenthaler A, Aebi C. Impact of the availability of an influenza virus rapid antigen test on diagnostic decision making in a pediatric emergency department. Pediatr Emerg Care. 2013 Jun;29(6):696-8.
- Horizon scan report 0022. Point-of-care tests for influenza in children March 2012. Oxford Diagnostic Horizon Scan Programme. http://madox.org/horizon-scanning-reports/20120022/point-of-care-tests-for-influenza-in-children.

APPENDIX 15
TECHNOLOGY: MICROFLUIDIC SYSTEMS FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING

Bottom line: *Microfluidic systems are a new innovation that could potentially aid point-of-care testing for antimicrobial susceptibility however they are in the early phase of development.*

Level of evidence:	
Microfluidic systems are in early phase development and currently have been shown to assess minimum inhibitory concentration values in 3-4 hours.	**Level 5: Laboratory evidence**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Due to their small size, the chips used in these assays can be incorporated into portable devices, which may facilitate antimicrobial susceptibility testing at the point of care.	**Medium**

1. Definition

These so-called 'lab on a chip' platforms utilize extremely small volumes of reagent and analyte (picolitres) for rapid detection of antibiotic susceptibility. They can be used in hospital/clinic settings and as point-of-care tests.

2. Summary of the evidence

Three studies were included in an expert review. One study using microfluidic agarose channels generated minimum inhibitory concentration (MIC) values in 3-4 hours. Another study utilizing electrochemical quantification of 16S rRNA generated results in 3.5 hours with 94% agreement with conventional antibiotic susceptibility testing methods. A third study which used microfluidic pH sensor technique generated susceptibility results in 2 hours.

3. Requirements for further research

Microfluidic systems are in the early stages of development and require further validation and accuracy studies to determine their role in testing for antimicrobial susceptibility.

4. References

- Pulido MR, García-Quintanilla M, Martín-Peña R, Cisneros JM, McConnell MJ. Progress on the development of rapid methods for antimicrobial susceptibility testing.J Antimicrob Chemother 2013; 68: 2710–2717

APPENDIX 16
TECHNOLOGY: PCR-BASED TECHNIQUES FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING

Bottom line: *PCR-based techniques for susceptibility testing are in the early phases of development and currently lack evidence from clinical trials in humans.*

Level of evidence	
Several PCR techniques have been tested and in some cases have the ability to detect MRSA directly from clinical samples in less than 2 hours.	**Level 3: Clinical trial**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
The tests can be carried out in a relatively short period of time. However, the presence of resistance may not always correlate with phenotypic resistance. PCR-based technologies which detect the presence of resistance genes are also unable to detect novel or uncharacterized mechanisms of resistance for which the genetic determinant is unknown.	**Low**

1. Definition

These techniques could be conventional or real-time, and rely on the sequence-specific amplification of nucleic acids. They can be used in hospital/clinic settings.

2. Summary of the evidence

An expert review reported that several PCR techniques have been tested with respect to MRSA, and in some cases have the ability to detect MRSA directly from clinical samples in less than 2 hours. The Cepheid Xpert MRSA showed high sensitivity compared with conventional culture. Vancomycin resistance was tested using PCR-based approaches with varying degrees of sensitivity and specificity – high false positives were reported in some studies.

3. Requirements for further research

Reducing the frequency of discordance between the presence of a resistance determinant and phenotypic resistance; clinical studies in humans are required.

4. References

- Pulido MR, García-Quintanilla M, Martín-Peña R, Cisneros JM, McConnell MJ. Progress on the development of rapid methods for antimicrobial susceptibility testing.J Antimicrob Chemother 2013; 68: 2710–2717

APPENDIX 17
TECHNOLOGY: MATRIX-ASSISTED LASER DESORPTION/IONIZATION TIME-OF-FLIGHT MASS SPECTROMETRY (MALDI-TOF MS) FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING

Bottom line: *MALDI-TOF MS is in the early phase of development and it remains to be seen if this approach offers sufficient sensitivity and specificity to affect clinical practice.*

Level of evidence	
MALDI-TOF MS may shorten the time required to detect resistance in bacterial pathogens; however, in many cases it remains to be determined whether these approaches provide sufficient sensitivity and specificity.	**Level 5: Laboratory studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
MALDI-TOF MS is extremely rapid and highly automated. The results obtained using this approach may not always directly correlate with phenotypic resistance and differences between strains that are unrelated to resistance can complicate the interpretation of results.	**Low**

1. Definition
MALDI-TOF MS identifies molecules based on their time of flight through a vacuum tube after laser irradiation of a matrix that is co-crystallized with the sample. MALDI-TOF MS aims to differentiate spectra from resistant and susceptible isolates using whole cells or crude extracts. The test can be conducted in hospital laboratories.

2. Summary of the evidence
MALDI-TOF MS has been validated in *Enterobacteriaceae* and *P. aeruginosa* with sensitivity and specificity of 96.7% and 97.8% respectively. Vancomycin-resistant *enterococci* have been demonstrated. However, MALDI-TOF MS is characterized by discordance between the presence of a resistance determinant and phenotypic resistance.

3. Requirements for further research
Validation studies; research into sensitivity and specificity and clinical trials are all warranted.

4. References

- Pulido MR, García-Quintanilla M, Martín-Peña R, Cisneros JM, McConnell MJ. Progress on the development of rapid methods for antimicrobial susceptibility testing.J Antimicrob Chemother 2013; 68: 2710–2717

APPENDIX 18
TECHNOLOGY: MICROARRAYS FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING

Bottom line: *Microarray utility is limited by a lack of clinical studies including information on sensitivity and specificity.*

Level of evidence	
Can detect large numbers of resistance genes in a single assay; however, at this current time the predictive value is uncertain.	**Level 5: Laboratory evidence**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Microarray has the ability to detect vast numbers of different resistance genes in a single assay. It used inefficiently, it could cause delays in susceptibility detection and underutilisation of resources.	**Low**

1. Definition

Microarrays identify the presence of specific nucleic acid sequences using complementary oligonucleotides; and have the ability to detect thousands of sequences in a single assay. They can be used in hospital/clinic laboratories.

2. Summary of the evidence

Several studies have employed microarrays for the detection of β-lactamases in Gram-negative bacteria, some of which can provide results in one working day. One study which combined microassays with real-time PCR showed high sensitivity and specificity. The results may not always correlate with phenotypic resistance and this approach does not provide data on MIC values. In addition, this technique may have limited ability to detect resistance in isolates harbouring novel or uncharacterized mechanisms of resistance.

3. Requirements for further research

Validation studies and research into sensitivity and specificity are needed.

4. References

- Pulido MR, García-Quintanilla M, Martín-Peña R, Cisneros JM, McConnell MJ. Progress on the development of rapid methods for antimicrobial susceptibility testing.J Antimicrob Chemother 2013; 68: 2710–2717

APPENDIX 19
TECHNOLOGY: CELL-LYSIS FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING

Bottom line: *Cell lysis techniques show good correlation with microdilution and E-test data, and have the ability to provide approximate MIC values. However, these techniques are yet to be tested using clinical samples.*

Level of evidence	
Currently studies have only assessed this technique using culture-purified bacteria and it remains to be determined if this approach can be used with clinical samples.	**Level 5: Laboratory studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
This approach produces results irrespective of the mechanism that is producing the resistance.	**Medium**

1. Definition

This approach is based on detecting bacterial cell lysis after incubation with the antibiotic being tested. The procedure can be carried out in 100 minutes and showed good correlation with microdilution and E-test data.

2. Summary of the evidence

Cell –lysis has been validated for the detection of quinolone and ampicillin resistance in *E. coli*, and recently for detecting carbapenem resistance in *A. baumannii*. It may have the ability to provide approximate MIC values. This technique has only been assessed using culture-purified bacteria and it remains to be determined if this approach can be used with clinical samples.

3. Requirements for further research

Direct testing with clinical samples warranted.

4. References

- Pulido MR, García-Quintanilla M, Martín-Peña R, Cisneros JM, McConnell MJ. Progress on the development of rapid methods for antimicrobial susceptibility testing.J Antimicrob Chemother 2013; 68: 2710–2717

APPENDIX 20
TECHNOLOGY: WHOLE-GENOME SEQUENCING FOR ANTIMICROBIAL SUSCEPTIBILITY TESTING

Bottom line: *whole genome sequences use in conventional clinical settings appears impractical. It is expensive compared with standard methods.*

Level of evidence	
Not cost-effective compared with conventional culture methods.	**Level 5: Laboratory studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It allows for the rapid synthesis of whole genome sequences. This could be of use in tracking outbreaks of infections (i.e. tracing).	**Low**

1. Definition

Whole genome sequencing approach combines very rapid sequencing of entire bacterial genomes with bioinformatic tools that can quickly assemble and analyse the huge data obtained from the sequencing runs. The tests could be carried out in hospital/clinic settings.

2. Summary of the evidence

High concordance rates (99.7%) have been demonstrated in the characterization of bacteria resistance profiles (Pulido et al, 2013). However, its use in conventional clinical settings appears impractical. It is expensive compared with standard methods. A number of studies describing whole-genome sequencing of small numbers of clinical isolates to characterize the genetic determinants of antibiotic resistance have been described (Snitkin et al, 2013; Tan et al, 2013; O'Neill et al, 2013; Huang et al, 2012).

Balance of benefit and harm

It allows for rapid synthesis of whole genome sequences. This could be of use in tracking outbreaks of infections (i.e. tracing).

3. Requirements for further research

Studies on how to improve cost-effectiveness are needed.

4. References

- Pulido MR, García-Quintanilla M, Martín-Peña R, Cisneros JM, McConnell MJ. Progress on the development of rapid methods for antimicrobial susceptibility testing. J Antimicrob Chemother 2013; 68: 2710–2717
- Snitkin ES, Zelazny A, Gupta J, et al. Genomic insights into the fate of colistin resistance and *Acinetobacter baumannii* during patient treatment. Genome Res 2013. doi:10.1101/gr.154328.112.
- Tan SY, Chua SL, Liu Y, et al Comparative genomic analysis of rapid evolution of an extreme-drug-resistant *Acinetobacter baumannii* clone. Genome Biol Evol 2013;5:807-18.
- O'Neill CE,Seth-Smith HM,Van Der Pol B et al. Chlamydia trachomatis clinical isolates identified as tetracycline resistant do not exhibit resistance in vitro: whole-genome sequencing reveals a mutation in porB but no evidence for tetracycline resistance genes. Microbiology 2013;159:748-56.
- Huang H, Yang ZL, Wu XM, et al. Complete genome sequence of *Acinetobacter baumannii* MDR-TJ and insights into its mechanism of antibiotic resistance. J Antimicrob Chemother 2012;67:2825-32.

APPENDIX 21
TECHNOLOGY: ANTIGEN DETECTION TESTS FOR DIAGNOSIS OF TB

Bottom line: *Antigen detection test for TB are unlikely to impact on antibiotic resistance. The poor quality of the available evidence limits their utility.*

Level of evidence	
Due to the limited number of studies targeting any specific antigen other than Lipoarabinomannan and concerns about methodological quality in a majority of studies, firm conclusions about the clinical usefulness of antigen detection tests cannot be drawn.	**Level 1: Systematic reviews**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Antigen detection with an easily obtained specimen can be exploited to develop a TB diagnostic test. However, pH and viscosity may affect diagnostic accuracy.	**Low**

5. Definition

This approach aims to detect circulating mycobacterial antigens in clinical specimens such as serum, sputum, urine, cerebrospinal spinal fluid and pleural fluid.

6. Summary of the evidence

A total of 47 studies (n = 5,036) were included. Overall, they were of poor quality. For pulmonary TB, sensitivity estimates ranged from 2% to 100% and specificity from 33% to 100%. Lipoarabinomannan (LAM) was the antigen most frequently targeted (23 studies, 49%). The pooled sensitivity of urine LAM was higher in HIV-infected than HIV-uninfected individuals (47% versus 14%); pooled specificity estimates were similar (96% and 97%, respectively). For extrapulmonary TB, sensitivity estimates ranged from 0% to 100% with specificity estimates from 62% to 100%. Five studies targeting LAM, ESAT-6, Ag85 complex, and the 65-kDa antigen in cerebrospinal fluid. When pooled they yielded the highest sensitivity (87%), but low specificity (84%).

7. Requirements for further research

Research to improve performance testing is needed.

8. References

- Flores LL, Steingart KR, Dendukuri N, Schiller I, Minion J, Pai M, Ramsay A, Henry M, Laal S. Systematic review and meta-analysis of antigen detection tests for the diagnosis of tuberculosis. Clin Vaccine Immunol. 2011 Oct;18(10):1616-27.

APPENDIX 22
TECHNOLOGY: COMMERCIAL SEROLOGICAL TESTS FOR THE DIAGNOSIS OF ACTIVE PULMONARY AND EXTRAPULMONARY TUBERCULOSIS

Bottom line: *Commercial serological tests continue to produce poor diagnostic accuracy (sensitivity and specificity). The quality of the evidence remains very low. The World Health Organization policy statement recommends against serological tests.*

Level of evidence	
They tests are characterized by inconsistency and imprecision of both sensitivity and specificity. The overall quality of evidence was graded very low for studies of pulmonary and extrapulmonary TB.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
By using ELISA, results could be available within hours and as a POCT it can replace microscopy. It may also be advantageous in children where sputum is difficult to obtain, and in patients with extra pulmonary TB. However, the tests are characterized by high proportions of false positives and false negatives.	**Low**

1. Definition

These are blood tests that detect the humoral immune (antibody) responses to *M. tuberculosis* antigens. They can be used as a point-of-care test (POCT) in hospital/clinic settings.

2. Summary of the evidence

A systematic review of commercial serological tests for the diagnosis of active pulmonary TB included 67 studies (n = 5,147) and 25 studies (n = 1,809) for extra pulmonary TB. The overall quality of evidence was low.

a. **Pulmonary TB:** For all tests, estimates were variable for sensitivity (0% to 100%) and specificity (31% to 100%). For anti-TB IgG, the only test with enough studies for meta-analysis, pooled sensitivity was 76% in smear-positive (7 studies) and 59% in smear-negative (4 studies) patients; pooled specificities were 92% and 91%, respectively. Compared with ELISA (pooled sensitivity 60%; pooled specificity 98%) immunochromatographic tests yielded lower pooled sensitivity (53%) and comparable pooled specificity (98%).
b. **Extrapulmonary TB:** For all tests, estimates were variable for sensitivity (0% to 100%) and specificity (59% to 100%).

3. Requirements for further research

Identification of new and/or alternative point-of-care tests with improved diagnostic accuracy that reduce the proportion of false positives and false negatives.

4. References

- Steingart KR, Flores LL, Dendukuri N, Schiller I, Laal S, Ramsay A, Hopewell PC, Pai M. Commercial serological tests for the diagnosis of active pulmonary and extrapulmonary tuberculosis: an updated systematic review and meta-analysis. PLoS Med. 2011 Aug;8(8):e1001062

APPENDIX 23
TECHNOLOGY: INTERFERON-GAMMA RELEASE ASSAYS FOR DIAGNOSIS OF MYCOBACTERIUM TUBERCULOSIS INFECTION IN CHILDREN

Bottom line: *Interferon Gamma Release Assays show promise for improving TB diagnosis in immunocompetent children aged over5 years in high income settings. However neither of the available tests can rule out nor confirm the certainty of diagnosis, and interpretation of results may be difficult.*

Level of evidence	
Overall, no better than tuberculin skin test (TST), but might help with improving diagnostic accuracy .	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
A reasonable choice for TB diagnosis in immunocompromised children >5 years.	**Low**

1. Definition
Interferon-gamma release assays (IGRAs) help with detection of circulating T-cells responsive to specific *Mycobacterium tuberculosis* antigens, which are absent in BCG and many non-tuberculosis mycobacteria. They can be used as a point-of-care test in hospital or clinic settings.

2. Summary of the evidence
The review included data from both high and low-income countries. In high income countries, QuantiFERON-TB Gold In Tube (QFT-G-IT) sensitivity was 79% for all studies, 78% including only studies performing a simultaneous three-way comparison and 86% considering only microbiologically confirmed studies. In the same analyses T-SPOT. TB (an interferon-gamma assay) sensitivity was 67% for all studies.

3. Requirements for further research
Diagnostic accuracy studies considering combined use with tuberculin skin testing (TST) in children <5 years.

4. References

- Sollai S, Galli L, de Martino M, Chiappini E. Systematic review and meta-analysis on the utility of Interferon-gamma release assays for the diagnosis of *Mycobacterium tuberculosis* infection in children: a 2013 update. BMC Infect Dis. 2014;14 Suppl 1:S6.

APPENDIX 24
TECHNOLOGY: GLUTARALDEHYDE TEST FOR DIAGNOSIS OF TB

Bottom line: *Along with conventional diagnostic tests, the glutaraldehyde test could be a rapid, easy, cost-effective and reliable test for the diagnosis TB, particularly in low resource settings.*

Level of evidence	
Useful for low-resource settings where many centres lack sputum culture capacity and sophisticated radiology facilities to aid diagnosis.	**Level 3: Observational studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It could be rapid, easy and cost-effective.	**Medium**

1. Definition

This approach uses blood glutaraldehyde gelification time (BGGT) for the diagnosis of pulmonary TB. This is based on the fact that whole blood of TB patients contains more fibrinogen normal subjects. It can be used in clinic/hospital settings.

2. Summary of the evidence

Three studies were identified. Mean gelification time was significantly reduced for subjects with pulmonary TB compared with those without TB (Mathur & Sachdev, 2005). Sensitivity was 85-89%, and 89-95% in two studies (Alavi-Naini et al, 2009; Larsson et al, 1990).

Balance of benefit and harm

It is fast, cost-effective, and reliable when combined with conventional techniques (Alavi-Naini et al, 2009). However the test does give rise to false negatives (Mathur & Sachdev, 2005).

3. Requirements for further research

Clinical trials are needed to improve performance testing (especially NPV) and investigate its role as an add-on test to other conventional tests.

4. References

- Alavi-Naini R, Hashemi M, Mohagegh-Montazeri M, Sharifi-Mood B, Naderi M. Glutaraldehyde test for rapid diagnosis of pulmonary tuberculosis. Int J Tuberc Lung Dis. 2009 May;13(5):601-5.
- Larsson S, Shrestha MP, Pokhrel BM, Upadhyay MP, Shrestha KB. The glutaraldehyde test as a rapid screening method for pulmonary tuberculosis: a preliminary report. Ann Trop Med Parasitol. 1990 Apr;84(2):111-7.
- Mathur ML, Sachdev R. Temperature affects the results of the glutaraldehyde test in the diagnosis of pulmonary tuberculosis. Int J Tuberc Lung Dis. 2005 Feb;9(2):200-5.

APPENDIX 25
TECHNOLOGY: KATG MUTATIONS FOR DIAGNOSIS OF ISONIAZID RESISTANCE IN TB

Bottom line: *Identification of katG mutations associated with high-level isoniazid resistance in Mycobacterium tuberculosis is in the early phase of development.*

Level of evidence	
This technology requires further testing and validation.	**Level 5: Laboratory study**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It may be useful when combined with conventional culture methods for detecting susceptibility.	**Low**

1. Definition

This approach involves sequencing katG of isoniazid (INH)-resistant MTB clinical isolates. It can be used in clinic/hospital settings.

2. Summary of the evidence

Nine novel katG mutants were identified after sequencing 108 IHN-resistant MTB isolates. All nine mutants showed significantly lower INH oxidase activities than the wild type.

3. Requirements for further research

This technology requires further development and testing.

4. References

- Ando H, Kondo Y, Suetake T, Toyota E, Kato S, Mori T, Kirikae T. Identification of katG mutations associated with high-level isoniazid resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2010 May;54(5):1793-9

APPENDIX 26
TECHNOLOGY: EVALUATION OF GENETIC MUTATIONS ASSOCIATED WITH MTB RESISTANCE TO AMIKACIN, KANAMYCIN AND CAPREOMYCIN

Bottom line: *Additional mutations appear to be associated with antibiotic resistance and could improve sensitivity and specificity of future diagnostics.*

Level of evidence	
The technology requires further testing and validation.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It could improve sensitivity and specificity of future diagnostics but unlikely to impact on resistance and diagnostics. More mutations in more genes will need to be included in order to accurately and sensitively detect resistance and cross-resistance to AMK, KAN and CAP for clinical decision-making purposes.	**Low**

1. Definition

This involves the use of rapid molecular diagnostic tests for faster detection of MTB resistance to second-line anti-TB drugs. The technique involves detecting gene mutations in TB resistant strains.

2. Summary of the evidence

The study reviewed mutation data for 1,585 unique clinical isolates from four continents and over 18 countries. Mutations in the rrs, tlyA, eis promoter and gidB genes were associated with AMK, KAN and/or CAP resistance. There was a possibility of misclassification errors.

Balance of benefit and harm

It may improve diagnostic accuracy for MTBDR to second-line drugs.

3. Requirements for further research

Further development and testing is required.

4. References

- Georghiou SB, Magana M, Garfein RS, Catanzaro DG, Catanzaro A, Rodwell TC. Evaluation of genetic mutations associated with Mycobacterium tuberculosis resistance to amikacin, kanamycin and capreomycin: a systematic review. PLoS One. 2012;7(3):e33275

APPENDIX 27
TECHNOLOGY: RAPID DIAGNOSTIC TESTS FOR THE DETECTION OF TUBERCULOSIS INFECTION

Bottom line: *Studies from low-prevalence countries strongly suggest that the RD1 antigen-based assays are more accurate than TST- and PPD-based assays for diagnosis of latent TB infection.*

Level of evidence	
Further testing and validation in a wide spectrum of patients using appropriate reference standards is required.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Rapid diagnostic tests could improve sensitivity and specificity of future diagnostics however robust clinical studies are needed.	**Low**

1. Definition

These tests comprise of nucleic acid amplification tests (NAAT), amplification molecular probe tests, serodiagnostic and biochemical assays, and phage-based tests.

2. Summary of the evidence

In a systematic review, 212 studies were included. NAAT accuracy was far superior when applied to respiratory samples as opposed to other body fluid. There was no evidence to support the use of adenosine deaminase (ADA) tests for the diagnosis of pulmonary TB; however, there is considerable evidence to support their use for diagnosis of pleural TB and, to a slightly lesser extent, for TB meningitis. Anti-TB antibody test performance was generally poor, irrespective of type of TB. Fully automated liquid culture methods were superior to culture on solid media, in terms of their speed and their precision. Assays based on the RD1 specific antigens ESAT-6 or CFP-10 correlate better with intensity of exposure, and therefore are more likely than tuberculin skin test (TST) or purified protein derivative (PPD) based assays to detect latent TB infection accurately. An additional advantage is that they are more likely to be independent of BCG vaccination status and HIV status. Studies from low-prevalence countries strongly suggest that the RD1 antigen-based assays are more accurate than TST- and PPD-based assays for diagnosis of latent TB infection.

3. Requirements for further research

Requires further performance testing to try to improve test accuracy. For active TB accuracy must be established, in a wide spectrum of patients, against an appropriate reference test.

4. References

- Dinnes J, Deeks J, Kunst H, Gibson A, Cummins E, Waugh N, Drobniewski F, Lalvani A. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. Health Technol Assess. 2007 Jan;11(3):1-196

APPENDIX 28
TECHNOLOGY: HIGH RESOLUTION MELTING CURVE ANALYSIS FOR RAPID DETECTION OF PYRAZINAMIDE RESISTANT MTB

Bottom line: *Rapid detection of pyrazinamide resistant Mycobacterium tuberculosis is in the early phase of assessment.*

Level of evidence	
Further testing and validation is required.	**Level 5: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It could offer a rapid and reliable screen for PZA-resistant MTB.	**Low**

1. Definition
This test involves the amplification of pncA gene fragments using the PCR method.

2. Summary of the evidence
Mutations were clearly detected in all PZA resistant samples by the high resolution melting curve (HRM) analysis, whereas all PZA susceptible samples showed no mutation in the pncA gene. Results were concordant with the drug susceptibility testing by using BACTEC MGIT 960 PZA kit and mutation detection by the DNA sequencing method

3. Requirements for further research
Further validation and performance testing is required.

4. References

- Watcharasamphankul W, Houpt ER, Foongladda S. Rapid detection of pyrazinamide resistant *Mycobacterium tuberculosis* by high resolution melting curve analysis. J Med Assoc Thai. 2013 Sep;96(9):1218-23.

APPENDIX 29
TECHNOLOGY: HIGH RESOLUTION MELTING CURVE ANALYSIS FOR RAPID DETECTION OF RIFAMPIN RESISTANT MTB

Bottom line: *HRMC might be a good alternative to conventional drug susceptibility tests in clinical practice, however its utility is limited by lack of clinical trial evidence.*

Level of evidence	
Rapid and simple to conduct, but performance testing is required.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It might be a good alternative to conventional methods as it has high sensitivity and specificity. However, performance on clinical specimens is yet to be determined.	**Low**

1. Definition
This novel method detects more mutations in DNA sequence with few probes or without probes.

2. Summary of the evidence
Overall quality of studies was good. The overall sensitivity of the HRMC analysis was 94% and the overall specificity was high at 99%. The values for the pooled positive likelihood ratio, negative likelihood ratio, and diagnostic odds ratio were 63, 0.06 and 892, respectively.

3. Requirements for further research
Cost-effectiveness and reliability studies are required.

4. References

- Yin X1, Zheng L, Liu Q, Lin L, Hu X, Hu Y, Wang Q. High-resolution melting curve analysis for rapid detection of rifampin resistance in *Mycobacterium tuberculosis*: a meta-analysis. J Clin Microbiol. 2013 Oct;51(10):3294-9.

APPENDIX 30
TECHNOLOGY: RAPID DIAGNOSTIC TESTS WITH PCR FOR THE DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN HOSPITALIZED PATIENTS

Bottom line: *The evidence regarding the use of rapid diagnostic tests with PCR for the detection of MRSA in hospitalized patients is insufficient for implementation.*

Level of evidence	
There is a shorter turnaround time for these tests but there is insufficient evidence on their clinical effectiveness.	**Level 1 evidence: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Other factors such as hygiene and restricted contact with infected patients should be considered.	**Low**

1. Definition

This is a molecular technique in which enzymatic replication is used to amplify a short sequence of DNA, and allows for faster detection of the presence of MRSA compared with culture-based methods. The test can be conducted in hospital settings.

2. Summary of the evidence

Nine studies compared PCR versus chromogenic agar for MRSA screening in a hospital setting, and two studies compared screening using PCR with no or targeted screening were identified. The quality of the studies was mixed. Some studies found lower MRSA colonization and acquisition, infection, and transmission rates in screening with PCR versus screening with chromogenic agar. The turnaround time for screening test results was lower for PCR. One study reported a lower number of unnecessary isolation days with screening using PCR versus screening with chromogenic agar, but the proportion of patients isolated was similar between both groups. The turnaround time for test results and number of isolation days were lower for PCR versus chromogenic agar for MRSA screening.

Balance of benefit and harm

The tests, especially the Xpert MRSA, may help with quicker detection of MRSA.

3. Requirements for further research

High quality studies are required.

4. References

- Polisena J, Chen S, Cimon K, McGill S, Forward K, Gardam M. Clinical effectiveness of rapid tests for methicillin resistant *Staphylococcus aureus* (MRSA) in hospitalized patients: a systematic review. BMC Infect Dis. 2011 Dec 12;11:336.

APPENDIX 31
TECHNOLOGY: RAPID DIAGNOSTIC TESTS WITH PCR FOR THE DETECTION OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) AT ADMISSION

Bottom line: *Rapid diagnostic tests for the detection of MRSA do not offer advantages over conventional methods of screening.*

Level of evidence	
Rapid screening tests are not associated with a significant decrease in MRSA acquisition rate compared to conventional methods.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Active screening is more important than type of test and rapid tests do not appear to confer any advantages over conventional culture methods.	**Low**

1. Definition

This is a molecular technique in which enzymatic replication is used to amplify a short sequence of DNA, and allows for faster detection of the presence of MRSA compared with culture-based methods. The test can be conducted in hospital settings.

2. Summary of the evidence

Ten studies (9 interventional studies and 1 unblinded, cluster-randomised, crossover trial) were reviewed. Quality of the included studies was fairly good. Compared with culture screening, use of rapid screening tests was not associated with a significant decrease in MRSA acquisition rate (risk ratio 0.87, 95% CI 0.61-1.24). Compared to wards that did not apply rapid screening tests, wards that did had a significantly decreased risk for MRSA bloodstream infections (0.54, 95% CI 0.41-0.71), but not for MRSA surgical-site infections (0.69, 95% CI 0.46-1.01). There was some heterogeneity between the studies.

3. Requirements for further research

High quality preferably focussed on targeted screening are required.

4. References

- Tacconelli E, De Angelis G, de Waure C, Cataldo MA, La Torre G, Cauda R. Rapid screening tests for meticillin-resistant *Staphylococcus aureus* at hospital admission: systematic review and meta-analysis. Lancet Infect Dis. 2009 Sep;9(9):546-54.

APPENDIX 32
TECHNOLOGY: RAPID DIAGNOSTIC TESTS WITH IMMUNOASSAY FOR THE DETECTION OF MRSA

Bottom line: *Rapid diagnostic tests with immunoassay have high sensitivity and specificity for detecting MRSA, however their use in clinical practice and the benefits over conventional detection methods remains to be elucidated.*

Level of evidence	
Further testing and development is required. The tests have high sensitivity and specificity.	**Level 3: Laboratory & observational studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Not validated for use but are rapid and are inexpensive.	**Medium**

1. Definition

These are novel rapid kits for (1) detecting MRSA penicillin-binding proteins; or (2) that utilize *Staphylococcus aureus*-specific lateral flow immunochromatography (LFI) test; or (3) combine Cycling Probe Technology (CPT) assay with a lateral-flow device (strip) for the detecting the mecA gene in MRSA cultures.

2. Summary of the evidence

One study which used EZ-Step MRSA rapid kit reported sensitivity of 94% and specificity 100%. Both LFI and CPT plus lateral-flow device were also reported to have positively detected MRSA in clinical isolates. An 11-month prospective observational study with LFI showed sensitivity of 98%, with 100% specificity for both MRSA and MSSA (methicillin-susceptible *S. aureus*).

3. Requirements for further research

Validation studies and clinical trials are required to test their diagnostic accuracy against conventional methods.

4. References

- Fong WK, Modrusan Z, McNevin JP, Marostenmaki J, Zin B, Bekkaoui F. Rapid solid-phase immunoassay for detection of methicillin-resistant *Staphylococcus aureus* using cycling probe technology. J Clin Microbiol. 2000 Jul;38(7):2525-9.
- Lindsey WC, Woodruff ES, Weed D, Ward DC, Jenison RD. Development of a rapid diagnostic assay for methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative Staphylococcus. Diagnostic Microbiology & Infectious Disease 61(3): 273-279.
- Shin KS, Song HG, Kim H, Yoon S, Hong SB, Koo SH, Kim J, Kim J, Roh KH. Direct detection of methicillin-resistant *Staphylococcus aureus* from blood cultures using an immunochromatographic immunoassay-based MRSA rapid kit for the detection of penicillin-binding protein 2a. Diagn Microbiol Infect Dis. 2010 Jul;67(3):301-3.
- Sinclair A, Mulcahy LE, Geldeard L, Malik S, Fielder MD, Le Gresley A. Development of an in situ culture-free screening test for the rapid detection of *Staphylococcus aureus* within healthcare environments. Org Biomol Chem. 2013 May 28;11(20):3307-13
- Trienski TL, Barrett HL, Pasquale TR, DiPersio JR, File TM Jr. Evaluation and use of a rapid *Staphylococcus aureus* assay by an antimicrobial stewardship program. Am J Health Syst Pharm. 2013 Nov 1;70(21):1908-12
- Tunsjø HS, Follin-Arbelet B, Clausen NM, Ness Y, Leegaard TM, Bemanian V. A rapid, high-throughput screening method for carriage of methicillin-resistant *Staphylococcus aureus*. APMIS. 2013 Sep;121(9):865-70

- Wiriyachaiporn S, Howarth PH, Bruce KD, Dailey LA. Evaluation of a rapid lateral flow immunoassay for *Staphylococcus aureus* detection in respiratory samples. Diagn Microbiol Infect Dis. 2013 Jan;75(1):28-36

APPENDIX 33

TECHNOLOGY: RAPID DIAGNOSTIC TESTS WITH CHEMILUMINESCENCE-BASED TECHNIQUE FOR THE DETECTION OF STAPHYLOCOCCUS AUREUS STRAINS WITH REDUCED SENSITIVITY TO VANCOMYCIN

Bottom line: *A rapid test that may supersede classical methods for testing susceptibility, however validation studies and clinical trials are needed to test and confirm diagnostic accuracy.*

Level of evidence	
Further testing and development is required.	**Level 5: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
A rapid test that may supersede classical methods for testing susceptibility.	**Medium**

1. Definition

This technique measures bacterial metabolic activity and distinguishes between vancomycin susceptible and resistant strains of *S. aureus*.

2. Summary of the evidence

Reported sensitivity and specificity were both greater than 95%.

3. Requirements for further research

Validation studies and clinical trials are required to test and confirm diagnostic accuracy.

4. References

- Tajima Y, Komatsu M, Ito T, Hiramatsu K. Rapid detection of *Staphylococcus aureus* strains having reduced susceptibility to vancomycin using a chemiluminescence-based drug-susceptibility test. J Microbiol Methods. 2007 Sep;70(3):434-41

APPENDIX 34
TECHNOLOGY: RAPID DIAGNOSTIC TESTS WITH MULTIPLEX PCR AND PULSED-FIELD GEL ELECTROPHORESIS FOR THE DETECTION OF PANTON-VALENTINE LEUCOCIDIN-POSITIVE COMMUNITY-ACQUIRED MRSA

Bottom line: *Rapid tests with multiplex PCR and pulsed-filed electrophoresis for the detection of PVA-positive CA-MRSA are in the early stage of development and are unlikely to impact on microbial resistance.*

Level of evidence	
Further testing and development is required.	**Level 5: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
The technique is not validated for use but may be superior to routine genotyping.	**Medium**

1. Definition

This technique uses multiplex PCR (m-PCR) and gel electrophoresis to target five genes for PVL, collagen adhesin, bone sialoprotein adhesin, methicillin resistance, and *S. aureus*-specific thermostable nuclease. It can be used in hospital laboratories.

2. Summary of the evidence

Compared to conventional PCR methods, the M-PCR/PFGE combination assay more precisely discriminated between PVL-positive ST30 community acquired MRSA (or its related clone) and PVL-positive community acquired MRSA belonging to other sequence types such as ST1, 8, 59, and 80, PVL-negative CA-MRSA, hospital-acquired MRSA, methicillin-susceptible *S. aureus*, or coagulase-negative *staphylococci* (CNS), including MRCNS.

3. Requirements for further research

Validation studies and clinical trials are required to test their diagnostic accuracy.

4. References

- Reva I, Higuchi W, Takano T, Singur O, Ozaki K, Isobe H, Yabe S, Saito K, Baranovich T, Enany S, Otsuka T, Potapov V, Nishiyama A, Yamamoto T. A rapid screening method for Panton-Valentine leucocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus* belonging to multilocus sequence type 30 and its related clone using a combination of multiplex PCR and pulsed-field gel electrophoresis. J Infect Chemother. 2009 Apr;15(2):75-83

APPENDIX 35
TECHNOLOGY: RAPID DIAGNOSTIC TESTS WITH BINAXNOW STAPHYLOCOCCUS AUREUS TEST FOR THE DETECTION OF GRAM-POSITIVE COCCI FROM VERSATREK BLOOD CULTURE BOTTLES

Bottom line: *A rapid test that is still in the early stage of development but does have high accuracy. It could shorten the time between the test and commencement of antimicrobial therapy.*

Level of evidence	
A rapid test that has high sensitivity and specificity.	**Level 5: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It could shorten the period between test the test being taken and the commencement of antimicrobial therapy.	**Low**

1. Definition

The rapid BinaxNOW *Staphylococcus aureus* (BNSA) immunochromatographic test accurately differentiates *S. aureus* from coagulase-negative staphylococci (CoNS) and other Gram-positive cocci (GPC) directly from VersaTREK blood culture bottles. The test can be conducted in hospital laboratories.

2. Summary of the evidence

The BNSA test was accurate for the detection and differentiation of *S. aureus* from CoNS and other GPC within 30 minutes from the time of blood culture positivity. It demonstrated a test sensitivity and specificity of 96% and 99.6%, respectively.

3. Requirements for further research

Validation studies are required to assess the utility as well as clinical studies assessing its cost-effectiveness.

4. References

- Dhiman N, Trienski TL, DiPersio LP, DiPersio JR. Evaluation of the BinaxNOW Staphylococcus aureus test for rapid identification of Gram-positive cocci from VersaTREK blood culture bottles. J Clin Microbiol. 2013 Sep;51(9):2939-42

APPENDIX 36
TECHNOLOGY: RAPID DIAGNOSTIC TESTS WITH FLUORESCENCE IN SITU HYBRIDIZATION (FISH) METHOD FOR SIMULTANEOUS IDENTIFICATION OF STAPHLOCOCCUS AUREUS AND COAGULASE-NEGATIVE STAPH DIRECTLY FROM BLOOD CULTURE

Bottom line: *Rapid diagnostic tests with the FISH method could improve diagnostic accuracy and shorten time to commencement of targeted antimicrobial therapy. However there is a lack of clinical trials and cost-effectiveness for targeted, rapid strategies for delivery of antimicrobial therapy.*

Level of evidence	
A rapid test which can detect organism within 30 minutes and has high sensitivity and specificity.	**Level 5: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It could improve diagnostic accuracy and shorten time to commencement of targeted antimicrobial therapy.	**Medium**

1. Definition
This method utilizes a microscope slide with pre-deposited positive- and negative-control organisms and a self-reporting 15 minute hybridization step, which eliminates the need for a wash step.

2. Summary of the evidence
The sensitivities for detection of *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) were 99.5% and 99%, respectively, and the combined specificity of the assay was 90% (Deck et al, 2012). The combined positive and negative predictive values of the assay were 99.7% and 71%, respectively. In another study using blood cultures from 104 patients, sensitivity was 100%, specificity 99.4%, PPV 99.2% and NPV 100% (González et al, 2004).

3. Requirements for further research
Clinical trials and cost-effectiveness studies in hospital populations are required.

4. References

- Deck MK, Anderson ES, Buckner RJ, Colasante G, Coull JM, Crystal B, Della Latta P, Fuchs M, Fuller D, Harris W, Hazen K, Klimas LL, Lindao D, Meltzer MC, Morgan M, Shepard J, Stevens S, Wu F, Fiandaca MJ. Multicenter evaluation of the Staphylococcus QuickFISH method for simultaneous identification of *Staphylococcus aureus* and coagulase-negative staphylococci directly from blood culture bottles in less than 30 minutes. J Clin Microbiol. 2012 Jun;50(6):1994-8.
- González V, Padilla E, Giménez M, Vilaplana C, Pérez A, Fernández G, Quesada MD, Pallarés MA, Ausina V. Rapid diagnosis of *Staphylococcus aureus* bacteremia using S. aureus PNA FISH. Eur J Clin Microbiol Infect Dis. 2004 May;23(5):396-8.

APPENDIX 37
TECHNOLOGY: POINT-OF-CARE TEST FOR C-REACTIVE PROTEIN

Bottom line: *The evidence for the use of POC CRP tests to guide antibiotic treatment is mixed. It can be a useful tool to aid clinical assessment but, at present, the evidence suggests it should not be used alone or routinely.*

Level of evidence	
The use of POC CRP has been shown to reduce antibiotic use and is cost-effective. However, the evidence is mixed and further research is required to further develop the evidence for implementation.	**Level 1a: Validating cohort studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
A small sample of blood is taken from the finger, and combined with a reagent containing anti-CRP antibodies. The antibodies form a complex with serum CRP and the resulting complexes can be measured using an automatic reader.	**High**

1. Definition

C-reactive protein (CRP) is a biomarker of inflammation that offers clinicians a guide to antibiotic prescribing decisions. Point-of-care (POC) CRP testing is increasingly used in primary care to assist GPs in the diagnostic workup for various complaints. POC CRP testing can be completed in 4 minutes from a finger-stick blood sample, it is a relatively pain-free procedure and would fit into the primary care consultation. POC CRP testing is used routinely in a number of European countries where benefits have been shown in reduction in prescribing and antimicrobial resistance. A number of POC devices have been identified to measure CRP levels, including Afinion CRP (Alere), QuikRead CRP (Orion Diagnostica); NycoCard II Reader (Axis-Shield); Modern health systems Ltd. C-reactive protein test; Eurolyser Smart POC instrument (Horizon scan report 0017).

2. Summary of the evidence

Recent draft NICE guidance states to consider a POC CRP test for patients presenting with lower RTI in primary care if it is not clear after clinical assessment whether antibiotics should be prescribed. The NICE guideline on Feverish Illness in Children (CG47, May 2007) also recommends the use of CRP in conjunction with full blood count, blood cultures and urine testing.

Huang et al (2013) conducted a systematic review to look at the association between POC CRP testing and antibiotic prescribing in RTIs. The review included 13 studies (10,005 patients) and showed POC CRP testing was associated with a significant reduction in antibiotic prescribing at the index consultation (RR 0.75, 95% CI 0.67 to 0.83), but was not associated with antibiotic prescribing at any time during the 28-day follow-up period (RR 0.85, 0.70 to 1.01) or with patient satisfaction (RR 1.07, 0.98 to 1.17).

Engel et al (2012) reviewed the available evidence for the role of POC CRP measurement in (i) guiding antibiotic prescription, (ii) predicting aetiology, (iii) prognosis and (iv) diagnosis of pneumonia in LRTI patients. The review included 12 articles, each answering one or more questions. (i) One of four studies showed a significant reduction in the antibiotic prescriptions when applying POC CRP measurement (RR 0.6, 95% CI 0.5-0.7).
(ii) Three studies on aetiology demonstrated that an elevated CRP was associated with bacterial (OR 2.46 to 4.8) and one with viral (OR 2.7) aetiology.
(iii) Results on the prognostic value were contradictory, providing evidence for faster symptom resolution (RR 1.16, 1.1 to 1.3), higher mortality rate (RR 2.5, 1.2 to 5.1) and no difference in outcome in patients with high CRP levels.

(iv)Four studies showed that CRP had limited value as a single predictor of pneumonia. When combined with clinical assessment, its value increased according to two of these studies (receiver operating characteristic area from 0.7 to 0.9). However, methodological flaws and/or wide CIs limit the generalisability of findings in all studies.

Joshi et al (2013) conducted a systematic review on the use of POC CRP tests in primary care. The outcomes assessed were antibiotic prescription, return visit, clinical recovery and diagnosis; POC CRP was effective across various outcomes in 9 of the studies. Four of the studies showed POC CRP testing to be useful in facilitating decision-making but no studies recommended it be used alone to diagnose infection and prescribe antibiotics. The authors concluded that POC CRP testing is a satisfactory procedure that can be used to detect inflammation and can reduce antibiotic prescription in primary care.

Minnaard et al (2013) compared analytical performance, agreement and user-friendliness of five POC CRP tests. Results were compared with those of a standard immunoturbidimetric method performed on a routine analyzer. Within-day CVs varied from 2.6% to 19.4% for low CRP values (< 20 mg/L), and 1.1% to 17.5% for high values (> 100 mg/L). Between-day CVs varied from 4.6% to 30.5% for low values and 4.0% to 18.0% for high values. With high CRP values (> 100 mg/L) agreement with the laboratory standard systematically decreased for all POC tests. Analytical performance, agreement, and user-friendliness of the POC CRP tests varied considerably, yet overall four devices showed adequate analytical performance and agreement.

Hunter et al (2012) conducted a cost-effectiveness analysis of the Afinion POC CRP test in primary care. The additional cost per patient of the CRP test is outweighed by the associated cost savings and QALY increment associated with a reduction in infections in the long term.

3. Requirements for further research

Further studies comparing rates of antibiotic prescription when using a POC CRP test are needed to evaluate the applications of the technology.

4. References

- Engel MF, et al., (2012). Evaluating the evidence for the implementation of C-reactive protein measurement in adult patients with suspected lower respiratory tract infection in primary care: a systematic review. *Family Practice* 29(4): 383-93.
- Huang Y, et al., (2013) Association between point-of-care CRP testing and antibiotic prescribing in respiratory tract infections: a systematic review and meta-analysis of primary care studies. *The British Journal of General* 63(616):787-94.
- Hunter, R (July 2014) Analysis of the cost-effectiveness of Afinion™ CRP test in Primary Care in the English National Health Service (NHS). To be submitted for publication.
- Joshi A, et al., (2013). Feasibility of using C-reactive protein for point-of-care testing. *Technology & Health Care* 21(3): 233-40.
- Minnaard MC, et al., (2013). Analytical performance, agreement and user-friendliness of five C-reactive protein point-of-care tests. *Scandinavian Journal of Clinical & Laboratory Investigation* 73(8): 627-34.
- NICE Pneumonia Guideline - Draft for consultation, June 2013
- NICE clinical guideline 69. Respiratory tract infections – antibiotic prescribing: Prescribing of antibiotics for self-limiting respiratory tract infections in adults and children in primary care. July 2008
- NIHR SPCR Horizon Scan Report 0017: Diagnostic Technology: Point-of-care test (POCT) for C-reactive protein (CRP). 28 July 2011

APPENDIX 38
TECHNOLOGY: IMMUNOCHROMATOGRAPHY TEST FOR RAPID AND DETECTION OF CLOSTRIDIUM DIFFICILE ANTIGEN AND TOXINS

Bottom line: *Immunochromotography tests for C. difficile infection could prove to be a reliable first-line method for detection of Clostridium difficile in stool specimens. Further clinical studies are warranted.*

Level of evidence	
The test may be beneficial in rapid diagnosis of *C. difficile* infection (CDI). It does not require special facilities. However there is a lack of clinical studies.	**Level 4: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Immunochromotography tests for CDI are rapid and have a high degree of accuracy. They are simple to perform and potentially cost-effective. They could prove to be a reliable first-line method for detection of *Clostridium difficile* in stool specimens.	**High**

1. Definition
The test detects both glutamate dehydrogenase (GDH) antigen and toxin A/B with one easy-to-use cartridge. The test can be used in hospital settings.

2. Summary of the evidence
One study used 608 consecutive loose stool specimens collected from the patients with suspected CDI. In comparison to the toxigenic cultures, the CD COMPLETE-toxin test had a sensitivity and specificity of 64% and 98% whilst the PPV and NPV were 76% and 96%. In comparison to the toxigenic cultures, the VIDAS CDAB test had a sensitivity and specificity of 76% and 97%, whilst the PPV and NPV were 73% and 98%. In comparison to the enriched *C. difficile* cultures, the sensitivity and specificity for the CD COMPLETE-GDH test were 91% and 92% with a PPV and NPV of 71% and 98%.

Another study tested 223 stool specimens from hospitalized patients with antibiotics-associated diarrhoea. The C. DIFF QUIK CHEK COMPLETE test had a sensitivity of 84% and specificity of 94% compared to PCR for Tox A/B (PPV 99%, NPV 92%). The Tox A/B EIA yielded corresponding values of 72% and 93%, with a PPV and NPV of 85% and 86%, respectively.

A third study compared immunoassay to both PCR and toxigenic culture. The results showed that this assay allows 88% of specimens to be accurately screened as either positive (both tests positive) or negative (both tests negative) for the presence of toxigenic *C. difficile* in less than 30 minutes and with minimal hands-on time. The sensitivity was 100% and specificity 99%.

A fourth study reported that some specimen may require retesting when results are discrepant.

3. Requirements for further research
Further clinical studies and cost-effectiveness analyses are required.

4. References

- Kim H, Kim WH, Kim M, Jeong SH, Lee K. Evaluation of a rapid membrane enzyme immunoassay for the simultaneous detection of glutamate dehydrogenase and toxin for the diagnosis of *Clostridium difficile* infection. Ann Lab Med. 2014 May;34(3):235-9.
- Quinn CD, Sefers SE, Babiker W, He Y, Alcabasa R, Stratton CW, Carroll KC, Tang YW. C. Diff Quik Chek complete enzyme immunoassay provides a reliable first-line method for detection of *Clostridium difficile* in stool specimens. J Clin Microbiol 2010; 48 (2): 603-5
- Samra Z, Madar-Shapiro L, Aziz M, Bishara J. Evaluation of a new immunochromatography test for rapid and simultaneous detection of *Clostridium difficile* antigen and toxins. Isr Med Assoc J. 2013 Jul;15(7):373-6

- Sharp SE, Ruden LO, Pohl JC, Hatcher PA, Jayne LM, Ivie WM. Evaluation of the C.Diff Quik Chek Complete Assay, a new glutamate dehydrogenase and A/B toxin combination lateral flow assay for use in rapid, simple diagnosis of *Clostridium difficile* disease. J Clin Microbiol 2010; 48 (6): 2082-6.

APPENDIX 39
TECHNOLOGY: MODIFIED MULTIPLE-LOCUS VARIABLE-NUMBER TANDEM-REPEAT ANALYSIS FOR RAPID IDENTIFICATION OF CLOSTRIDIUM DIFFICILE DURING INSTITUTIONAL OUTBREAKS

Bottom line: *Modified MLVA has high set-up costs, requires technical expertise and currently lacks clinical trial evidence.*

Level of evidence	
High set-up costs, requires technical expertise and lacks clinical trial evidence.	**Level 5: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Highly discriminatory detection and typing; enables simultaneous detection of CDI and identification of hypervirulent epidemic strains, as well as providing typing information which enables rapid identification of clusters of outbreak isolates in institutions; expensive although it has a shorter turnaround time compared with MLVA, it does not have significant advantages over other PCR methods.	**Low**

1. Definition
Modified MLVA (MMLVA) differs from traditional MVLA* though its ability to isolate the epidemic strain NAP1/027. It requires laboratory staff with technical expertise.

2. Summary of the evidence
By adding the 18-bp NAP1 tcdC deletion to the MMLVA analysis, the NAP1/027 strain responsible for all outbreaks was immediately separated from non-NAP1/027 strains. This included NAP1/027 variants with single-nucleotide polymorphisms (SNPs) upstream of the deletion. Good correlation between MMLVA and pulsed-field gel electrophoresis (PFGE) was observed. MMLVA technique was able to further segregate four clusters within 19 NAP1/027 isolates and two NAP7 isolates forming a cluster, as well as four other non-NAP1 pairs.

3. Requirements for further research
Long-term cost-effectiveness and clinical trials are needed.

4. References

- Broukhanski G, Low DE, Pillai DR. Modified multiple-locus variable-number tandem-repeat analysis for rapid identification and typing of *Clostridium difficile* during institutional outbreaks. J Clin Microbiol. 2011 May;49(5):1983-6

*Traditional MLVA is technically challenging, requires considerable expertise beyond the medical laboratory technologist level, and relies on prior culture of the organism, resulting in significant delays in reporting during institutional outbreak investigation.

APPENDIX 40
TECHNOLOGY: NEW SELECTIVE CULTURE MEDIA AND FISH FOR THE DETECTION OF CLOSTRIDIUM DIFFICILE IN STOOL SAMPLES

Bottom line: *Allows C. difficile strain typing and resistance testing, which allows for species identification and toxin detection within the same day. However the technology currently lacks clinical trial data and cost-effectiveness studies.*

Level of evidence	
It may be of value in *C. difficile* strain typing and resistance testing; allows for species identification and toxin detection within the same day.	**Level 5: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
A rapid, sensitive and cheap test that has high sensitivity and specificity. It showed no cross-reaction to related species.	**High**

1. Definition
This method utilizes two new selective agars, the modified *C. difficile* Selective Agar (CDSB) from Oxoid and the *C. difficile* Selective Agar (CDSA) from BD for the detection of CDI. For fluorescent in situ hybridization (FISH), the authors designed a *C. difficile* -specific 16S rRNA-based probe (Cd-198 m: 5 9 -CATCCTGTACTGGCTCAC), labelled with Cy3 (Thermo Hybaid), and used it in conjunction with the eubacterial FITC-conjugated probe EUB. It can be used in hospital settings.

2. Summary of the evidence
For the culture-positive samples, the sensitivities were 84%, 43% and 90%, for CDSB, CDSA and conventional culture respectively. The FISH method in this design requires hand-on time of only 10 minutes.

3. Requirements for further research
Further testing in clinical trials is warranted along with cost-effectiveness analysis.

4. References

- Bloedt K, Riecker M, Poppert S, Wellinghausen N. Evaluation of new selective culture media and a rapid fluorescence in situ hybridization assay for identification of *Clostridium difficile* from stool samples. J Med Microbiol. 2009 Jul;58(Pt 7):874-7.

APPENDIX 41
TECHNOLOGY: RAPID MOLECULAR DIAGNOSTIC TESTS FOR THE DETECTION OF CLOSTRIDIUM DIFFICILE

Bottom line: *PCR is a rapid useful test for diagnosing, with high sensitivity and specificity, but is expensive.*

Level of evidence	
PCR is highly sensitive and specific, however the accuracy of loop-mediated isothermal amplification (LAMP) is uncertain.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
PCR is a useful diagnostic test for detecting *C. difficile* infection (CDI) but is expensive.	**Medium**

1. Definition
PCR techniques generate copies of particular DNA sequences, while loop-mediated isothermal amplification (LAMP) amplifies DNA.

2. Summary of the evidence
The systematic review included 25 PCR studies, and 6 LAMP studies (O'Horo et al, 2012). Compared with toxigenic culture, pooled sensitivity and specificity were 92% and 94%, respectively. Using cytotoxicity as a standard, pooled sensitivity was 87% whilst specificity was 97%. Heterogeneity was substantial. The PCR results confirm the results of an earlier review, which also showed high specificity and sensitivity (Deshpande et al, 2011).

Balance of benefit and harm
The test is rapid and has high sensitivity and specificity; overall accuracy is dependent on the prevalence of CDI.

3. Requirements for further research
Detection of asymptomatic colonization (PCR); better quality studies needed (LAMP).

4. References

- O'Horo JC, Jones A, Sternke M, Harper C, Safdar N. Molecular Techniques for Diagnosis of *Clostridium difficile* Infection: Systematic Review and Meta-analysis. Mayo Clin Proc. 2012 Jul;87(7):643-51.
- Deshpande A, Pasupuleti V, Rolston DD, Jain A, Deshpande N, Pant C, Hernandez AV. Diagnostic accuracy of real-time polymerase chain reaction in detection of *Clostridium difficile* in the stool samples of patients with suspected *Clostridium difficile* Infection: a meta-analysis. Clin Infect Dis. 2011;53(7):e81-e90.

APPENDIX 42
TECHNOLOGY: COMBINATION OF A SHORT MULTI-CAPILLARY GAS CHROMATOGRAPHY COLUMN WITH METAL OXIDE SENSOR DETECTION FOR DETECTION OF CLOSTRIDIUM DIFFICILE

Bottom line: *Short multi-capillary chromatography column is in the early phase of development. The accuracy of the technology requires improvement.*

Level of Evidence	
This technology is rapid, but its sensitivity and specificity is less than 90%.	**Level 5: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It may be useful as a point-of-care test but it will lead to substantial false positive and false negative test results unless accuracy is improved.	**Low**

1. Definition

This technique uses a combination of short multi-capillary chromatography column (SMCC), highly sensitive heated metal oxide semiconductor sensor and artificial neural network software. It can be used in clinic settings.

2. Summary of the evidence

The diagnostic potential of the prototypes was assessed using 50 *C. difficile* positive and 50 negative samples. The prototypes showed similar capability of discriminating between positive and negative samples with a sensitivity of 85% and a specificity of 80%.

Balance of benefit and harm

It is a quick method. However, use of this technique can result in false positives and false negatives.

3. Requirements for further research

Validation testing is required.

4. References

- McGuire ND, Ewen RJ, de Lacy Costello B, Garner CE, Probert CSJ, Vaughan K, Ratcliffe NM. Towards point of care testing for *C. difficile* infection by volatile profiling, using the combination of a short multi-capillary gas chromatography column with metal oxide sensor detection. Meas. Sci. Technol. 2014; 25 065108

APPENDIX 43
TECHNOLOGY: ALTERNATIVE BIOMARKERS AND BIOMARKER COMBINATIONS IN THE DIAGNOSIS OF INFECTION AND SEPSIS

Bottom line: *Combining biomarkers might be a useful strategy for improving diagnosis, however there is a lack of clinical studies and cost effectiveness, which is preventing uptake.*

Level of evidence	
Combining several biomarkers, as well as investigating alternative biomarkers, shows promise but further studies are required.	**Level 3: Laboratory & observational studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Several new biomarkers are being assessed for their relevance to the diagnosis of infection, as well as the utility of combining markers, however this requires further clinical validation.	**Medium**
Combining CRP, PCT and temperature may be an approach to improve detection of nosocomial infection in the ICU.	**Medium**
NLCR showed promise as a cheap and rapid marker to differentiate bacterial bloodstream infection patients.	**Medium**

1. Definition

While biomarkers such as CRP and procalcitonin have been investigated individually for their utility in aiding diagnosis of infection, with current technology the possibility exists to combine biomarker measures. It is unclear what the added benefit of this strategy might be.

2. Summary of the evidence

One recent study assessed the utility of C-reactive protein (CRP), neutrophil-lymphocyte count ratio (NLCR), procalcitonin (PCT) and soluble urokinase-type plasminogen activator receptor (suPAR) in the diagnosis of infection in patients presenting to an emergency care unit (Loonen et al). The study reported that the receiver operating characteristic curves of the 4 biomarkers for differentiating bacteraemia from non-bacteraemia showed the highest area under the curve (AUC) for PCT (0.806). NLCR, suPAR and CRP resulted in an AUC of 0.770, 0.793, and 0.485, respectively. However as PCT is an expensive biomarker, the study suggested that NLCR showed promise as a cheap and rapid marker to differentiate bacterial bloodstream infection patients for subsequent pathogen identification.

A prospective observational cohort study of ICU patients assessed a composite score combining procalcitonin, CRP, and temperature for its accuracy in the diagnosis of intensive care-acquired infections (Robriquet et al). For temperature the sensitivity and specificity were 76% and 94%, respectively; for PCT, they were 68% and 91%; and for CRP, they were 92% and 70%. This led to a composite score - $(0.068 \times D0\ PCT + 0.005 \times D0\ CRP + 0.7 \times \text{temperature})$ - which was more highly specific than each component. The composite score had an AUC of 0.90, a sensitivity of 80% and a specificity of 97%.

A conference abstract reported a study investigating the clinical utility of a novel neonatal sepsis diagnostic platform (ASCMicroPlat). The platform, which combines CRP and PCT, was used on 350 infants being evaluated for sepsis. The preliminary conclusion of the study was that this technology may have clinical utility in sepsis diagnosis, (McAllister et al).

Another conference abstract on the influence of the availability of interleukin-6 and CRP results at the point of care on clinical decision making in neonatal sepsis (web-based questionnaire of hypothetical neonatal sepsis scenarios with hypothetical biomarker results) suggested that both point-of-care IL-6 and CRP results helped confirm a diagnosis of sepsis, however IL-6 was not useful in ruling out sepsis

(Babarao et al). New tests for the rapid quantitative measurement of procalcitonin, which alongside other laboratory findings may aid risk assessment for progression to severe sepsis are also available (e.g. Siemens ADVIA Centaur systems PCT assay).

Markers which may show promise for the management of antibiotic therapy in acute infections include:

- Soluble Triggering Receptor Expressed on Myeloid cells-1 (sTREM-1),
- Soluble urokinase-type Plasminogen receptor (suPAR),
- Proadrenomedullin (ProADM),
- Presepsin (Dupuy et al).

However, the current evidence base for these markers is limited and further research is required to establish their accuracy and clinical utility, particularly in children. In addition, markers of endothelial cell activation are also being investigated as potentially clinically informative markers to improve diagnosis, prognosis and evaluation of patients with sepsis, however a systematic review has concluded that currently the evidence for their clinical utility is lacking with few studies reporting clinically-relevant measures (Xing et al.).

Research into multi-parameter point-of-care-testing, for example TNFalpha, PCT and CRP, is also under way but currently appears to be at the proof-of-concept stage (Krämer et al).

3. Requirements for further research

Several new biomarkers are under investigation, as well as multi-platform systems simultaneously measuring more than one biomarker, however these require further investigation and clinical validation.

4. References

- Babarao S, Miall L. Does Availability of Interleukin-6 Results Influence Clinical Decision Making in Neonatal Sepsis? Arch Dis Child 2012;97:A339-A340
- Dupuy AM, Philippart F, Péan Y, Lasocki S, Charles PE, Chalumeau M, Claessens YE, Quenot JP, Guen CG, Ruiz S, Luyt CE, Roche N, Stahl JP, Bedos JP, Pugin J, Gauzit R, Misset B, Brun-Buisson C; Maurice Rapin Institute Biomarkers Group. Role of biomarkers in the management of antibiotic therapy: an expert panel review: I - currently available biomarkers for clinical use in acute infections. Ann Intensive Care. 2013 Jul 9;3(1):22.
- Krämer PM, Kess M, Kremmer E, Schulte-Hostede S. Multi-parameter determination of TNFα, PCT and CRP for point-of-care testing. Analyst. 2011 Feb 21;136(4):692-5.
- Loonen AJ, de Jager CP, Tosserams J, Kusters R, Hilbink M, Wever PC, van den Brule AJ. Biomarkers and molecular analysis to improve bloodstream infection diagnostics in an emergency care unit. PLoS One. 2014 Jan 27;9(1):e87315.
- McAllister K, Sheridan-Pereira M, O'Sullivan N, O'Kelly R, Mark D, Czilwik G, Martin C, Sheils O, O'Leary J. Clinical utility of using C-reactive protein and procalcitonin as biomarkers for a novel neonatal sepsis diagnostic platform (ASCMicroPlat). Crit Care. 2012; 16(Suppl 3): P106.
- Robriquet L, Séjourné C, Kipnis E, D'Herbomez M, Fourrier F. A composite score combining procalcitonin, C-reactive protein and temperature has a high positive predictive value for the diagnosis of intensive care-acquired infections.BMC Infect Dis. 2013 Apr 2;13:159
- Xing K, Murthy S, Conrad Liles W, Singh JM. Clinical utility of biomarkers of endothelial activation in sepsis-a systematic review. Crit Care. 2012; 16(1): R7.

APPENDIX 44
TECHNOLOGY: REAL-TIME PCR TESTS FOR THE DIAGNOSIS OF SEPSIS

Bottom line: *A systematic review of the accuracy of real-time PCR tests for multiple pathogens in the diagnosis of sepsis is currently in process and further research should await the results of this review.*

Level of evidence	
This technology may have some clinical utility but further prospective studies are required (depending on the results of the systematic review in progress).	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Real-time PCR tests for detecting multiple pathogens, both bacterial and fungal, may have utility in the diagnosis of sepsis in the hospital setting, A systematic review of the accuracy of real-time PCR tests for multiple pathogens in the diagnosis of sepsis is currently in process and further research should await the results of this review.	**High**

1. Definition

Real-time PCR tests to diagnose sepsis by determining the presence of several pathogens have been developed, for example the Magicplex Sepsis test (Seegene), SepsiTest (Molzym) and SeptiFast (Roche). These are broad range tests identifying several bacteria and fungi, and in some cases, resistance genes.

2. Summary of the evidence

A systematic review of the accuracy of the SeptiFast multiplex PCR system for the detection of pathogens in patients with presumed sepsis assessed 34 studies with over 6,000 patients with suspected sepsis. It reported an overall sensitivity of 75% and specificity of 92% to detect bacteraemia or fungaemia (Chang et al). The study concluded that the SeptiFast was a good rule-in test for early detection of septic patients.

A recent study of patients presenting the ED with suspected sepsis assessed SepsiTest and MagicPlex Sepsis Test (Loonen et al). When compared to blood cultures, the sensitivity and specificity, for SepsiTest was 11% and 96%, respectively. For the MagicPlex Sepsis Test, the sensitivity and specificity was 37%, and 77%, respectively. The study concluded that these tests had several limitations and were not yet suitable for implementation.

A NIHR HTA systematic review of *"Rapid detection of healthcare-associated bloodstream infection in critical care using multi-pathogen real-time polymerase chain reaction (RT-PCR) technology: a diagnostic accuracy study and systematic review*" is currently under way, with the expected publication date of April 2015 (http://www.nets.nihr.ac.uk/projects/hta/081316). Further assessment of these technologies should await the publication of this report.

3. Requirements for further research

A systematic review of the accuracy of real-time PCR tests for multiple pathogens in the diagnosis of sepsis is currently in process and further research should await the results of this review.

4. References

- Chang SS, Hsieh WH, Liu TS, Lee SH, Wang CH, Chou HC, Yeo YH, Tseng CP, Lee CC. Multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis - a systemic review and meta-analysis. PLoS One. 2013 May 29;8(5):e62323.
- Loonen AJ, de Jager CP, Tosserams J, Kusters R, Hilbink M, Wever PC, van den Brule AJ. Biomarkers and molecular analysis to improve bloodstream infection diagnostics in an emergency care unit. PLoS One. 2014 Jan 27;9(1):e87315.

APPENDIX 45
TECHNOLOGY: BIOSENSOR PLATFORM FOR RAPID ANTIMICROBIAL SUSCEPTIBILITY TESTING

Bottom line: *Biosensor systems could have a role in improving the rapid detection of UTI. Research aimed at integrating biosensors with microfluidic technology and automation of sample processing for point of care application is required.*

Level of evidence	
This technology can significantly improve the sensitivity of the assay compared with standard absorbance spectroscopy.	**Level 4: Prospective studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It has been tested for UTI diagnosis in patients at risk of polymicrobial infection. It can detect organisms and WBCs within an hour.	**High**

1. Definition

The system comprises of a recognition system and a signal transducer. It works by detecting a biological entity, ranging from lateral flow test strips for pregnancy testing to cell-based sensors using B-lymphocytes to direct pathogens. The magnitude of the signal correlates with the concentration of the analyte.

2. Summary of the evidence

In a clinical validation study with 368 urine samples, the biosensor based antimicrobial susceptibility test showed 94% accuracy compared to standard microbiological analysis (Liao et al, 2006). Another clinical validation study reported 89% sensitivity and 97% specificity (Mach et al, 2009). There was also a significant correlation between lactoferrin concentration measured by the biosensor and while blood cells (WBC) and leukocyte esterase ($p<0.001$ for both). A third study reported 100% sensitivity and 98% specificity for identification of gram-negative bacteria (Mach et al, 2011). A fourth study reported a sensitivity of 89% and specificity 100% (Mohan et al, 2011).

3. Requirements for further research

Research aimed at integrating biosensors with microfluidic technology and the automation of sample processing for point of care application is required.

4. References

- Liao JC, Mastali M, Gau V, Suchard MA, Møller AK, Bruckner DA, Babbitt JT, Li Y, Gornbein J, Landaw EM, McCabe ER, Churchill BM, Haake DA. Use of electrochemical DNA biosensors for rapid molecular identification of uropathogens in clinical urine specimens. J Clin Microbiol. 2006; 44:561–570.
- Mach KE, Du CB, Phull H, Haake DA, Shih MC, Baron EJ, Liao JC. Multiplex pathogen identification for polymicrobial urinary tract infections using biosensor technology: a prospective clinical study. J Urol. 2009; 182:2735–2741.
- Mach KE, Mohan R, Baron EJ, Shih MC, Gau V, Wong PK, Liao JC. A Biosensor Platform for Rapid Antimicrobial Susceptibility Testing Driectly from Clinical Samples. J Urol. 2011 Jan;185(1):148-53
- Mohan R, Mach KE, Bercovici M, Pan Y, Dhulipala L, Wong PK, Liao JC. Clinical validation of integrated nucleic acid and protein detection on an electrochemical biosensor array for urinary tract infection diagnosis. PLoS One. 2011;6(10):e26846.

APPENDIX 46
TECHNOLOGY: CELL PHONE-BASED MICROPHOTOMETRIC SYSTEM FOR RAPID ANTIMICROBIAL SUSCEPTIBILITY TESTING

Bottom line: *Gas-permeable microwell arrays are simple to use and likely to be a cost effective for antimicrobial susceptibility testing and require further clinical studies to assess their role in clinical care.*

Level of evidence	
Gas-permeable microwell arrays can significantly improve the sensitivity of the assay compared with standard absorbance spectroscopy.	**Level 5: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
A simple to use and cost-effective POCT.	**High**

1. Definition

This technique works by incorporating gas-permeable microwell arrays, a colorimetric cell viability reagent, and a cell phone–based microphotometric system. The bacteria are cultured in gas-permeable microwell arrays to allow rapid bacterial growth without the requirement of oxygenation or external agitation. It can be used in low-resource settings such as hospitals and field environments.

2. Summary of the evidence

Urine samples with bacterial concentration from 10^1 to 10^6 cfu/mL can be tested directly by adjusting the incubation time. Higher absorbance values were observed for the uropathogens in urine when iPhotometer was used.

Balance of benefit and harm

This is a rapid test and does not require complicated sample preparation procedures because it directly determines the antibiotic resistance of bacterial in physiological samples. A larger variation in the absorbance value could result in misleading results.

3. Requirements for further research

Optimization of the pre-coating procedure and then further accuracy and clinical studies are required.

4. References

- Kadlec MW, You D, Liao JC, Wong PK. A Cell Phone-Based Microphotometric System for Rapid Antimicrobial Susceptibility Testing. J Lab Autom. 2013.

APPENDIX 47
TECHNOLOGY: RAPID URINE TESTS (DIP STICKS & MICROSCOPY) FOR URINARY TRACT INFECTION IN CHILDREN

Bottom line: *Studies assessing the incremental accuracy of a rapid test for UTI in children compared with a clinical investigation alone are required.*

Level of evidence	
Rapid tests are negative in around 10% of children with UTI and cannot replace urine culture. If resources allow, microscopy with Gram stain should be the single rapid test used.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Microscopy alone is not more accurate than dipstick for diagnosis of UTI.	**Medium**

1. Definition

This technology uses urine dipsticks and microscopy of urine to estimate white blood cells (WBC) and bacteria. They are not designed to replace urine culture because they neither identify the causal pathogen nor establish antibiotic sensitivities that guide the choice of antibiotic. Dipsticks can be used as a point-of care test.

2. Summary of the evidence

The systematic review included 95 studies (n = 95,703).

Test	*Sensitivity*	*Specificity*
Microscopy for gram-stained bacteria	91%	96%
Microscopy for unstained bacteria	88%	92%
Microscopy for urine white cells	74%	86%
Leucocyte esterase or nitrite positive dipstick	88%	79%
Nitrite-only positive dipstick	49%	98%

Microscopy for bacteria with Gram stain had higher accuracy than other laboratory tests with relative diagnostic odds ratio compared with bacteria without Gram stain of 8·7 (95% CI: 1·8–41·1), WBC of 14·5 (95% CI: 4·7–44·4), and nitrite of 22·0 (95% CI: 0·7–746·3).

Balance of benefit and harm

It is a rapid test but does give rise to false negative test results.

3. Requirements for further research

Studies assessing the incremental accuracy of a rapid test after clinical investigation compared with a clinical investigation alone are needed.

4. References

- Williams GJ, Macaskill P, Chan SF, Turner RM, Hodson E, Craig JC. Absolute and relative accuracy of rapid urine tests for urinary tract infection in children: a meta-analysis. Lancet Infect Dis. 2010 Apr;10(4):240-50.

APPENDIX 48
TECHNOLOGY: RAPID URINE TESTS (DIP STICKS) FOR URINARY TRACT INFECTION

Bottom line: *Urine dipstick test alone seems to be useful in all populations to exclude the presence of infection if the results of both nitrites and leukocyte-esterase are negative.*

Level of evidence	
The usefulness of the dipstick test alone to rule in infection is uncertain, even with high pre-test probabilities.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Urine dipstick test alone seems to be useful in all populations to exclude the presence of infection if the results of both nitrites and leukocyte-esterase are negative.	**High**

1. Definition
These use urine dipsticks estimate white cells and bacteria. Dipsticks can be used as point-of-care test.

2. Summary of the evidence
The systematic review included 70 articles (72 studies). Diagnostic accuracy of nitrites was high in pregnant women (OR = 165) and elderly individuals (OR = 108). PPVs were ≥80% in elderly and in family medicine. Diagnostic accuracy of leukocyte-esterase was high in studies in urology patients (OR = 276). Sensitivities were highest in family medicine (86%). NPVs were high in both tests in all patient groups and settings, except for in family medicine. The combination of both test results showed an important increase in sensitivity. Diagnostic accuracy was high in studies in urology patients (OR = 52), in children (OR = 46), and if clinical information was present (OR = 28). Sensitivity was highest in studies carried out in family medicine (90%). Predictive values of combinations of positive test results were low in all other situations.

Balance of benefit and harm
It is rapid although it can have false positives and low specificity.

3. Requirements for further research
Double-blinded clinical trials are required; such studies should also clearly define their inclusion and exclusion criteria.

4. References

- Devillé WL, Yzermans JC, van Duijn NP, Bezemer PD, van der Windt DA, Bouter LM. The urine dipstick test useful to rule out infections. A meta-analysis of the accuracy. BMC Urol. 2004 Jun 2;4:4

APPENDIX 49
TECHNOLOGY: FLEXICULT SSI-URINARY KIT FOR URINARY TRACT INFECTION DIAGNOSIS AND SUSCEPTIBILITY TESTING

Bottom line: *The use of a UTI diagnosis kit with susceptibility testing could aid diagnosis and, more importantly, antimicrobial treatment in UTI. When available, results of a recently completed RCT could influence how we manage UTI. The study aims to quantify the costs and effects of an optimised point-of-care test, guided diagnostic and treatment regime for symptoms of uncomplicated UTI.*

Level of evidence	
It is suitable for POCT and susceptibility testing of uncomplicated UTI.	**Level 4: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
This technology could be beneficial in point-of-care test in primary care settings. It is rapid, easy to handle and read but cannot identify isolates.	**High**

1. Definition

Flexicult SSI urinary kit is designed as an ordinary Petri dish divided into six compartments: a large one for quantitative analysis and five smaller ones for susceptibility testing. The agar in each small compartment contains one of five antimicrobials (trimethoprim, sulfamethoxazole, ampicillin, nitrofurantoin and mecillinam) at a concentration adjusted to the breakpoint, and growth in these compartments indicates resistance. It can be used as a point-of-care test.

2. Summary of the evidence

The kit was tested in-house with 116 urinary tract pathogens and by 19 general practitioners in a field trial with 121 diagnostic urine specimens. In-house validation determined the detection limit for the kit to be approximately 5 x 10^2 cfu/ml. In the field trial, the quantitation had an overall error rate of 4% and correctly determined susceptibility in 93% of the tested bacteria.

We identified a randomised interventional and observational trial completed on 31/07/2014*. The study aims to quantify the costs and effects of an optimised point-of-care test, guided diagnostic and treatment regime for symptoms of uncomplicated UTI.

3. Requirements for further research

Further development is required and the RCT will require repeating in other settings.

4. References

- Blom M, Sørensen TL, Espersen F, Frimodt-Møller N. Validation of FLEXICULT SSI-Urinary Kit for use in the primary health care setting. Scand J Infect Dis. 2002;34(6):430-5.

* Point of care testing for urinary tract infection in primary care: Stages 3 & 4. http://www.controlled-trials.com/ ISRCTN65200697/ [Accessed 22nd October, 2014]

APPENDIX 50
TECHNOLOGY: MICROFLUIDICS FOR URINARY TRACT INFECTION DIAGNOSIS AND SUSCEPTIBILITY TESTING

Bottom line: *The use of microfluidics with susceptibility testing could aid diagnosis and, more importantly, antimicrobial treatment in UTI. RCTs are required to determine patient benefit.*

Level of evidence	
It is suitable for POCT and susceptibility testing of UTI.	**Level 4: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Susceptibility can be determined as rapidly as 2 hours compared to days in standard clinical procedures.	**High**

1. Definition

Some utilize gas permeable polydimethylsiloxane (PDMS) microchannels that have a large surface-to-volume ratio. Others are composed of a microfluidic chip, laser excitation, high-voltage supply, and a custom-made point confocal fluorescence system. This allows for detection of bacterial 16S ribosomal RNA (rRNA) without the need to perform nucleic acid amplification steps such as PCR. They can be used as point-of-care tests.

2. Summary of the evidence

One study reported growth rates that could be clearly distinguishable after 2 hours (Chen et al, 2010). The system sustained the growth of *E. coli* to over 10^9 cfu/mL without external agitation or oxygenation.

Two other studies showed PCR-free quantitative detection of bacterial rRNA from infected human urine samples, at clinically relevant concentrations (Riahi et al, 2011; Bercovici et al, 2011).

Balance of benefit and harm

It is a rapid technology that can be easily integrated.

3. Requirements for further research

Accuracy studies followed by clinical trials to determine its effectiveness in clinical practice are required.

4. References

- Chen CH, Lu Y, Sin ML, Mach KE, Zhang DD, Gau V, Liao JC, Wong PK. Antimicrobial susceptibility testing using high surface-to-volume ratio microchannels. Anal Chem. 2010 Feb 1;82(3):1012-9
- Riahi R, Mach KE, Mohan R, Liao JC, Wong PK. Molecular detection of bacterial pathogens using microparticle enhanced double-stranded DNA probes. Anal Chem. 2011 Aug 15;83(16):6349-54
- Bercovici M, Kaigala GV, Mach KE, Han CM, Liao JC, Santiago JG. Rapid detection of urinary tract infections using isotachophoresis and molecular beacons. Anal Chem. 2011 Jun 1;83(11):4110-7

APPENDIX 51
TECHNOLOGY: POINT-OF-CARE TESTS FOR NEISSERIA GONORRHOEAE

Bottom line: *The utility of these point-of-care tests in the UK setting is currently limited and based on current research, it is unclear if such tests would provide any benefit compared to current standard practice.*

Level of evidence	
Point-of-care tests for *N. gonorrhoeae* may have utility in high prevalence settings, however currently do not appear to provide benefit in the UK setting compared to usual care.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Rapid point-of-care tests for *N. gonorrhoeae* may aid in the diagnosis and inform appropriate antibiotic prescribing, while avoiding unnecessary treatment of patients whose symptoms are due to other causes.	**Medium**

1. Definition

Rapid, point-of-care tests for *Neisseria gonorrhoeae* include immunochromatographic test strips (ICT) (vaginal or cervical swabs; test time 15-30 minutes) and leucocyte esterase (LE) dipsticks (first void urine or vaginal/cervical swabs; test time under 2 minutes).

2. Summary of the evidence

A systematic review (Watchirs et al) of the accuracy of point-of-care tests for *N. gonorrhoeae* reported a median sensitivity for LE dipsticks of 71% (range 23–85%), median specificity of 70% (33–99%), median positive predictive value (PPV) of 19% (5–40%) and median negative predictive value (NPV) of 95% (56–99%). The median sensitivity of immunochromatographic tests (ICT) was 70% (60–94%), median specificity was 96% (89–97%), median PPV was 56% (55–97%) and median NPV was 93% (92–99%). However, due to heterogeneity between the studies and the use of different cut-offs a meta-analysis could not be performed. Overall the review concluded that in high prevalence settings both tests may provide some advantages over management based on symptoms alone, however they are limited by their lack of specificity in diagnosing gonorrhoea.

3. Requirements for further research

The utility of these point-of-care tests in the UK setting is currently limited and, based on current research, it is unclear if such tests would provide any benefit compared to current practice. In addition the emergence of multi-drug resistant strains, antibiotic susceptibility will be important in treatment decisions (Unemo et al.).

4. References

- Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. Clin Microbiol Rev. 2014 Jul;27(3):587-613.
- Watchirs Smith LA, Hillman R, Ward J, Whiley DM, Causer L, Skov S, Donovan B, Kaldor J, Guy R. Point-of-care tests for the diagnosis *of Neisseria gonorrhoeae* infection: a systematic review of operational and performance characteristics. Sex Transm Infect. 2013 Jun;89(4):320-6.

APPENDIX 52
TECHNOLOGY: POINT-OF-CARE TESTS FOR CHLAMYDIA TRACHOMATIS

Bottom line: *Current evidence suggests that the laboratory-based PCR tests are still the most accurate and cost-effective for diagnosing chlamydia and there is currently no robust evidence to support the use of POC chlamydia tests.*

Level of evidence	
Rapid point-of-care tests for chlamydia currently do not appear to provide advantages over usual care.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Accuracy for point of care tests for chlamydia is only moderate when compared to vaginal swabs. Economic analysis indicated that both point-of-care tests would be more costly and less effective than the current laboratory PCR-based tests.	**Medium**

1. Definition

Rapid point-of-care tests for *Chlamydia trachomatis* can be performed using either first void urine, urethral or vaginal swab specimens.

2. Summary of the evidence

A systematic review of the accuracy of point-of-care tests for *C. trachomatis* included thirteen studies with 8,817 participants (Hislop et al). In the pooled estimates for the Chlamydia Rapid Test (CRT), sensitivity (95% CI) was 80% (73% to 85%) for vaginal swab specimens and 77% (59% to 89%) for first void urine (FVU) specimens. Specificity was 99% (99% to 100%) for vaginal swab specimens and 99% (98% to 99%) for FVU specimens. In the pooled estimates for another point-of-care test (Clearview Chlamydia), sensitivity was 52% for vaginal, cervical and urethral swab specimens combined, and 64% for cervical specimens alone. Specificity was 97% for vaginal, cervical and urethral swab specimens combined, and 97% for cervical specimens alone. Economic analysis indicated that both point-of-care tests would be more costly and less effective than the current laboratory PCR-based tests.

3. Requirements for further research

Current evidence suggests that the laboratory-based PCR tests are still the most accurate and cost-effective for diagnosing chlamydia and there is currently no robust evidence to support the use of the above POC chlamydia tests. Given reports of antibiotic resistant chlamydia, further research should also await the results of a Cochrane systematic review assessing the effects and safety of antibiotic treatment for *Chlamydia trachomatis* genital infection in terms of microbiological and/or clinical cure (Paez-Canro et al).

4. References

- Carol Paez-Canro, Fernando Martinez-Martinez, Juan Pablo Alzate, Anne Lethaby and Hernando G Gaitán. Antibiotics for treating genital chlamydia trachomatis infection in men and non-pregnant women. Cochrane Systematic Review Protocol. Online Publication Date: December 2013.
- Hislop J, Quayyum Z, Flett G, Boachie C, Fraser C, Mowatt G. Systematic review of the clinical effectiveness and cost-effectiveness of rapid point-of-care tests for the detection of genital chlamydia infection in women and men. Health Technol Assess. 2010 Jun;14(29):1-97, iii-iv.

APPENDIX 53
TECHNOLOGY: RAPID NUCLEIC ACID AMPLIFICATION TEST FOR THE DIAGNOSIS OF CHLAMYDIA AND GONORRHOEAE

Bottom line: *Point-of-care NAAT testing in clinics could reduce cost and clinician time and may lead to more appropriate and rapid care of patients, reducing number of patients lost to follow-up; however, utility and implementation studies are currently lacking.*

Level of evidence	
The rapid PCR-based tests for chlamydia and gonorrhoea appear to show sufficient accuracy and modelling suggests they may be more cost-effective, however utility and implementation studies are lacking.	**Level 5: Laboratory evidence & modelling**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Rapid PCR-based testing in clinics is accurate. It may lead to shorter patient pathways and be less expensive, while reducing overtreatment and therefore potentially improving antimicrobial stewardship.	**Medium**

1. Definition

A real-time PCR nucleic acid amplification (NAAT) test for both *Chlamydia trachomatis* and *Neisseria gonorrheae* (Cepheid GeneXpert CT/NG assay; FDA approved December 2012) using urine or cervical/vaginal swabs can provide results in 90 minutes and could have utility in a genitourinary medicine (GUM) clinic, for example.

2. Summary of the evidence

A study assessing the accuracy of the Cepheid GeneXpert CT/NG assay compared to currently approved laboratory-based PCR tests in samples from 1,722 female and 1,387 male volunteers reported the following: For chlamydia in females, sensitivities for endocervical, vaginal, and urine samples were 97%, 99%, and 98%, respectively, and a sensitivity of 98% was reported for urine samples from males. All chlamydia specificity estimates were over 99.3%. Results for gonorrhoea in females demonstrated sensitivities for endocervical, vaginal, and urine samples of 100%, 100%, and 96%, respectively, and for male urine samples, a sensitivity of 98% was reported. All gonorrhoea estimates of specificity were above 99.7% (Gaydos et al.).

A recent UK study mapping the patient pathways and resource use comparing this rapid NAAT test to current standard testing in four GUM clinics reported that using the rapid NAAT test resulted in a shorter patient pathway that was less expensive than current pathways. The study concluded that point-of-care NAAT testing in clinics could reduce cost and clinician time and may lead to more appropriate and rapid care of patients (Adams et al).

3. Requirements for further research

Further research should await the results of a Cochrane systematic review assessing the effectiveness and safety of home-based management strategy (including self-taken samples) for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* compared with clinic-based management strategy (Fajardo-Bernal et al).

4. References

- Adams EJ, Ehrlich A, Turner KM, Shah K, Macleod J, Goldenberg S4, Meray RK, Pearce V, Horner P. Mapping patient pathways and estimating resource use for point of care versus standard testing and treatment of chlamydia and gonorrhoea in genitourinary medicine clinics in the UK. BMJ Open. 2014 Jul 23;4(7):e005322.
- Gaydos CA, Van Der Pol B, Jett-Goheen M, Barnes M, Quinn N, Clark C, Daniel GE, Dixon PB, Hook EW 3rd; CT/NG Study Group. Performance of the Cepheid CT/NG Xpert Rapid PCR Test for

Detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. J Clin Microbiol. 2013 Jun;51(6):1666-72.

- Luisa Fajardo-Bernal , Edith Angel-Müller , Johanna Aponte-Gonzalez , Carlos Rincon , Hernando G Gaitán and Nicola Low .Home-based versus clinic-based management strategy for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: Cochrane Systematic Review Protocol. Online Publication Date: September 2014.

APPENDIX 54
TECHNOLOGY: POINT-OF-CARE WHITE BLOOD CELL COUNTS

Bottom line: *The role of point-of-care white blood cell count devices in diagnosis of serious infection and their use to inform antibiotic prescribing (perhaps in conjunction with other markers) requires further research.*

Level of evidence	
Point-of-care white blood cell count devices show adequate performance, however their use in antibiotic prescribing decisions is unclear.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Although POC WBC devices appear to have reasonable accuracy, the evidence suggests that their diagnostic value in identifying serious infection in children is limited and insufficient evidence exists to ascertain their use in antibiotic prescribing decisions.	**High**

1. Definition

There are several point-of-care white blood cell count (WBC) devices available, which in some cases can also measure red blood cells, 3-part differentials and haemoglobin (Hb) (e.g. HemoCue WBC and Chempaq XBC).

2. Summary of the evidence

Limited published data on the accuracy of these devices is available. A comparison of Chempaq XBC with laboratory-based methods showed that whilst Hb, granulocytes and lymphocytes showed good correlation, monocyte counts correlated poorly. The HemoCue system was shown to provide reliable results in the range of 0.4–30.0 × 10^9/l and initial results regarding accuracy appear promising (Briggs et al; Diagnostic Horizon Scan report 0011).

A low or normal WBC is usually associated with viral illness. One study evaluating the reliability of POC WBC count devices in assisting antibiotic prescribing decisions in 120 acutely ill children found that correlation between POC and laboratory-based WBC measurements was high (Casey et al.). Of the children that had a low blood count and therefore did not receive antibiotics, 3% returned within 30 days and received an antibiotic. The authors concluded that WBC may assist in judicious antibiotic prescribing. However, a systematic review of the diagnostic value of laboratory tests, including WBC, in identifying serious infections in febrile children concluded that measuring WBC is less useful for ruling in serious infection and not useful for ruling out serious infection (Van den Bruel et al.).

3. Requirements for further research

The role of these devices in diagnosis of serious infection and their use to inform antibiotic prescribing (perhaps in conjunction with other markers) require further research.

4. References

- Briggs C, Kimber S, Green L. Where are we at with point-of-care testing in haematology? Br J Haematol. 2012 Sep;158(6):679-90.
- Casey JR, Pichichero ME A comparison of 2 white blood cell count devices to aid judicious antibiotic prescribing. Clin Pediatr (Phila). 2009 Apr;48(3):291-4.
- Point-of-care tests for total white blood cell count. Primary Care Diagnostic Horizon Scan Report 0011. October 2010. http://madox.org/horizon-scanning-reports/201011/point-of-care-test-for-total-white-blood-cell-count
- Van den Bruel A, Thompson MJ, Haj-Hassan T, Stevens R, Moll H, Lakhanpaul M, Mant D. Diagnostic value of laboratory tests in identifying serious infections in febrile children: systematic review. BMJ. 2011 Jun 8;342:d3082.

APPENDIX 55
TECHNOLOGY: DNA MICROARRAY ASSAY FOR THE DETECTION OF MYCOPLASMA PNEUMONIAE

Bottom line: *The ArrayStrip microarray is easy to use with potential high throughput, high information content, and affordability. However, single test assays are unlikely to prove useful in clinical practice*.

Level of evidence	
The ArrayStrip microarray is easy to use with potential high throughput, high information content and affordability.	**Level 5: Laboratory**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
High specificity; requires further testing.	**High**

1. Definition
This method utilizes a DNA microarray carrying 70 oligonucleotide probes derived from the 23S rRNA gene and 86 probes from the tuf gene target regions.

2. Summary of the evidence
The analytical sensitivity of the test is comparable to that of real-time PCR and allows examination of field samples without the requirement for culture. The ArrayStrip microarray is easy to use with potential high throughput, high information content, and affordability.

3. Requirements for further research
Testing of more *Mycoplasma* species is required. Optimization research needed.

4. References

- Schnee C, Schulsse S, Hotzel H, Ayling RD, Nicholas RA, Schubert E, Heller M, Ehricht R, Sachse K. A novel rapid DNA microarray assay enables identification of 37 Mycoplasma species and highlights multiple Mycoplasma infections. PLoS One. 2012;7(3):e33237.

APPENDIX 56
TECHNOLOGY: LOOP-MEDIATED ISOTHERMAL AMPLIFICATION FOR THE DETECTION OF MYCOPLASMA PNEUMONIAE

Bottom line: *Loop mediated isothermal amplification has high specificity, is easy to use and can rapidly detect Mycoplasma; however single test assays are unlikely to prove useful in clinical practice.*

Level of evidence	
The technology is simple to use and reduces the time to detection; may be cost-effective.	**Level 3: Non-randomized**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It can be used for rapid detection. Whilst it has high specificity, the sensitivity is low.	**High**

1. Definition

This technique uses a set of primers targeting the SDC1 repetitive element of the *M. pneumoniae* genome. It can be used in areas of low-resource.

2. Summary of the evidence

One study reported sensitivity of 100% among 39 *M. pneumoniae* isolates, and specificity of 100% among 9 members of other Mycoplasma and 12 common respiratory pathogens (Zhao et al, 2013).

In a prospective study including 368 paediatric hospital patients, sensitivity was 78%, specificity 97%, PPV 76% and NPV 98% (Gotoh et al, 2012). In a further study among 43 paediatric patients (median age 9 years) 96% were positive on the LAMP assay and had a fourfold or greater increase in *M. pneumonia* titres (Aizawa et al, 2014); the median interval for diagnosis versus serology was 7 vs 13 days.

A fourth study reported rapid and specific amplification for all the strains of *M. pneumoniae* types I and II within 1 hour at 65ºC in 204 clinical samples – sputum, throat swabs. No cross-reactivity with the most common causative organisms causing bacterial pneumonia was observed (Yoshino et al, 2008).

3. Requirements for further research

Detection of Mycoplasma in asymptomatic patients; cost-effectiveness studies are required.

4. References

- Zhao F, Liu Z, Gu Y, Yang Y, Xiao D, Tao X, Meng F, He L, Zhang J. Detection of *Mycoplasma pneumoniae* by colorimetric loop-mediated isothermal amplification. Acta Microbiol Immunol Hung. 2013 Mar;60(1):1-9.
- Gotoh K, Nishimura N, Ohshima Y, Arakawa Y, Hosono H, Yamamoto Y, Iwata Y, Nakane K, Funahashi K, Ozaki T. Detection of *Mycoplasma pneumoniae* by loop-mediated isothermal amplification (LAMP) assay and serology in pediatric community-acquired pneumonia. J Infect Chemother. 2012 Oct;18(5):662-7.
- Aizawa Y, Oishi T, Tsukano S, Taguchi T, Saitoh A. Clinical utility of loop-mediated isothermal amplification for rapid diagnosis of *Mycoplasma pneumoniae* in children. J Med Microbiol. 2014 Feb;63(Pt 2):248-51.
- Yoshino M, Annaka T, Kojima T, Ikedo M. [Sensitive and rapid detection of *Mycoplasma pneumoniae* by loop-mediated isothermal amplification]. [Article in Japanese]. Kansenshogaku Zasshi. 2008 May;82(3):168-76.

APPENDIX 57
TECHNOLOGY: PCR FOR THE DETECTION OF MYCOPLASMA PNEUMONIA

Bottom line: *PCR is expensive and offers little benefit over serology. It is not suitable for use in routine laboratories.*

Level of evidence	
PCR should not replace serology; expensive	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
PCR is rapid and has high specificity, but lower sensitivity; not suitable in routine laboratories	**High**

1. Definition
PCR tests involve the amplification of nucleic acids. They can be used in hospital settings.

2. Summary of the evidence
The systematic review compared PCR with serology, and included 14 studies. No study reported blinded interpretation of test results. Overall diagnostic accuracy for sensitivity was 62% and specificity was 96%. Heterogeneity was substantial. Targeting the 16s rDNA in adult subjects with real-time PCR showed higher performance on test results.

3. Requirements for further research
Further studies investigating the detection of Mycoplasma in asymptomatics and assessing cost-effectiveness are required.

4. References

- Zhang L, Zong ZY, Liu YB, Ye H, Lv XJ. PCR versus serology for diagnosing *Mycoplasma pneumoniae* infection: a systematic review & meta-analysis. Indian J Med Res. 2011 Sep;134:270-80.

APPENDIX 58
TECHNOLOGY: MICROFLUIDICS FOR DETECTION OF MYCOPLASMA PNEUMONIA

Bottom line: *Microfluidics tests are easy to use with potential high throughput and affordability; however single test assays are unlikely to prove useful in clinical practice.*

Level of evidence	
Microfluidics tests are three times faster than conventional PCR.	**Level 3: Comparative**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It is as equally sensitive as PCR, but cheaper and three times quicker at detection. It could be used as POCT for mycoplasma.	**High**

1. Definition

Microfluidics technology uses discrete unit-sized droplets that are individually positioned and manipulated by using an array of electrodes formed on a printed circuit board (PCB) substrate. It can be used as a point-of-care test in both hospital and outpatient settings.

2. Summary of the evidence

The study compared microfluidics PCR versus conventional PCR. Three of 59 (5.0%) nasopharyngeal washes were positive, and agreement between the methods was 98%. The time required to run 50 PCR cycles on the microfluidic platform was 60 minutes versus 165 minutes on the conventional platform.

3. Requirements for further research

Optimization research is needed.

4. References

- Wulff-Burchfield E, Schell WA, Eckhardt AE, Pollack MG, Hua Z, Rouse JL, Pamula VK, Srinivasan V, Benton JL, Alexander BD, Wilfret DA, Kraft M, Cairns CB, Perfect JR, Mitchell TG. Microfluidic platform versus conventional real-time polymerase chain reaction for the detection of *Mycoplasma pneumoniae* in respiratory specimens. Diagn Microbiol Infect Dis. 2010 May;67(1):22-9.

APPENDIX 59
TECHNOLOGY: RAPID MYCOPLASMA CULTURE FOR THE DETECTION OF MYCOPLASMA PNEUMONIAE

Bottom line: *Rapid culture is expensive and seems to offer little benefit given it still takes one to three days to detect mycoplasma.*

Level of evidence	
Culture time is reduced from 7-10 days to 1-3 days, however this does not provide substantial benefit and the method is expensive.	**Level 3: Non-randomized**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
It may be useful in early diagnosis of *Mycoplasma* infection.	**High**

1. Definition
This method utilizes a specific liquid culture medium. It can be performed in hospital settings.

2. Summary of the evidence
The positive rate of rapid culture for *Mycoplasma* was slightly higher than that by *Mycoplasma* antibody assay, but the diagnostic accordance was good between these two methods (P>0.05). The positive rates of *Mycoplasma* culture were higher in children aged 3-5 years and adults older than 70 years, 54% and 32%, respectively, compared with other age groups. Using this method *Mycoplasma* pneumonia could be detected in 1-3 days, compared with 7-10 for the conventional culture method.

Balance of benefit and harm
It is rapid but expensive and not suitable for use in routine laboratories.

3. Requirements for further research
The ability of the technology to detect *Mycoplasma* in asymptomatic patients should be tested. The cost-effectiveness requires evaluation.

4. References

- Ma LD, Chen B, Dong Y, Fan J, Xia L, Wang SZ, Liu Q, Jiang L. Rapid mycoplasma culture for the early diagnosis of Mycoplasma pneumoniae infection." Journal of Clinical Laboratory Analysis 2010:24(4): 224-229.

APPENDIX 60
TECHNOLOGY: MULTIPLEX MOLECULAR PLATFORMS FOR THE DIAGNOSIS OF RESPIRATORY VIRUSES AND BACTERIA

Bottom line: *Rapid detection of respiratory viruses may aid diagnosis of respiratory infections and inform decisions on antibiotic prescribing thus avoiding overuse of antibiotics. However, an adequately powered trial with antibiotic use as an outcome is currently needed. Current evidence for diagnostic testing for influenza, respiratory syncytial virus and Streptococcus pneumoniae infection on the management of acute admissions in the elderly and high-risk 18 to 64 year olds reported no evidence that POCTs for influenza or S. pneumonia or PCR for influenza or RSV influenced antimicrobial prescribing or clinical outcomes.*

Level of evidence	
Regarding rapid viral detection, systematic review has shown no impact on antibiotic prescribing. Regarding multiplex platforms, several cohort studies assessing accuracy are available, but a systematic review is lacking.	**Level 1: Systematic review, RCT**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Rapid detection of respiratory viruses may aid diagnosis of respiratory infections and inform decisions on antibiotic prescribing, avoiding overuse of antibiotics. These tests are laboratory-based tests and may reduce turnaround time of testing.	**High**

1. Definition

Several tests have been developed to aid in the diagnosis of respiratory viruses, for example:

a. The mariPOC® point-of-care test system tests for eight respiratory viruses from a single nasopharyngeal swab: influenza A and B, respiratory syncytial virus A/B (RSV), adenovirus, human metapneumovirus (hMPV) and parainfluenza type 1, 2 and 3. The same system also includes a test for *Streptococcus pneumonia*. Results in 2 hours.
b. The Nanosphere Verigene Respiratory Virus Plus (RV+) test (Northbrook, IL) test detects influenza A virus, influenza B virus, RSV A, and RSV B while also providing influenza A virus-specific typing results for 2009 H1N1, H3, and H1. Results in 2.5 hours.
c. Prodesse ProFLU+/FAST+ (GenProbe): ProFLU+ enables the detection and differentiation of influenza A and B virus, and RSV types A and B. ProFAST is designed for the qualitative detection and discrimination of influenza A virus subtypes: seasonal A/H1, seasonal A/H3, and 2009 H1N1.
d. FilmArray RP respiratory panel simultaneous identification of adenovirus, bocavirus, coronavirus, human metapneumovirus, influenza A and B, parainfluenza virus, rhinovirus/enterovirus, RSV, *Bordetella pertussis*, *Chlamydophila pneumoniae*, and *Mycoplasma pneumoniae*.
e. Respiratory Multiplex Array (Randox) allows for the simultaneous detection of the 22 most prevalent viral and bacterial respiratory pathogens within 5h.
f. Iquum, recently purchased by Roche.

2. Summary of the evidence

Several studies have evaluated the mariPOC system with similar results. For example, one study compared mariPOC to laboratory RT-PCR testing in a paediatric emergency department in 158 children with respiratory symptoms. Sensitivities and specificities of the mariPOC test for influenza A, 71% and 100%; influenza B, 86% and 98%; RSV, 89% and 100%; adenovirus, 25% and 97%; and for human metapneumovirus, 50% and 100%, respectively. The study concluded that this system may provide a rapid option for detecting multiple respiratory viruses, showing high specificity for all the tested viruses, however sensitivity was moderate for RSV and low for adenovirus (Ivaska et al).

One study assessing the accuracy of the Verigene RV+ test compared to standard laboratory PCR testing using retrospective and prospective nasopharyngeal samples. It reported overall sensitivities and specificities for RV+ of 96.6% and 100% for influenza A virus, 100% and 99.7% for influenza B virus, and

100% and 100% for respiratory syncytial virus (RSV) (Alby et al). A study comparing FilmArray RP, Verigene RV+ and ProFLU+/FAST+ reported that FilmArray RP and Prodesse ProFLU+/FAST+ assays were convenient to perform with regard to sensitivity, ease of use, and low percentages of invalid results (Van Wenedbeeck et al).

Several other tests are available and there appears to be a large body or research on these tests, but we were not able to identify a systematic review on this topic. One study assessed the implementation of FilmArray RP in a core pathology laboratory and reported an average median turnaround time of 1.4-1.6 hours, compared to 6.5-7 hours before implementation of this test (Xu et al). However, a 2014 updated Cochrane systematic review on the use of rapid viral detection for children with acute respiratory infection in the emergency department concluded that rapid viral testing resulted in a trend toward decreased antibiotic use in the ED, but this was not statistically significant. They reported lower rates of chest radiography (RR 0.77, 95% CI 0.65 to 0.91) in the rapid viral testing group, but no effect on length of ED visits, or blood or urine testing in the ED. The authors found insufficient evidence regarding the reduction of antibiotic prescribing rates and recommended that an adequately powered trial with antibiotic use as an outcome is needed (Doan et al). A 2014 UK randomised controlled trial and health economic evaluation of the impact of diagnostic testing for influenza, respiratory syncytial virus and *Streptococcus pneumoniae* infection on the management of acute admissions in the elderly and high-risk 18 to 64 year olds reported that they found no evidence that POCTs for influenza or *S. pneumonia* or PCR for influenza or RSV influenced antimicrobial prescribing or clinical outcomes and their analysis did not support routine use of POCTs, however viral culture should be replaced by PCR (Nicholson et al.).

3. Requirements for further research

Several multiplex platforms to diagnose respiratory viruses have been developed (only some are mentioned here) and several studies have assessed their comparative accuracy, however a systematic review is required. Regarding impact on antibiotic prescribing, an adequately powered trial with antibiotic use as an outcome is needed, as recommended by the recent systematic review. A 2014 HTA report has suggested that for adults the hospital setting POCTs for influenza or pneumococcal antigen did not influence antibiotic prescribing.

4. References

- Alby K, Popowitch EB, Miller MB. Comparative evaluation of the Nanosphere Verigene RV+ assay and the Simplexa Flu A/B & RSV kit for detection of influenza and respiratory syncytial viruses. J Clin Microbiol. 2013 Jan;51(1):352-3.
- Doan Q, Enarson P, Kissoon N, Klassen TP, Johnson DW. Rapid viral diagnosis for acute febrile respiratory illness in children in the Emergency Department. Cochrane Database Syst Rev. 2014 Sep 15;9:CD006452.
- Ivaska L1, Niemelä J, Heikkinen T, Vuorinen T, Peltola V. Identification of respiratory viruses with a novel point-of-care multianalyte antigen detection test in children with acute respiratory tract infection. J Clin Virol. 2013 Jun;57(2):136-40.
- Nicholson KG, Abrams KR, Batham S, Medina MJ, Warren FC, Barer M, Bermingham A, Clark TW, Latimer N, Fraser M, Perera N, Rajakumar K, Zambon M.Randomised controlled trial and health economic evaluation of the impact of diagnostic testing for influenza, respiratory syncytial virus and *Streptococcus pneumoniae* infection on the management of acute admissions in the elderly and high-risk 18- to 64-year-olds. Health Technol Assess. 2014 May;18(36):1-274, vii-viii.
- Van Wesenbeeck L, Meeuws H, Van Immerseel A, Ispas G, Schmidt K, Houspie L, Van Ranst M, Stuyver L. Comparison of the FilmArray RP, Verigene RV+, and Prodesse ProFLU+/FAST+ multiplex platforms for detection of influenza viruses in clinical samples from the 2011-2012 influenza season in Belgium. J Clin Microbiol. 2013 Sep;51(9):2977-85
- Xu M, Qin X, Astion ML, Rutledge JC, Simpson J, Jerome KR, Englund JA, Zerr DM, Migita RT, Rich S, Childs JC, Cent A, Del Beccaro MA. Implementation of filmarray respiratory viral panel in a core laboratory improves testing turnaround time and patient care. Am J Clin Pathol. 2013 Jan;139(1):118-23.

APPENDIX 61
TECHNOLOGY: POINT-OF-CARE TESTS FOR SYPHILIS

Bottom line: *Point-of-care syphilis tests appear to show adequate accuracy; however their utility and cost-effectiveness in informing antibiotic prescribing strategies in the UK setting requires assessment.*

Level of evidence	
Point-of-care syphilis tests appear to show adequate accuracy, but their role in the UK setting requires assessment.	**Level 1: Systematic reviews**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
The utility of point-of-care syphilis testing in the UK setting and its role in antimicrobial prescribing has not been assessed thus far.	**High**

1. Definition

Several rapid blood and serum tests for *Treponema pallidum* to diagnose syphilis have been developed, which can be performed within 20 minutes at the point of care.

2. Summary of the evidence

A 2010 systematic review of the accuracy of immunochromatographic point-of-care strip tests for syphilis reported median sensitivity and specificity of 86% and 99%, respectively (Tucker et al). Another systematic review of the accuracy of point-of-care tests for syphilis, which included data from 33 publications, reported sensitivities for whole blood ranging from 74% to 86% and specificities ranging from 95% to 99%. The study concluded that POC treponemal tests showed sensitivity and specificity estimates comparable to laboratory-based tests and may be useful for screening for syphilis, particularly in resource-limited settings (Jafari et al. 2013). A systematic review on the implementation research outcomes for point-of-care diagnostics for syphilis assessed reporting of preference, acceptability, feasibility, barriers and challenges, impact and prevalence. The study concluded that implementation outcomes were generally poorly defined and evaluated. Some studies highlighted that the tests were easy to use and acceptable to patients and clinicians, and significantly increased testing and treatment. Barriers to implementation included lack of trust in the results by clinicians and patients (Jafari et al. 2014). No specific mention was made of the effect of point-of-care testing on antibiotic prescribing strategies.

3. Requirements for further research

The utility and cost-effectiveness of point-of-care syphilis testing in informing antibiotic prescribing strategies in the UK setting requires assessment. The current NICE Clinical Knowledge Summary on management of suspected syphilis in primary care (last revised in September 2014) recommends laboratory serology testing.

4. References

- Jafari Y, Peeling RW, Shivkumar S, Claessens C, Joseph L, Pai NP. Are Treponema pallidum specific rapid and point-of-care tests for syphilis accurate enough for screening in resource limited settings? Evidence from a meta-analysis. PLoS One. 2013;8(2):e54695.
- Jafari Y, Johri M, Joseph L, Vadnais C, Pant Pai N. Poor Reporting of Outcomes Beyond Accuracy in Point-of-Care Tests for Syphilis: A Call for a Framework. AIDS Res Treat. 2014;2014:465932.
- Tucker JD, Bu J, Brown LB, Yin YP, Chen XS, Cohen MS. Accelerating worldwide syphilis screening through rapid testing: a systematic review. Lancet Infect Dis. 2010 Jun;10(6):381-6.

APPENDIX 62
TECHNOLOGY: RAPID DIAGNOSTIC TESTS FOR THE DETECTION OF INTESTINAL PATHOGENS

Bottom line: *Rapid diagnostic assays, especially PCR, for Salmonella, Campylobacter and E. coli O157 are highly accurate; however evidence regarding the relative cost of implementing these tests in practice is currently sparse and unclear.*

Level of evidence	
A systematic review of several rapid laboratory-based tests for various intestinal pathogens shows they appear to be accurate, however cost-effectiveness is lacking.	**Level 1: Systematic review**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
The systematic review did not assess antimicrobial prescribing as an outcome, but rapid testing may inform judicious prescribing. The multiplex systems require further assessment for their utility in informing antibiotic prescribing.	**High**

1. Definition
Several rapid laboratory-based tests are available for the identification of gastrointestinal pathogens, including bacteria, viruses and parasites, including multiplex systems.

2. Summary of the evidence
A systematic review of the accuracy and cost-effectiveness of rapid diagnostic tests for food-borne pathogens performed summary receiver operating characteristic (SROC) analyses for each of the point-of-care tests and reported:

- *Campylobacter*, evaluating PCR for the 16s rRNA gene, the area under the curve (AUC) was 0.987. For the ProSpecT immunoassay (Alexon-Trend), the overall AUC was 0.862 (95% CI 0.568 to 1.000).
- For *Salmonella,* test methods included PCR, Wellcolex Colour agar, MUCAP test, Wampole Bactigen and AutoMicroBic identification system. Combining 2,134 samples (from seven studies), the AUC value for PCR was 0.995; however, publication bias was evident. Other tests exhibited very high diagnostic odds ratios (DORs), ranging from 264 (95% CI 116.9 to 597.6) (Wampole Bactigen) to 2,951 (95% CI 710.9 to 12,000) (Wellcolex Colour).
- For *E. coli* SROC analysis for PCR assays showed very high diagnostic accuracy (AUC 0.996); however, publication bias was evident, compared with VTEC-Screen reverse passive latex agglutination (RPLA) results (AUC 0.994), which was not affected by publication bias. The Premier enterohaemorrhagic *Escherichia coli* (EHEC) immunoassay had high pooled sensitivity and specificity values (0.935 and 0.997, respectively). Other entrohaemorrhagic *E. coli* tests evaluated included ProSpecT, Duopath Verotoxin, ImmunoCard Stat and RidaScreen Verotoxin.

The study concluded that rapid diagnostic assays, especially PCR, for *Salmonella*, *Campylobacter* and *E. coli* O157 are highly accurate, however evidence regarding the relative cost of implementing these tests in practice is sparse and unclear.

3. Requirements for further research
The cost-effectiveness and utility in practice of these tests remains unclear, and with the emergence of multiplex tests and DNA microarrays (See reports elsewhere in this document) these require assessment regarding effectiveness and cost-effectiveness to establish the optimal testing pathway.

4. References

- Abubakar I, Irvine L, Aldus CF, Wyatt GM, Fordham R, Schelenz S, Shepstone L, Howe A, Peck M, Hunter PR. A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food. Health Technol Assess. 2007 Sep;11(36):1-216.

APPENDIX 63
TECHNOLOGY: POINT-OF-CARE TESTS FOR CAMPYLOBACTER

Bottom line: *Isolated point-of-care tests, which test for only one pathogen are unlikely to prove effective given the range of intestinal pathogens and the current evidence of their effectiveness is lacking.*

Level of evidence	
The point-of-care rapid *Campylobacter* tests may show good accuracy profiles, however studies addressing predictive value and utility in practice are lacking.	**Level 5: Laboratory studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
The role of a rapid Campylobacter test in informing antibiotic prescribing remains to be assessed.	**Low**

1. Definition

Rapid immunochromatographic tests have been developed to directly detect the *Campylobacter* species directly in stool samples. They can be performed at the point-of-care with results being available in 5 minutes.

2. Summary of the evidence

One study assessed the accuracy of the rapid immunochromatography test ImmunoCard STAT CAMPY compared to laboratory-based PCR and conventional bacteriological methods. It reported sensitivity between 86% and 90% (depending on the reference standard used) and specificity of 100% (Dey et al). Another study compared the accuracy of the ImmunoCard STAT CAMPY test to laboratory-based immunoassays. Sensitivity and specificity of 98.5% and 98.2%, respectively, were reported (Granato et al).

3. Requirements for further research

The current evidence is solely laboratory-based accuracy studies. Studies reporting predictive value and utility in the setting where they would be implemented are required. There was also no evidence for their role in antibiotic prescribing

4. References

- Dey SK, Nishimura S, Okitsu S, Hayakawa S, Mizuguchi M, Ushijima H. Comparison of immunochromatography, PCR and culture methods for the detection of Campylobacter bacteria. J Microbiol Methods. 2012 Dec;91(3):566-8.
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APPENDIX 64
TECHNOLOGY: BIOFIRE FILM ARRAY RESPIRATORY PANEL (RP) FOR THE DETECTION OF BACTERIAL AND VIRAL PATHOGENS

Bottom line: *Implementation of a biofilm array panel reduces the time to test result and increase the proportion of results received in ED before admission, but currently has no significant impact on antibiotic prescription and use.*

Level of evidence	
There is some evidence regarding the accuracy of this panel, however most based on retrospective laboratory analyses, rather than prospective studies of consecutively recruited patients.	**Level 3: Cohort studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
This test is efficient and is capable of detecting a wide range of viral and bacterial pathogens, which could reduce antibiotic usage if positioned correctly in the pathway.	**High**

1. Definition

This is a multiplex PCR molecular panel for the detection of both bacterial and viral respiratory pathogens in nasopharygeal swabs. It detects 20 pathogens simultaneously (17 viruses and 3 bacteria: *Bordetella pertussis, Chlamydophila pneumonia, Mycoplasma pneumonia*) and has a turnaround time of 1 hour. It has been approved the FDA. A new version of the FilmArray RP assay (version 1.7) with improved sensitivity for the adenovirus was released in 2013.

2. Summary of the evidence

There have been a number of studies that have evaluated the accuracy of this panel. Sensitivity is estimated to be between 84-100% and specificity between 98-100% (Zumla et al, 2014).

Wang et al. compared the laboratory input required for FilmArray RP and direct fluorescent antibody (DFA) staining. The FilmArray RP proved to be more efficient, requiring far less hands-on and turn-around time, in addition to detecting a greater number of organisms (20 viruses and bacteria vs 7 viruses for DFA staining).

Rogers et al. (2014) evaluated whether implementation of the FilmArray RP resulted in different outcomes for children admitted to the hospital with an acute respiratory tract illness. Implementation was found to significantly reduce the time to test result and increase the proportion of results received in ED before admission, but failed to have any significant impact on antibiotic prescription and use.

3. Requirements for further research

This panel has recently been updated and therefore more evidence is required based on a representative sample of consecutive patients suspected of RTI to explore the effect on accuracy and antibiotic prescriptions.

4. References

- Rogers BB, Shankar P, Jerris RC, Kotzbauer D, Anderson EJ, Watson JR et al. Impact of a Rapid Respiratory Panel Test on Patient Outcomes. Arch Pathol Lab Med. 2014 Aug 25 [Epub ahead of print].
- Wang J, Simons DB, Adams JL, Jerris RC, Rogers BB. Multiplex viral polymerase chain reaction testing using the FilmArray device compared with direct fluorescent antibody testing. Lab Med. 2014 Winter; 45(1):62-4.
- Zumla A, Al-Tawfiq JA, Enne VI, Kidd M, Drosten,C Breuer J et al. Rapid point of care diagnostic tests for viral and bacterial respiratory tract infections — needs, advances, and future prospects. The Lancet Infectious Diseases. 2014, 14(11): 1123-1135.

APPENDIX 65
TECHNOLOGY: RAPID ANTIGEN DETECTION TESTS (RADTS) FOR STREPTOCOCCAL INFECTIONS

Bottom line: *There has been extensive investment from the HTA on evaluating the benefit of using RADTs in clinical practice, including the production of RCT, cost-effectiveness, and physician-acceptability evidence. Further validation of the symptom-based clinical score (FeverPAIN) is required. Additional research on RADTs is not required until the variability in sensitivity is reduced and the detection of non-group A strains that commonly cause Streptococcal throat are developed.*

Level of evidence	
There is comparative accuracy evidence available and one large RCT-based program of work which directly evaluated the impact of RADT use on antibiotic prescribing, its cost-effectiveness and clinician-acceptability.	**Level 4: RCT and cost-effectiveness**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Trial evidence indicates that a simple symptom-based clinical score is effective in reducing unnecessary antibiotic prescription, and that RADT use offered little benefit.	**High**

1. Definition

Several tests have been developed to aid in the diagnosis of bacterial pharyngitis caused by Group A *streptococci* (GAS). Some examples are:

- OSOM Ultra Strep A (Bio-Stat Limited, Stockport, UK): QuickVue Dipstick Strep A test (TK Diagnostic, Oxford, UK):
- Streptatest (DECTRA PHARM, Strasbourg, France)
- Clearview Exact (Inverness Medical Professional Diagnostics, Bedford, UK)
- IMI TestPack Plus Strep A (Inverness Medical, Bedford, UK)

These tests are based on immunochromatographic assays that detect Group A *Streptococcus* directly from throat swab specimens. They produce two colour qualitative results in around 5 minutes.

2. Summary of the evidence

A recent study compared the accuracy and ease of use of five UK RADTs (listed above) for group A beta-haemolytic streptococcus (GABHS). All of the tests were 100% specific. Sensitivity was highly dependent on GABHS concentration. At the highest concentration, Clearview had a sensitivity of 62% (95% confidence interval (CI) 51% to 72%) and OSOM Ultra and IMI TestPac a sensitivity of 95% (95% CI 88% to 98%). The IMI TestPack was the easiest to use and recommended as the best option for use in primary care.

Cohen et al (2013) found that clinical spectrum, inoculum size and physician characteristics directly influenced the sensitivity of RADTs. Sensitivity was higher for children with pharyngitis compared to asymptomatic children (89% vs. 41%) and those with heavy vs. light inoculum (94% vs. 53%). RADT sensitivity was also influenced by the physician performing the test (range 56–96%, p=0.01) and was higher for physicians with hospital-based clinical activity.

A recent RCT compared using RADTs in combination with a clinical score to using the clinical score alone. No significant benefit on antibiotic use was found (Little, 2014). Qualitative and cost-effectiveness studies were nested within the RCT. The cost-effectiveness analysis found that using a clinical score alone instead of one in combination with RADTs is more cost-effective. The qualitative study revealed multiple concerns about RADT use in clinical practice, including the validity of the tests, and highlighted that their clinical implementation is unlikely until these concerns are met and clinicians have direct experience of using them (Leydon, 2014).

3. Requirements for further research

Further validation of the symptom-based clinical score (FeverPAIN) is required. Additional research on RADTs is not required until the variability in sensitivity is reduced and the detection of non-group A strains that commonly cause Streptococcal throat are developed.

4. References

- J. F. Cohen, M. Chalumeau, C. Levy, P. Bidet, M. Benani, M. Koskas, E. Bingen, R. Cohen. Effect of clinical spectrum, inoculum size and physician characteristics on sensitivity of a rapid antigen detection test for group A streptococcal pharyngitis. European Journal of Clinical Microbiology & Infectious Diseases. June 2013, Volume 32, Issue 6, pp 787-793
- Leydon G, McDermott L, Moore M, Williamson I, Hobbs FDR, Little P et al. A qualitative study of general practitioner, nurse practitioner and patient views about the use of rapid streptococcus antigen detection tests in primary care: 'swamped with sore throats?' Health Technol Assess. 2014;18(6)
- Little P, Hobbs FR, Moore M, Mant D, Williamson I, McNulty C, et al. PRImary care Streptococcal Management (PRISM) study: in vitro study, diagnostic cohorts and a pragmatic adaptive randomised controlled trial with nested qualitative study and cost-effectiveness study. Health Technol Assess. 2014;18(6)

APPENDIX 66
TECHNOLOGY: BAC T/ALERT 3D SYSTEM FOR BLOODSTREAM INFECTIONS

Bottom line: *The Bac T/Alert 3D test is reliable, time-saving, cost-effective and might reduce mortality. Randomized comparisons are required to confirm the important findings of the comparative studies.*

Level of evidence	
Compared with conventional culture, automation was superior in terms of recovery and time to detect pathogens.	**Level 2: Comparative study**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
The Bac T/Alert system is reliable, time-saving and cost-effective and might reduce mortality.	**High**

1. Definition

New blood culture media containing antibiotic-binding beads have been developed for the Bac T/ Alert 3D (bioMérieux), which is a fully automated colorimetric blood culture system. These media absorb antimicrobial agents or other substances that may inhibit growth, allowing for better and faster detection of microorganisms.

2. Summary of the evidence

Several comparative studies have shown that the new aerobic and anaerobic media allowed earlier identification of coagulase-negative staphylococcal growth and were superior for the identification of Gram-positive cocci, *Escherichia coli* and Gram-negative bacilli compared to standard methods. The system appears to be a reliable, timesaving tool for the detection of blood stream infections. Combined with mass spectrometry and simple lysis-based methods, microorganisms can potentially be directly identified within an hour after blood culture positivity, resulting in significant time saving which could impact on clinical management of antibiotic treatment.

3. Requirements for further research

Randomized comparisons with cost effectiveness analyses are required to confirm the findings of the comparative studies.

4. References

- Fiori B, D'Inzeo T, Di Florio V, De Maio F, De Angelis G, Giaquinto A, Campana L, Tanzarella E, Tumbarello M, Antonelli M, Sanguinetti M, Spanu T. Performance of Two Resin-Containing Blood Culture Media in Detection of Bloodstream Infections and in Direct Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS) Broth Assays for Isolate Identification: Clinical Comparison of the BacT/Alert Plus and Bactec Plus Systems. J Clin Microbiol. 2014 Oct;52(10):3558-67.
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- Mestas J, Felsenstein S, Dien Bard J. Direct identification of bacteria from positive BacT/ALERT blood culture bottles using matrix-assisted laser desorption ionization-time-of-flight mass spectrometry. Diagn Microbiol Infect Dis. 2014 Jul 31. [Epub ahead of print]

APPENDIX 67
TECHNOLOGY: RAPID TESTS FOR THE DETECTION OF GROUP B STREPTOCCUS (GBS)

Bottom line: *The role of rapid tests for the detection of Group B strep is unclear. Cost effectiveness analyses are required to understand whether such tests offer any value over current screening methods.*

Level of evidence	
A limited number of cross-sectional accuracy studies are available, but more are needed in the intended clinical population.	**Level 2/3: Cohort & case-control studies**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Rapid tests for Group B Streptococcus could reduce unnecessary antibiotic use in women going into labour who have either failed to get screened or have gone into pre-term labour.	**Medium**

1. Definition

Rapid tests that offer a faster turnaround for Group B *Streptococcus* detection than standard culture testing (which takes around 48-72hrs) are now available. Two examples are:

- Nanologix QuickTest kit: an immunoblot-based diagnostic test for GBS. An additional feature of this test is that it simultaneously determines antimicrobial susceptibility. Turnaround time is 6.5 hours.
- Cepheid Xpert group B streptococcus: an in vitro PCR diagnostic test for rapid intrapartum GBS testing. The manufacturer reports turnaround of 35 minutes for a positive test and 52 minutes for a negative test.

2. Summary of the evidence

Faro et al (2013) compared the accuracy of the Nanologix QuickTest Kit to standard GBS culture in 356 women being routinely screened for GBS colonisation at 35-37 weeks gestation. A sensitivity of 97% and a specificity of 88% were reported, in addition to inter-observer reliability of 88%.

Park et al. (2013) evaluated the accuracy of the Xpert GBS test in 175 pregnant women who were undergoing routine screening between 35-39 weeks of gestation. Using culture results as a reference, the sensitivity and specificity of the Xpert GBS assay were 87% and 96%, respectively. This test has also been used to test amniotic fluid (Bourgeois-Nicolaos, 2013), providing a sensitivity and specificity of 92% and 85%, respectively (when any intrapartum positive result from the Xpert GBS or culture was considered a true positive).

3. Requirements for further research

Further cross-sectional studies assessing the accuracy of these tests are required. Clearer definition of the target clinical population is also required: are these replacement tests for standard culture screening tests or are they only of value for those who have missed out on screening?

4. References

- Bourgeois-Nicolaos N, Cordier AG, Guillet-Caruba C, Casanova F, Benachi A, Doucet-Populaire F. Evaluation of the Cepheid Xpert GBS assay for rapid detection of group B Streptococci in amniotic fluids from pregnant women with premature rupture of membranes. *J Clin Microbiol*. 2013 Apr;51(4):1305-6.
- Jonathan P. Faro, Karen Bishop, Gerald Riddle, et al. Accuracy of an Accelerated, Culture-Based Assay for Detection of Group B Streptococcus. *Infectious Diseases in Obstetrics and Gynecology*, vol. 2013, Article ID 367935.
- Park JS, Cho DH, Yang JH, Kim MY, Shin SM, Kim EC, Park SS, Seong MW. Usefulness of a rapid real-time PCR assay in prenatal screening for group B streptococcus colonization. *Ann Lab Med.* 2013 Jan;33(1):39-44.

APPENDIX 68
TECHNOLOGY: POINT-OF-CARE ASSAY FOR TRICHOMONAS VAGINALIS

Bottom line: *The rapid PCR-based test for T. vaginalis is at the early stages of development and requires further accuracy studies, as well as evidence regarding utility and cost-effectiveness.*

Level of evidence	
The rapid PCR-based test for *T. vaginalis* is at the early stages of development and requires further accuracy studies, as well as evidence regarding utility and cost-effectiveness.	**Level 5: Laboratory evidence**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
A rapid test for *T. vaginalis* in clinics may lead to shorter patient pathways, aid diagnosis and inform appropriate antibiotic prescribing.	**Medium**

1. Definition

A rapid PCR-based test for Trichomonas vaginalis using vaginal swab samples, designed for use in conjunction with the Atlas io PoC platform, can provide results within 30min and could have utility in a genitourinary medicine (GUM) clinic, for example.

2. Summary of the evidence

A preliminary performance study of a prototype *T. vaginalis* assay using 90 clinical vaginal swab samples, which had been previously assessed using a laboratory PCR assay, reported both sensitivity and specificity of 95%. However, although these results indicate that the assay is comparable to existing laboratory PCR-based assays, this is an early-phase study which requires substantial further validation and assessment.

3. Requirements for further research

This rapid PCR-based assay, which has potential use at the point of care, is in the very early stages of development and requires substantial further validation and assessment of clinical utility and cost-effectiveness.

4. References

- Pearce DM, Styles DN, Hardick JP, Gaydos CA. A new rapid molecular point-of-care assay for Trichomonas vaginalis: preliminary performance data. Sex Transm Infect. 2013 Sep;89(6):495-7.

APPENDIX 69
TECHNOLOGY: BREATH TESTS FOR THE DIAGNOSIS OF INFECTIOUS DISEASES

Bottom line: *Breath tests for the diagnosis of infectious agents are at the proof-of-concept stage and require substantial further validation, assessment of accuracy and clinical utility.*

Level of evidence	
Breath tests for the diagnosis of infectious agents are at the proof-of-concept stage and require substantial further research.	**Level 5: Laboratory evidence**

A summary of the implications for practice with regard to anti-microbial resistance	**Clinical Relevance**
Breath tests could provide a non-invasive alternative to blood tests in the diagnosis of, for example, respiratory pathogens or sepsis; however they require substantial further research and validation.	**Medium**

1. Definition

Tests measuring volatile organic compounds in exhaled breath could be a simple, non-invasive technique to diagnose various infectious diseases, including respiratory diseases (e.g. tuberculosis, aspergillosis) and sepsis.

2. Summary of the evidence

Research on breath analysis in the diagnosis of disease has largely focused on conditions such as cancer, asthma, cystic fibrosis and diabetes, however there is some indication that it may also be of use in infectious diseases, including respiratory, gastrointestinal and urinary tract infections (Sethi et al; Konvalina et al). A study assessing breath fungal secondary metabolite signature in breath samples from patients with suspected invasive fungal pneumonia reported a sensitivity and specificity of 94% and 93% to measure volatile metabolites specific to *Aspergillus fumigatus* (Koo et al). Early animal studies suggest this method may also aid in diagnosis of sepsis (Barbour et al).

3. Requirements for further research

Breath tests for the diagnosis of infectious diseases are in the early proof-of-concept stage and require substantial further validation, accuracy and clinical utility studies.

4. References

- Barbour AG, Hirsch CM, Ghalyanchi Langeroudi A, Meinardi S, Lewis ER, Estabragh AS, Blake DR. Elevated carbon monoxide in the exhaled breath of mice during a systemic bacterial infection. PLoS One. 2013 Jul 31;8(7):e69802.
- Konvalina G and Haick H. Sensors for breath testing: from nanomaterials to comprehensive disease detection. Accounts of chemical research. 2014, 47(1):66-76
- Koo S, Thomas HR, Daniels SD, Lynch RC, Fortier SM, Shea MM, Rearden P, Comolli JC, Baden LR, Marty FM. A Breath Fungal Secondary Metabolite Signature to Diagnose Invasive Aspergillosis. Clin Infect Dis. 2014 Oct 22. pii: ciu725. [Epub ahead of print]
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