ORIGINAL ARTICLE

Cross-sectional study to evaluate *Trichomonas vaginalis* positivity in women tested for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*, attending genitourinary medicine and primary care clinics in Bristol, South West England

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ABSTRACT

**Background** Highly sensitive, commercial nucleic acid amplification tests (NAAT) for *Trichomonas vaginalis* have only recently been recommended for use in the UK. While testing for *T. vaginalis* is routine in symptomatic women attending genitourinary medicine (GUM) clinics, it is rare in asymptomatic women or those attending primary care. The aim of this study was to evaluate the positivity of *T. vaginalis* using a commercial NAAT, in symptomatic and asymptomatic women undergoing testing for chlamydia and gonorrhoea in GUM and primary care settings.

**Methods** Samples from 9186 women undergoing chlamydia and gonorrhoea testing in South West England between May 2013 and Jan 2015 were also tested for *T. vaginalis* by NAAT alongside existing tests.

**Results** *T. vaginalis* positivity using NAAT was as follows: in GUM 4.5% (24/530, symptomatic) and 1.7% (27/1584, asymptomatic); in primary care 2.7% (94/3499, symptomatic) and 1.2% (41/3573, asymptomatic). Multivariable regression found that in GUM older age, black ethnicity and deprivation were independent risk factors for *T. vaginalis* infection. Older age and deprivation were also risk factors in primary care. Testing women presenting with symptoms in GUM and primary care using TV NAATs is estimated to cost £260 per positive case diagnosed compared with £716 using current microbiological tests.

**Conclusions** Aptima TV outperforms existing testing methods used to identify *T. vaginalis* infection in this population. An NAAT should be used when testing for *T. vaginalis* in women who present for testing with symptoms in primary care and GUM, based on test performance and cost.

**BACKGROUND**

*Trichomonas vaginalis* is the most common non-viral STI worldwide with an estimated 276 million new cases annually.1 In the USA, 3.15% of women of reproductive age are estimated to be infected, corresponding to 2.31 million prevalent infections.2–4 *T. vaginalis* infection is associated with female gender, non-Hispanic black race/ethnicity, older age, a greater number of lifetime sex partners, lower educational level and poverty.2–4

There is increasing recognition that *T. vaginalis* increases the likelihood of HIV acquisition, HIV shedding and onward transmission.5 6–8 This interaction with HIV could increase the cost-effectiveness of *T. vaginalis* testing in areas or populations with moderate to high HIV incidence.2 10 11

Highly sensitive nucleic acid amplification tests (NAATs) for *T. vaginalis*, approved by the FDA, are available in Europe and the USA.4 12 13

Data from Public Health England in 2015 showed 6396 new diagnoses of *T. vaginalis* compared with >200 000 chlamydia diagnoses.14 The Natsal-3 study estimated the British general population *T. vaginalis* positivity as 0.3%.13

At the start of this study, routine clinical practice in the UK was to test symptomatic women attending genitourinary medicine (GUM) clinics for *T. vaginalis* infection, using culture and wet mount microscopy. In 2014, updated BASHH guidelines recommended *T. vaginalis* NAAT testing in GUM for symptomatic women, where resources allow, however, availability remains limited.

One such NAAT, the Aptima *T. vaginalis* transcription-mediated amplification test (Aptima TV; Hologic, San Diego, USA), has shown acceptable performance characteristics in the UK and USA.12 17 18 It is not known which other patient groups could benefit from *T. vaginalis* testing using NAAT or whether testing would be considered good value for money.

In this study, we evaluate the positivity of *T. vaginalis* in symptomatic and asymptomatic women undergoing testing for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in GUM and primary care. We compare the new test with existing testing practice and validate self-collected vaginal swabs.

Finally, we consider the economic implications in each clinical setting of changing testing protocol, to inform how best to implement *T. vaginalis* NAAT nationally.

**METHODS**

**Setting**

Bristol has a population of 442 500,19 20 with 16% black minority ethnic. We recruited patients from the Bristol GUM clinic (Bristol Sexual Health Centre) and primary care practices in central and South Bristol, Bath and Weston-Super-Mare.

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Study period
Data were collected from May 2013 to January 2015.

Eligibility
All females undergoing C. trachomatis and N. gonorrhoeae NAAT testing were eligible. We excluded women who were pregnant or under 18 years old.

Recruitment
GUM clinic
Women presenting for testing in GUM were asked about symptoms (vaginal discharge irritation, dysuria, pain).

Primary care
Women were recruited from GP practices served by University Hospitals Bristol, Weston General Hospital or Royal United Hospital Bath laboratories.

Healthcare professionals submitting an electronic request for chlamydia and gonorrhoea NAAT were offered Aptima TV automatically. The test was offered consistently across all practices served by the above laboratories for the duration of the study. However, it was not possible to obtain information about patients who opted out.

Testing of samples
Aptima swab samples were tested for C. trachomatis and N. gonorrhoeae using the Aptima Combo 2 C. trachomatis and N. gonorrhoeae test (Hologic). The residual sample was tested using Aptima TV. All test kits were provided free of charge by Hologic.

Genital swabs in standard microbiological media were tested for T. vaginalis using wet mount and culture according to existing testing protocols: All symptomatic patients with GUM had a wet mount and culture for T. vaginalis. Samples from primary care were tested using wet mount and culture, prepared from charcoal transport swabs, dependent on the clinical details provided according to routine laboratory practice.

Consent
Written consent was obtained from patients in group 1, because an additional sample was collected for validation of self-collection swabs. Patients in groups 2–4 were notified of the study using posters in the waiting rooms and clinical areas and were included unless they opted out of the study.

Ethics
Ethical approval for this study was obtained from the NHS Health Research Authority NRES Committee South West, Cornwall and Plymouth (REC Reference: 12/SW/0181).

Data governance
All patient identifiable data were converted to suitable proxy variables and then removed from the study database prior to statistical analysis.

Analysis
Information on age, symptoms (present/absent) and the results of all diagnostic tests performed for T. vaginalis, C. trachomatis and N. gonorrhoeae was collected for all participants. In addition, ethnicity and symptom details were collected for GUM participants only (groups 1 and 2).

The index of multiple deprivation (IMD) is a composite score indicating relative socioeconomic disadvantage, published by the UK Office for National Statistics (ONS),21 used under open license (http://www.nationalarchives.gov.uk/doc/open-government-licence/version/3/).

Deprivation scores were assigned to study individuals by matching the LSOA codes to IMD scores from ONS data. For GUM (groups 1 and 2) participants, the LSOA code was derived from postcode of residence and for GP (groups 3 and 4) participants on their GP practice postcode.

All analyses were performed in STATA V14 (Stata LP).

Sample size
We used group 1 to examine the diagnostic accuracy of Aptima TV compared with wet mount and culture. We estimated the number of positive cases required (n=24) to show with 95% power at an α of 0.05 that the diagnostic accuracy of Aptima TV was the same as that of wet mount and culture. We calculated the sample size (n=800 tests) based on the estimated number positive and the positivity of T. vaginalis in this population by existing tests prior to the study (3%). Since the interim analysis in December 2014 indicated a higher observed positivity of T. vaginalis (4.6%), we revised our sample size calculation (n=510 tests).

Collection of samples for groups 2–4 continued until we had recruited the required number from group 1.

Statistical analysis
The following multivariable logistic regression analyses of factors associated with T. vaginalis-positive test result were performed:

1. All participants: independent variables: age, setting (GP or GUM), symptoms (presence/absence), chlamydia diagnosis (positive/negative), gonorrhoea diagnosis (positive/negative)
2. GUM only (groups 1 and 2) as above, IMD score (based on postcode of residence, ethnic group)
3. Primary care only (groups 3 and 4) as above, IMD score (based on practice postcode)
4. Performance of existing tests was compared with Aptima TV using the \( \chi^2 \) test.

Economic evaluation
The costs of current and new testing methods were estimated. These estimates took into account reagents and staff costs. Two scenarios of costs for implementing T. vaginalis NAAT testing were considered:

Scenario 1: using same NAAT platform,
additional test added to chlamydia and gonorrhoea NAAT, where Aptima NAAT platform is in use (assumes Aptima TV test cost £7.62).

Scenario 2: using different NAAT platform, stand-alone test with a different sample from that used for chlamydia and gonorrhoea NAAT, where another NAAT platform is in use (assumes Aptima TV cost £15.19).

The number of additional diagnoses, number of tests performed, total cost of testing, cost per positive and cost per additional positive in each group were calculated.
RESULTS
A total of 9241 women were recruited to the study: 9220 were eligible and 9186 had complete data on age and definitive test results for *T. vaginalis*, *C. trachomatis* and *N. gonorrhoeae* summarised in table 1. Study recruitment and exclusions are shown in online supplementary appendix figure A1.

During the study period May 13 to January 15, there were a total of 46 188 chlamydia/gonorrhoea NAATs performed on female patients in the three laboratory areas. A total of 14 367 tests were performed in GUM of which 2114 (14.7%) were included in the analysis and 31 821 were performed in primary care of which 7072 (22.2%) were included in the analysis. The positivity of *T. vaginalis*, *C. trachomatis* and *N. gonorrhoeae* is shown in figure 1 and online supplementary appendix table A1.

Overall, the *T. vaginalis* positivity was 2.0% (95% CIs 1.75% to 2.33%) compared with *N. gonorrhoeae* 0.4% (95% CIs 0.26% to 0.52%) and *C. trachomatis* 2.7% (95% CIs 2.4% to 3.08%).

The observed *T. vaginalis* positivity was highest in symptomatic patients with GUM 4.5% (24/530), followed by symptomatic women attending primary care (2.7%, 27/1584). In symptomatic women attending primary care, the positivity of *T. vaginalis* (2.7%) was higher than *C. trachomatis* (2.1%).

Risk factor analysis
In multivariable logistic regression of risk factors (n=9186), the presence of symptoms, attendance at GUM, age over 35 and chlamydia diagnosis were all significantly associated with diagnosis of *T. vaginalis* at 5% significance level (table 2).

In subgroup analysis in GUM, black ethnicity was associated with increased odds of diagnosis with *T. vaginalis* (adjusted OR 5.28, CI 2.65 to 10.50, p<0.001) compared with white ethnicity (see online supplementary appendix table A3). The effect of deprivation was significant in both settings (GUM and primary care) with more deprived (higher) IMD scores associated with higher risk of *T. vaginalis*.

Performance of new test compared with existing testing practice
A total of 3424 (of the total 9186) patients were tested both by Aptima TV and existing testing methods (either wet mount microscopy, culture or both). The majority were symptomatic (group 1: 485, group 2: 17, group 3: 2133, group 4: 789).

In the GUM clinic, where wet mount microscopy is undertaken on site in addition to culture, existing testing methods were 56.5% (13/23) sensitive compared with the Aptima TV test.

In primary care, sensitivity was 25.7% (19/74), which may reflect deterioration of samples in transit. There were no cases identified by existing test methods which were not also identified by Aptima TV which significantly outperformed existing testing methods in GUM and primary care (p<0.001, χ² test) see online supplementary appendix table A4.

Clinician and self-collected swabs had equivalent performance (details in online supplementary appendix table A5).

Economic evaluation
Table 3 shows the number of tests performed for *T. vaginalis* in symptomatic and asymptomatic women in primary care and GUM clinics (Row B), number of diagnoses (A) compared with numbers tested under current testing policy (E) and diagnosed by Aptima TV (C) or wet mount/culture (D). These results were used to calculate the positivity and number of additional diagnoses and the cost implications of different testing strategies.

Compared with baseline estimates, for women currently tested for *T. vaginalis* (3424) using Aptima TV would result in an additional 45 diagnoses (97 compared with 32). If all women who are currently tested for chlamydia/gonorrhoea (9186) were also tested for *T. vaginalis*, this would result in 186 diagnoses (2.0% positivity).

Scenario 1 (using same testing platform) compared with baseline
The total cost of universal testing for *T. vaginalis* in women currently receiving chlamydia/gonorrhoea testing assuming use of existing NAAT platform is estimated at £69 997 over 21 months, equating to approximately £40 000 per year,
depending on test volume. The overall cost per positive is £376, compared with £849 per positive using current tests (table 3). If only symptomatic women are tested (ie, combining group 1 and 3), the cost per positive would be £260 = (530 + 3499) × £7.62 / (24 + 94) compared with £716 = (485 + 2133) × £7.93 / (12 + 17) using current tests.

Scenario 2 (using a different testing platform) compared with baseline

For clinics with different testing platforms, the total cost of universal testing for \textit{T. vaginalis} is £139 535 (21 months study period) or £79 700 per year. Correspondingly, the cost per positive is also nearly doubled.

This only includes the test cost and excludes equipment purchase, lab overheads, training and other opportunity costs associated with implementing a new laboratory test which should also be considered.

DISCUSSION

Summary of main findings

In women attending primary care and at risk of STIs, positivity of \textit{T. vaginalis} infection was 2.7% (symptomatic) and 1.1% (asymptomatic), using the Aptima TV test.

In women attending GUM, the positivity of \textit{T. vaginalis} was 4.5% (symptomatic) and 1.7% (asymptomatic) which is consistent with comparable UK NAAT estimates.\textsuperscript{22}

Aptima TV outperformed existing testing methods, identifying additional cases of \textit{T. vaginalis} in GUM and primary care settings.

\textit{T. vaginalis} positivity was 11.9% (16/134) in patients of black ethnicity in GUM and 1.6% (29/1789) in those who self-identified as white. However, as the absolute number of cases was higher in women of white ethnicity, 69% (35/51) of \textit{T. vaginalis} NAAT-positive cases in GUM would not have been identified had testing been targeted based on black ethnicity alone.
T. vaginalis infection was independently associated with deprivation, and positivity was higher in older women in all clinical settings.

The current cost of wet mount/culture is comparable to the cost of an additional test in the existing testing platform in the Bristol clinic, and the higher detection rate makes it relatively more cost-effective as well as more accurate, especially for patients in primary care.

**Strengths and weaknesses**

The study is a large, cross-sectional study of T. vaginalis infection diagnosed with NAATs in over 9000 women undergoing STI testing and is the first to report on T. vaginalis positivity in primary care in the UK.

**Study limitations**

An important limitation of the study is that it does not include any information about patient’s sexual behaviour, their partners and their risks. It would have been useful to know partner’s ethnicity for those patients testing positive for T. vaginalis. In primary care, ethnicity information is not consistently recorded at patient or practice level.

Patients tested for STIs in primary care are likely at increased risk compared with the general population and positivity would be expected to be higher. In primary care, opportunistic chlamydia screening is recommended for sexually active women under 25 years. Older women are not routinely screened and might be more likely to present in the event of symptoms or perceived risk. A limitation of this study is that it does not distinguish between patients in primary care who present in response to symptoms or a perceived need to test and those who are screened opportunistically. It was only possible to distinguish those who presented with and without symptoms.

We only included women over 18 so cannot comment on the risk in younger women. We did not recruit patients from community sexual health clinics, where positivity might be lower than GUM but higher than primary care.

We do not have information on women who withheld consent so cannot define the representativeness of the women in each study group. We used opt-out consent method, other than in symptomatic patients with GUM, which should reduce participation bias.

The economic evaluation did not consider factors such as indirect effects on population prevalence, such as reducing risk of outcomes or the effect on HIV transmission. Local commissioning decisions are likely to be based on pragmatic considerations of cost and detection rate, which was the focus of this study.

**Findings compared with other studies**

In the USA in 2001–2004, the National Health and Nutrition Examination Survey observed that 3.1% (n=3754) of women of reproductive age were infected with T. vaginalis, with highest rates in women of non-Hispanic black ethnicity (13.3%). Additional factors associated with infection were older age (especially in black women), lower educational achievement and poverty.

In the Netherlands, a comparative cohort study observed T. vaginalis infection in women in 1.6% of a general practice cohort (n=554) and 0.8% of a nationally representative chlamydia screening study (n=566). In Australia, a retrospective analysis of community samples tested with NAAT found 1.5% (n=37 137) of women positive for T. vaginalis. Indigenous referrals accounted for 48% of positive cases in this sample.

The positivity in a general practice population is comparable with the Netherlands and Australian findings and somewhat lower than in USA, as expected. Findings from the GUM study groups were also consistent with other comparable populations in the UK.

The positivity is much higher than that found in the recently published Natsal-3 data which showed a 0.3% positivity in

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**Table 3 Economic implications for use of nucleic acid amplification test (NAAT) technology in different clinic settings (May 2013 to January 2015, 21 months)**

<table>
<thead>
<tr>
<th>Scenario 1</th>
<th>TMA costs £7.62 using existing diagnostic platform</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Positive, TMA</td>
<td>Total tested</td>
<td>Cost per positive (£7.93)</td>
</tr>
<tr>
<td>A</td>
<td>24 (4.5%)</td>
<td>£335</td>
</tr>
<tr>
<td>B</td>
<td>27 (1.7%)</td>
<td>£135</td>
</tr>
<tr>
<td>C</td>
<td>94 (2.7%)</td>
<td>£995</td>
</tr>
<tr>
<td>D</td>
<td>41 (1.1%)</td>
<td>£127</td>
</tr>
<tr>
<td>Total</td>
<td>186 (2.0%)</td>
<td>£321</td>
</tr>
<tr>
<td>Difference in diagnoses</td>
<td>F=A–D</td>
<td>£168</td>
</tr>
<tr>
<td>TMA test</td>
<td>G=C/E</td>
<td>£168</td>
</tr>
<tr>
<td>Cost per additional positive</td>
<td>H=D/E</td>
<td>£447</td>
</tr>
<tr>
<td>Cost per positive</td>
<td>J=I/D</td>
<td>£844</td>
</tr>
<tr>
<td>Total cost (TMA test)</td>
<td>K=B×£7.62</td>
<td>£4039</td>
</tr>
<tr>
<td>Current cost</td>
<td>I=Ex£7.93</td>
<td>£3846</td>
</tr>
<tr>
<td>Cost per positive (current situation)</td>
<td>J=I/D</td>
<td>£135</td>
</tr>
<tr>
<td>Difference in cost</td>
<td>L=K–I</td>
<td>£127</td>
</tr>
<tr>
<td>Cost per additional positive</td>
<td>M=J/L</td>
<td>£565</td>
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<tr>
<td>Cost per positive</td>
<td>N=K/A</td>
<td>£321</td>
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<tr>
<td>Total cost (TMA test)</td>
<td>O=B×£7.93</td>
<td>£8051</td>
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<tr>
<td>TMA costs £15.19, different test platform</td>
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<td></td>
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<tr>
<td>Total tested</td>
<td>P=O×£15.19</td>
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<tr>
<td>Difference in cost</td>
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<tr>
<td>Cost per additional positive</td>
<td>R=Q/F</td>
<td>£350</td>
</tr>
<tr>
<td>Cost per positive</td>
<td>S=R/A</td>
<td>£335</td>
</tr>
</tbody>
</table>

TMA, transcription-mediated amplification.

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4396 urine samples obtained from men and women aged 16–44 years. This suggests that the population who present for testing in primary care is at higher risk than the general population.

The Aptima TV test outperformed wet mount and culture in the GUM clinic and particularly in primary care. Sensitivity of wet mount and culture was 56.5% compared with NAAT when performed in the GUM clinic but was only 25.7% in primary care. This could be due to deterioration of samples in transit, calling into question the use of traditional microbiological testing methods for *T. vaginalis* outside the GUM setting where near patient wet mount microscopy is routinely available.

As has been shown previously for gonorrhoea and chlamydia, self-collected swabs are as good as clinician-taken swabs for *T. vaginalis* NAAT testing, and this is the preferred method for sample collection.25

The association with black ethnicity has been documented previously in the UK and USA.22 26 The findings are consistent with previous UK surveillance data which shows a higher proportion of the absolute number of *T. vaginalis* diagnoses in those of white ethnicity, but much higher rate of infection in those of black ethnicity.

What this study means?

The positivity of *T. vaginalis* in women with symptoms in GUM and in primary care was higher than anticipated using Aptima TV. Opportunities for diagnosis of *T. vaginalis* may be being missed in GUM and in primary care, and this could have implications for onward transmission and population positivity.

Use of sensitive NAATs such as Aptima TV will identify additional cases, and is likely to be cost-effective for symptomatic patients, especially when performed using the same sample and diagnostic platform as that used for chlamydia and gonorrhoea testing.

Use of NAATs in asymptomatic patients is more expensive. Complex testing strategies based on a combination of risk factors could help to optimise detection of *T. vaginalis* in the community. These would need to be easy to implement in practice.

Local epidemiology and locally relevant cost data will influence commissioning decisions regarding *T. vaginalis* testing in future in the UK.

Key messages

- **Positivity of Trichomonas vaginalis** determined by nucleic acid amplification tests (NAATs) is high in women with symptoms presenting for STI testing in genitourinary medicine (GUM) (4.5%) and in primary care (2.7%).
- In this UK population, deprivation increases the risk of *T. vaginalis* independent of ethnicity.
- Based on cost, NAATs for *T. vaginalis* are recommended for symptomatic women presenting for STI testing both in GUM and primary care.

Handling editor Jackie A Cassell

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Contributors JEN, PJH and PM conceived the study idea; wrote the protocol with input from KMET, MTM and JM. MTM calculated sample sizes based on data from KG and had overview of the statistical analysis. JEN, KG and PM ran the study in the lab and in primary care. JEN and PJH ran the study in the GUM clinic. RF wrote the CE programme to offer Aptima TV testing and record consent in primary care. PN was responsible for extracting and summarising the data from LIMS throughout the study. KT performed the data linkage and data analysis on data from PN and MTM provided statistical support. KMET undertook the economic evaluation. JN wrote the first draft with input from KMET, PJH and PM. Subsequent drafts followed critical review by all authors. All authors approved the final version.

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Competing interests Hologic provided test kits free of charge during the study. In addition, PJH, JEN and KMET received expenses (travel, conference fee and accommodation) to attend ISSTDR 2015 (Brisbane). JEN received expenses to attend ECCMID 2016 (Amsterdam) and RICAI 2017 (Paris). No other consultancy fees or payments were received for this study and Hologic was not involved in the study design or analysis.

All authors provided ICME from to generate the following statement: JEN reports personal fees and non financial support from Hologic during the conduct of the study. PJH reports non-financial support from Hologic, during the conduct of the study, personal fees from Crown Prosecution service, personal fees from British Association for Sexual Health and HIV, grants from Mast Group, outside the submitted work. In addition, PJH has a patent A sidialisate test spot to diagnose bacterial vaginosis issued to University of Bristol. PM reports non-financial support from Hologic, during the conduct of the study; grants, personal fees, non-financial support from Hologic, grants from Innovate UK, grants and non-financial support from Mast Group and Elthec UK outside the submitted work. KMET reports personal fees from Hologic, during the conduct of the study; personal fees from Aquarius Population Health, outside the submitted work. MTM reports non-financial support from Hologic, during the conduct of the study. Statement provided (KG) confirmed by email to JEN (RF and PN). The views expressed are those of the authors and not those of the NHS, NIHR, PHE or Hologic.

Ethics approval NRES Committee South West Cornwall and Plymouth.

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