Frequencies and patterns of microbiology test requests from primary care in Oxfordshire, UK, 2008–2018: a retrospective cohort study of electronic health records to inform point-of-care testing

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ABSTRACT

Objectives To inform point-of-care test (POCT) development, we quantified the primary care demand for laboratory microbiology tests by describing their frequencies overall, frequencies of positives, most common organisms identified, temporal trends in testing and patterns of cotesting on the same and subsequent dates.

Design Retrospective cohort study.

Setting Primary care practices in Oxfordshire.

Participants 393 905 patients (65% female; 49% aged 18–49).

Primary and secondary outcome measures The frequencies of all microbiology tests requested between 2008 and 2018 were quantified. Patterns of cotesting were investigated with heat maps. All analyses were done overall, by sex and age categories.

Results 1 596 752 microbiology tests were requested. Urine culture±microscopy was the most common of all tests (n=673 612, 42%), was mainly requested without other tests and was the most common test requested in follow-up within 7 and 14 days. Of all urine cultures, 180 047 (27%) were positive and 172 651 (26%) showed mixed growth, and Escherichia coli was the most prevalent organism (132 277, 73% of positive urine cultures). Antenatal urine cultures and blood tests in pregnancy (hepatitis B, HIV and syphilis) formed a common test combination, consistent with their use in antenatal screening.

Conclusions The greatest burden of microbiology testing in primary care is attributable to urine culture±microscopy; genital and routine antenatal urine and blood testing are also significant contributors. Further research should focus on the feasibility and impact of POCTs for these specimen types.

INTRODUCTION

Viral, bacterial and parasitic infections are associated with a large burden of morbidity and mortality worldwide. Rapid and accurate identification of pathogens causing the infection could lead to quicker selection of therapy, improve prognosis and reduce transmission. This may also facilitate antibiotic stewardship by ensuring antibiotics are only prescribed when appropriate.

Recent technological advances and their potential benefits have contributed to some POC tests becoming available in primary care, despite doubts about their cost-effectiveness.

In the UK, antimicrobial prescribing guidelines for primary care are produced locally and can occasionally also incorporate

Strengths and limitations of this study

We analysed a very comprehensive dataset with detailed data for all microbiology test requests and results over a decade by a large clinical microbiology laboratory.

Coding of tests may have changed over time, but we reviewed 95% of all codes and grouped similar ones to avoid missing relevant tests.

The results of our study may not apply to other regions of the UK or other countries, where patterns of testing and prevalence of organisms may differ.

It was not always possible to distinguish between test combinations done together as a standard of practice by the laboratory from those that were requested together for clinical reasons.

suggested diagnostics. These change over time according to national and local changes in resistance and guidelines.

Due to limited resources and technical development in some cases, and also partly to the variability in specimens received by the laboratory (e.g., urine, blood, stool, sputum), which guides the processing and culture medium needed, clinical microbiology continues to rely on traditional methods such as specimen-specific cultures to identify microorganisms. In the last decade, molecular methods including PCR, microarray and nucleic acid sequencing have started to take a prominent place in clinical microbiology. There are examples of rapid tests for HIV, hepatitis C, influenza, syphilis and urinary tract infections.

Multiplex tests that permit the identification of different pathogens in the same specimen are also now available. For example, there are various multiplex molecular panels that can detect bacteria, viruses and parasites in stool samples. In secondary care, BioFire FilmArray panels can be used to detect bacterial or viral pathogens and antimicrobial resistance genes when investigating respiratory tract infections.

Despite the potential advantages of POC testing in primary care, barriers to uptake include concerns about their clinical utility and technical performance, over-reliance on results, undermining of clinical skills and cost. Identifying which individual tests and combinations are most frequently requested from primary care, as has already been noted for biochemistry laboratory blood tests, could inform test development and adoption of POC tests by general practitioners (GPs). Although microbiology testing in primary care in the UK has been examined in terms of regional inequalities for a limited number of tests, a comprehensive assessment of current demand for microbiology testing from primary care is currently lacking.

The aim of this study was to describe the frequencies of the most commonly requested microbiology tests, individually and in combination, from primary care practices in the publicly funded National Health Service Oxfordshire Clinical Commissioning Group. We also explored the yearly usage of these tests and described the most common organisms identified in positive results.

METHODS
Study setting and population
The Oxford University Hospitals Microbiology laboratory processes all samples taken from primary care GP surgeries in Oxfordshire. We conducted a retrospective cohort study using the Infections in Oxfordshire Research Database (IORD), including all microbiology tests requested by 74 active and 20 closed/merged GP surgeries in Oxfordshire between January 2008 and May 2018.

Test grouping
As our aim was to summarise frequently occurring tests, we decided to exclude infrequent tests which were requested less than 1000 times a year. This rule covered for 95% of all test codes. Some of the included tests may show a lower frequency due to elimination of duplicates and grouping of test codes. Tests routinely performed together as part of standard operating procedures were grouped (online supplemental table 1). For example, urine microscopy is reserved for few specific indications and usually accompanied by urine culture (but not necessarily vice versa), so formed a single category. Faecal test was similarly grouped.

This created eight groups of culture±microscopy test requests: urine, genital, surface swab, faecal, antenatal urine, dermatophyte, pus and respiratory tract. Gastrointestinal PCR bacterial panel tests (BD MAX Enteric Panel, Becton Dickinson, New Jersey, USA), for the identification of Salmonella spp, Campylobacter spp, Shigella spp, and shigatoxigenic Escherichia coli in faeces, were also grouped. Other tests targeted individual organisms/infections (online supplemental table 1). For each of hepatitis B, hepatitis C and HIV, serology and molecular tests (antigen, antibody, ±DNA or RNA) were grouped.

We excluded a small number of tests that are no longer routinely requested or tests misclassified as microbiological, such as semen analysis for male fertility.

Nearly all test codes (99%) were included, the remaining excluded due to being too infrequent. Results were classified as positive or negative, as appropriate for the test type. For example, a culture was considered positive if it met the laboratory standard defined in standard operating procedures (eg >10^5–10^9 CFUs/mL of a pathogenic organism in urine cultures); mixed growth and equivocal results were reported separately.

Statistical analysis
The frequency of the most common microbiology tests was described. We also reported the number of patients with at least one test during the study period, and the frequency of positive results. For each test, we reported the five the most common organisms identified, as percentages of the total number of tests and of the total number of positives.

Data were reported overall, by sex, and by age category. We used heat maps to investigate test combinations requested on the same date, and within 7 and 14 days after an initial request, since tests within this time period are more likely to be requested for the same medical condition. Statistical analyses were conducted in R (V.3.6.0) using the ‘ComplexHeatmap’ package.

Patient and public involvement
Patients and the public were not involved in the design, conduct or reporting of this research.
RESULTS
The dataset included 1596752 test requests (average 145,000/year), corresponding to 1,207518 request dates among 393,905 patients. For comparison, the mid-2018 population estimate for Oxfordshire was 687,524. Most patients were female (257,367, 65.3%), and the age distribution was similar to that of Oxfordshire (online supplemental table 2).

Frequencies of testing
Table 1 shows the frequencies of the most commonly requested test groups. Urine culture±microscopy was the most common (65,000/year), accounting for 42% of tests and 63% of patients with at least one test during the study period. The most common targeted test was hepatitis B virus (11,000/year, primarily surface antigen tests) accounting for 7% of all tests and 20% of all patients. Respiratory tract cultures accounted only for 0.20% of all tests and 0.42% of all study participants.

Of all tests, 79% were from females, and among included patients, females had two times as many tests per person as males (mean 4.9 vs 2.5) (table 2), mainly due to more urine and genital cultures and antenatal tests in women aged 18–49. Conversely, surface swabs, faecal tests, dermatophyte, pus and respiratory tract cultures were the most common in males. Proportionally more urine and Clostridioides difficile tests were conducted in older individuals.
Cryptosporidium/Giardia

Faecal culture±microscopy were often accompanied by antenatal tests, which were often requested in combination. Urine tests were mainly requested in isolation. In children aged 14–17, genital culture±microscopy were the most common combination in children aged 14–17 years, and in older adults.

Patterns of testing

Figure 1 shows combinations of tests requested on the same date. Urine tests were mainly requested in isolation. Antenatal tests were often requested in combination. Faecal culture±microscopy were often accompanied by Cryptosporidium/Giardia, C. difficile and gastrointestinal bacterial PCR tests. Many genital cultures were accompanied by a chlamydia PCR test.

Online supplemental figures 1–6 show test combination frequencies by age. In all age groups, urine culture±microscopy remained the most frequent request in isolation. Faecal culture±microscopy, Cryptosporidium/Giardia, gastrointestinal bacterial PCR and C. difficile tests were the most common combination in children aged 0–13. In children aged 14–17, genital culture±microscopy and chlamydia tests became more common. In the 50–64 age group, Helicobacter pylori was the second most common test. In the two oldest groups, surface swabs were the second most common test, and faecal tests, Cryptosporidium/Giardia, gastrointestinal bacterial and C. difficile formed the most common combination. Overall, 18% (71 572/393 905) and 23% (91 483/393 905) of all patients were retested on 102 108 and 154 528 occasions within 7 and 14 days, respectively. Urine (including antenatal) tests were a common reason for retesting within 7 days, often in combination with rubella, hepatitis B, syphilis or HIV (figure 2). Of the gastrointestinal bacterial panel, 13% were followed by faecal culture or microscopy within 7 days. Similar patterns were seen for 14 days (online supplemental figure 7). Repeated testing more often followed a mixed growth result than a positive or negative result: 7% of mixed growth urine cultures were retested on 108 and 154 occasions within 7 and 14 days, respectively.

Test results

Table 1 shows percentages of tests that yielded a positive result. Urine cultures were positive, mixed growth and equivocal in 27%, 26% and 3% of cases, respectively. Antenatal urine cultures were less often positive (7%) but mixed growth (25%) remained common. Positive results occurred more often for surface swabs (41%) and pus

(online supplemental table 3). Cryptosporidium/Giardia tests were done mostly in children aged 15 or younger. Respiratory tract cultures were more likely done in children aged 14–17 years, and in older adults.

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(35%) cultures. Most respiratory tract cultures were positive for at least one organism (94%).

The most common organism detected in urine culture was *E. coli* 20% of all urine cultures, 73% of positive urine cultures and 48% of positive antenatal urine cultures (table 3). *Enterococcus* spp (primarily *Enterococcus faecalis*) were more common in positive antenatal urine cultures (33%) than in positive general urine cultures (7%). Particular organisms predominated in other groups: *Candida* spp in 72% of positive genital cultures, *Staphylococcus* spp in 60% of positive surface swab cultures and 62% of positive pus cultures, *Campylobacter* spp in 85% of positive faecal cultures, and *Trichophyton* spp in 89% of positive dermatophyte cultures.

Urine cultures were more likely to return positive results in females than in males (29% vs 20%), while positive dermatophyte cultures were more common in males (32%) than in females (22%) (online supplemental table 4). Urine cultures were more often positive in older individuals, and *Proteus* spp were more common in children and older adults (online supplemental tables 5 and 6).

In surface swab cultures, *Staphylococcus* spp prevalence increased with age. In dermatophyte cultures, *Trichophyton* spp became less prevalent and *Candida* spp more prevalent with increasing age.

Most serological tests performed in the antenatal screen returned negative results; for example, hepatitis B surface antigen was detected in 0.6% of samples, and 96% were positive for rubella antibodies, consistent with previous vaccination/infection (table 1). Among non-antenatal serological tests, *H. pylori* antibodies were detected in 20% of samples. Of the Epstein-Barr virus group, 71% were positive for Epstein-Barr virus Nuclear Antigen (EBNA) IgG (suggesting previous exposure), 48% for Viral Capsid Antigen (VCA) IgG and 27% for VCA IgM (consistent with acute infection). Positive results for *H. pylori* and Epstein-Barr virus were more common at older ages (online supplemental table 6). For non-culture faecal investigations, positive results occurred in 16% of gastrointestinal PCR tests, 6% of *C. difficile* tests and 2% of Cryptosporidium/Giardia tests.

Longitudinal trends in testing
For most tests, the number of requests per year remained roughly constant over time (online supplemental figures 8 and 9). Antenatal urine requests increased between 2008 and 2011 in line with the National Institute for Health and Care Excellence (NICE) guidance to offer women screening for asymptomatic bacteriuria early in pregnancy to reduce the risk of pyelonephritis. Genital testing declined slightly after 2015 as swabs without specific clinical indication are no longer recommended in the NICE guidance. Genital testing declined slightly after 2015 as swabs without specific clinical indication are no longer recommended in the NICE guidance. Rubella IgG and *C. difficile* tests have decreased, as NICE guidelines did not advocate
rubella screening in pregnancy after April 2016, along-side a national decline in \textit{C. difficile}-associated infection.\textsuperscript{26} \textit{H. pylori} testing has gradually increased, and gastrointestinal PCR tests were not conducted until 2016, when the BD MAX Enteric Bacterial Panel was introduced.

**DISCUSSION**

**Summary of findings**

In this analysis of microbiology testing patterns in primary care in Oxfordshire, we have shown that the greatest burden of testing is attributable to urine tests (42\% of all tests). The burden was even greater in the older age groups (57\%–81\% of all tests in these age groups). This is understandable as NICE guidance recommends samples to be sent for urine cultures in women with suspected urinary tract infection if they are pregnant, are older than 65, had a positive urine dipstick or had symptoms persisting after antibiotic treatment.\textsuperscript{7,27} Antenatal urine cultures and blood tests, which are part of national antenatal screening NICE guidelines,\textsuperscript{28} are the second largest contributor, but are much less frequent (5\%–7\% of all tests) than urine cultures. Of note, 26\% of all urine cultures were reported as mixed growth, consistent with poor sample quality reflecting perineal contamination.

Antenatal urine cultures were less likely to be positive (7\%) than urine cultures in other individuals (27\%), many of whom would be expected to be symptomatic. NICE guidance advocates treatment of asymptomatic bacteriuria in pregnancy as this may be a risk factor for pyelonephritis, low birth weight and premature delivery.\textsuperscript{29} While \textit{E. coli} was the predominant organism in positive urine cultures, the proportion containing \textit{Enterococcus} spp or \textit{Streptococcus} spp (predominantly Group B) was higher in antenatal cultures. Novel POC urine tests should therefore be capable of identifying a range of targets, including Group B streptococci in pregnant women.

For several tests, results may have reflected the prevalence of normal flora or sample contamination, so those classified as ‘positive’ were not necessarily pathogenic and may not have changed empiric management.\textsuperscript{30,31} Examples include \textit{Candida} spp in genital cultures and \textit{Staphylococcus} spp in surface swab cultures. The apparent high positivity rate in respiratory tract cultures was caused by a range of organisms, of which some may be pathogenic but may also be part of the commensal microbiota.\textsuperscript{30,32} Among faecal specimens, positive culture results were less common overall (10\% of faecal samples), with \textit{Campylobacter} spp being the most common organism detected, consistent with national trends.\textsuperscript{33} We observed gastrointestinal PCR tests and faecal cultures are often requested on the same and subsequent dates. Since 2016, the most
common bacterial pathogens have been tested with PCR and if positive for *Shigella* spp, and/or shigatoxigenic *E. coli*, they are confirmed with faecal culture and reference laboratory testing. For *Salmonella* spp, a culture plate is usually set up in parallel with PCR. In the UK, respiratory tract infections are a common reason for consultation in primary care although are in most cases caused by a virus and do not need antibiotic prescription. Guidelines recommend further investigation only if symptoms deteriorate or do not resolve after 3 weeks. Respiratory tract cultures were very uncommon in our study, although commoner among males, in children aged 14–17 years, and in older adults. Respiratory tract cultures are requested by primary care doctors to assist in the diagnosis of rare respiratory conditions such as cystic fibrosis or in the management of acute exacerbations of bronchiectasis or chronic obstructive pulmonary disease. Due to their being used significantly less than other culture types, they are unlikely to be a useful candidate for the development of new POC tests.

**Strengths and limitations**

The main advantage of our investigation is the availability of a comprehensive dataset including all microbiology test requests and results recorded over a decade by a large clinical microbiology service, minimising selection or sampling bias.

Our study has also limitations. First, test coding may have changed over time, but we reviewed 95% of all codes and grouped similar ones to avoid missing relevant tests. Second, as our study was done in a single county, we cannot extrapolate to other regions where patterns of

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**Table 3** Frequency of the five most common organisms by test group in Oxfordshire primary care practices between 2008 and 2018

<table>
<thead>
<tr>
<th>Test group organism detected</th>
<th>N positive</th>
<th>% of positive specimens</th>
<th>% of all tested specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>132227</td>
<td>73.4</td>
<td>19.6</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp</td>
<td>13093</td>
<td>7.27</td>
<td>1.94</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp</td>
<td>7050</td>
<td>3.92</td>
<td>1.05</td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp</td>
<td>6783</td>
<td>3.77</td>
<td>1.01</td>
</tr>
<tr>
<td><em>Proteus</em> spp</td>
<td>6728</td>
<td>3.74</td>
<td>1.00</td>
</tr>
<tr>
<td>Other</td>
<td>14177</td>
<td>7.87</td>
<td>2.10</td>
</tr>
<tr>
<td><strong>Genital</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida</em> spp</td>
<td>21767</td>
<td>71.9</td>
<td>20.0</td>
</tr>
<tr>
<td>Metronidazole-sensitive anaerobes</td>
<td>5316</td>
<td>17.6</td>
<td>4.88</td>
</tr>
<tr>
<td><strong>Streptococcus</strong> spp</td>
<td>4421</td>
<td>14.6</td>
<td>4.06</td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp</td>
<td>676</td>
<td>2.23</td>
<td>0.62</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>393</td>
<td>1.30</td>
<td>0.36</td>
</tr>
<tr>
<td>Other</td>
<td>41</td>
<td>0.14</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Surface swab</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp</td>
<td>16798</td>
<td>59.9</td>
<td>24.6</td>
</tr>
<tr>
<td><strong>Streptococcus</strong> spp</td>
<td>8495</td>
<td>30.3</td>
<td>12.4</td>
</tr>
<tr>
<td><em>Candida</em> spp</td>
<td>3227</td>
<td>11.5</td>
<td>4.73</td>
</tr>
<tr>
<td>Metronidazole-sensitive anaerobes</td>
<td>1109</td>
<td>3.96</td>
<td>1.62</td>
</tr>
<tr>
<td><em>Haemophilus</em> spp</td>
<td>1001</td>
<td>3.57</td>
<td>1.47</td>
</tr>
<tr>
<td>Other</td>
<td>797</td>
<td>2.84</td>
<td>1.17</td>
</tr>
<tr>
<td><strong>Faecal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Campylobacter</em> spp</td>
<td>5631</td>
<td>84.7</td>
<td>8.25</td>
</tr>
<tr>
<td><em>Salmonella</em> spp</td>
<td>608</td>
<td>9.14</td>
<td>0.89</td>
</tr>
<tr>
<td><em>Giardia</em> lamblia</td>
<td>288</td>
<td>4.33</td>
<td>0.42</td>
</tr>
<tr>
<td><em>Shigella</em> spp</td>
<td>83</td>
<td>1.25</td>
<td>0.12</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>82</td>
<td>1.23</td>
<td>0.12</td>
</tr>
<tr>
<td>Other</td>
<td>4</td>
<td>0.06</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Antenatal urine</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1796</td>
<td>47.6</td>
<td>3.13</td>
</tr>
<tr>
<td><em>Enterococcus</em> spp</td>
<td>1250</td>
<td>33.1</td>
<td>2.18</td>
</tr>
<tr>
<td><strong>Streptococcus</strong> spp†</td>
<td>325</td>
<td>8.61</td>
<td>0.57</td>
</tr>
<tr>
<td><em>Staphylococcus</em> spp</td>
<td>193</td>
<td>5.12</td>
<td>0.34</td>
</tr>
<tr>
<td><em>Candida</em> spp</td>
<td>100</td>
<td>2.65</td>
<td>0.17</td>
</tr>
<tr>
<td>Other</td>
<td>109</td>
<td>2.89</td>
<td>0.19</td>
</tr>
<tr>
<td><strong>Dermatophyte</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Trichophyton</em> spp</td>
<td>5746</td>
<td>89.1</td>
<td>23.8</td>
</tr>
<tr>
<td><em>Candida</em> spp</td>
<td>577</td>
<td>8.94</td>
<td>2.39</td>
</tr>
<tr>
<td><em>Fusarium</em> spp</td>
<td>94</td>
<td>1.46</td>
<td>0.39</td>
</tr>
<tr>
<td><em>Acremonium</em> spp</td>
<td>68</td>
<td>1.05</td>
<td>0.28</td>
</tr>
<tr>
<td><em>Scopulariosis</em> spp</td>
<td>44</td>
<td>0.68</td>
<td>0.18</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
<td>0.12</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Pus</strong></td>
<td>1725</td>
<td>100</td>
<td>35.0</td>
</tr>
</tbody>
</table>

*The number of tests positives for all organisms within a test group may not add up to the total number of positives, as some specimens may be positive for more than one organism.†Predominantly Group B streptococcus.
testing and prevalence of organisms may differ.\(^8\) Thirdly, it was not always possible to distinguish test combinations performed together by default from those which were requested together for clinical reasons, and therefore it is unclear which elements of, for example, faecal PCR would be a clinical priority. Relatedly, we cannot be certain whether some test groups were requested in response to symptoms or as part of routine management. The latter appears likely for the antenatal test group, as typically antenatal urine tests and hepatitis B, HIV and syphilis blood tests would be requested together at booking, and so if these appeared on different dates it may have been an artefact of how data are recorded or reporting delays. Finally, we have considered the demand from primary care to inform prioritisation of the development of new POC tests, but we could not consider the likely costs of these new POC tests, their acceptability by primary care doctors and patients and other factors relevant for their adoption.\(^16\)

**Comparison with other literature**

A previous study investigated the demand for biochemistry laboratory blood tests in the community in Oxfordshire.\(^7\) In comparison, microbiology tests form a smaller number of overall requests from primary care (approximately 145,000 per year vs 3.6 million per year), but microbiology tests were more frequently repeated within 7 days (18% vs less than 3% for most specific blood tests). This might be explained by the number of urine cultures that returned inconclusive or mixed growth results. The balance between total demand and the ability to perform rapid repeat testing should therefore be considered when setting priorities for POC test development. Consideration should also be given to improving sample quality for urine tests, whether performed at POC or in the laboratory.

**Implications for research and practice**

Our results suggest that tests targeting urine infection diagnostics should have high priority for POC test development, based on the high frequency of requests made. The figures presented here underestimate the likely demand for total number of urine investigations performed in primary care. Urine dipsticks taken at the point of care are more commonly used to diagnose urinary tract infection than urine cultures in the UK and other European countries.\(^8\),\(^9\) This is particularly the case among non-pregnant and non-menopausal women. The diagnostic performance of urine dipsticks is inferior to bacteriological urine culture, which are often used to confirm positive urine dipsticks, and remain the ‘gold standard’ for investigating urinary tract infections.\(^9\) Viable POC tests should be able to detect the range of organisms described here, and reduce the need for repeat testing, caused in part by mixed growth results. Further work should aim to assess factors that might affect uptake of such POC tests in practice, including cost-benefit considerations, as well as the clinical impact of tests becoming available.

Our analysis has also highlighted the potential value of a diagnostic for other specimen types that have a high burden of testing, notably genital samples and tests for antenatal screening.

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