**Targeted Point-of-Care Testing Compared to Syndromic Management of Urogenital Infections in Women: a Cross-Sectional Screening and Diagnostic Accuracy Study**

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**APPENDIX**

**Informed consent procedures**

All participants provided written informed consent. The age of majority for Rwandan women was lowered from 21 to 18 years in November 2016, and we also obtained parent/guardian consent for non-married participants aged 18-20 years until this was no longer required. Participants and/or parents/guardians with insufficient literacy could sign by thumbprint but the informed consent process was observed by an independent witness who co-signed the informed consent form. The witness could not be a Rinda Ubuzima staff member, but could be another participant.

**Selection of POCTs for evaluation in WISH**

We chose POCTs that comply with the WHO ASSURED criteria (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment-free, and Deliverable to end users) as much as possible1,2, and that are feasible to conduct and interpret by primary care clinic staff in African settings. For example, some might argue that microscopy is an ‘almost ASSURED’ diagnostic method for diagnosing bacterial vaginosis (BV). Microscopy is not equipment-free, but microscopes are much more readily available than other types of equipment. However, our experience is that microscopy is not ‘user-friendly’. While the acts of preparing a slide and viewing it under a microscope might be feasible after sufficient practice, the actual recognition of human cell types and micro-organisms requires a much higher level of biology training than primary care clinic staff in African settings have typically received. Also, Gram staining requires a laboratory with dedicated sinks and running water for safety reasons, and it is not practical to stain one slide at a time.

The development of ASSURED POCTs for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) has been ongoing for years. Unfortunately, lateral flow tests based on CT/NG-specific antibody, antigen, or enzyme detection to date have had low sensitivity.3,4 The GeneXpert CT/NG assay (Cepheid, Sunnyvale, USA) was the first CT/NG POCT based on nucleic acid amplification testing (NAAT) and has excellent performance compared to other validated CT/NG NAATs.5,6 However, it is not ASSURED because it requires equipment, is expensive (we paid 18·25 USD for consumables per test), and takes 90 minutes to return results. We chose to use this test anyway because it was the best CT/NG POCT available at the time of WISH study implementation, and because GeneXpert machines are widely available in Rwanda for tuberculosis testing. The machines are modular and can accommodate different types of test cartridges. Cepheid is currently considering developing next-generation GeneXpert CT/NG assays with shorter turn-around times but development has not yet been initiated (personal communication with representatives of Cepheid Europe in July 2018). Furthermore, additional NAAT-based POCTs for CT and/or NG are under development and prices might decline as a result of market competition.7–9 In the WISH study, the Rwandan laboratory technicians were trained by a Cepheid representative based in Nairobi, Kenya. The representative visited the Rinda Ubuzima laboratory, assisted the team with equipment set-up, and conducted a one-day training using this equipment so that any start-up problems could be resolved immediately. The University of Liverpool investigators also attended this training to be able to assist the Rwandan technicians with potential future problems. However, we did not experience any problems during the study. Also, the percentage of invalid test results throughout the study was in line with those expected by Cepheid (fewer than 5% of the tests overall).

For *Trichomonas vaginalis* (TV), we chose the TV OSOM test (Sekisui Diagnostics, Lexington, USA) because experts consider it ASSURED and best in its class of lateral flow tests.10 It is easy and quick to perform, has sensitivities of 83-86% compared with NAATs,10 and is cheaper than NAATs (we paid 6·35 USD for consumables per test). POC NAATs that will likely have improved sensitivities are available or under development, including a module that can be used on the GeneXpert machine, but those are not ASSURED and more expensive.10

BV and vulvovaginal candidiasis (VVC) are not infections by a single pathogen but changes from an immuno-tolerant lactobacilli-dominated vaginal microbiological ecology towards a proinflammatory ecology. Knowledge about the vaginal ecology has significantly increased in recent years.11 From a microbiological perspective, BV is a bacterial dysbiosis, and VVC is a dysbiosis characterised by an overgrowth of yeasts/fungi. BV is characterised by a persistent depletion of lactobacilli and an increased concentration of other bacteria, most commonly – but not exclusively – a highly diverse mixture of anaerobes including *Gardnerella vaginalis* and *Atopobium vaginae*. From a clinical perspective, the gold standard BV diagnosis is a Gram stain Nugent score of 7-10,12 but the Amsel criteria are also often used.13 Currently, only symptomatic BV in women seeking care is treated, even though asymptomatic BV has been associated with pregnancy complications and other complications.14 There is no consensus yet about which types and levels of vaginal bacterial dysbiosis should be treated to prevent complications.15 The Amsel criteria include a high vaginal pH as one of the criteria, and in a recent study at Rinda Ubuzima, we found that a vaginal pH≥5·0 predicted a Nugent score of 7-10 with 71·0% sensitivity and 76·0% specificity (unpublished data). Vaginal pH can be measured easily and cheaply. We therefore decided to use the EcoCare vaginal pH swab (Merete Medical, Luckenwalde, Germany) with pH ≥5·0 as the cut-off to diagnose BV in the WISH study. Some BV POCTs detecting enzymes or metabolites produced by *G. vaginalis* and/or other anaerobic bacteria (e.g. sialidase, proline aminopeptidase, or amines) have been developed,16 but did not significantly outperform the vaginal pH results that we obtained in our previous study (unpublished data). VVC POCTs detecting *Candida* antigens/antibodies have either shown inadequate sensitivity and specificity,17–19 or have not been compared to gold standard NAAT or culture assays.20 Molecular tests targeting lactobacilli and/or key BV-associated bacteria and/or *Candida* species are available or being developed, but are expensive and not ASSURED.21–25

**Other POCTs offered to WISH participants**

We wanted to offer WISH participants a complete sexual health screening package and therefore offered them HIV, syphilis, pregnancy, and urinary tract infection (UTI) POCTs as a service. We did not evaluate the performance of these POCTs because they are either known to have good performance (HIV, syphilis, pregnancy), and/or we did not have suitable gold standard test results available to us to compare the POCT results to (syphilis, UTI).

Validated ASSURED1,2 POCTs with good performance for HIV, syphilis, and pregnancy have been available for many years, including in low and middle-income countries.26,27 In WISH, we used locally available POCTs that were recommended by the Rwanda Ministry of Health at the time of the study (see legend of Manuscript, figure 1).

UTIs in women can be caused by multiple pathogens. *Escherichia coli* is the most common cause (>70% of cases28), but other causes include *Enterococcus* species, *Klebsiella pneumoniae*, *Proteus mirabilis*, and STI pathogens such as NG. By far the most commonly used UTI POCTs are lateral flow tests detecting leukocytes, nitrites and other bacterial metabolites. These are readily available and cheap, but have modest performance when compared to urine culture.28 Lateral flow tests to detect *E. coli* in urine are being developed.29 The WISH study focussed on genital infections and we therefore elected to use a locally available urinalysis dipstick assessing leukocytes and nitrites for UTI POC testing (figure 1). While we did not formally evaluate the performance of this urinalysis dipstick, we compared dipstick findings with urine *E. coli* qPCR findings (see ‘gold standard testing’ below) in this Appendix (Appendix, table 5).

**Gold standard testing**

*CT/NG GeneXpert at Rinda Ubuzima, Kigali, Rwanda*

The CT/NG GeneXpert assay was considered a gold standard because of its high performance compared to other validated CT/NG NAATs (see above). While only women who had a positive CT/NG score were tested that same day, GeneXpert swabs were also taken from all other women and tested in batches. All GeneXpert assays were performed in the onsite Rinda Ubuzima laboratory in Kigali, Rwanda.

*Assays conducted at the Institute of Tropical Medicine in Antwerp, Belgium*

All other NAATs were conducted in the STI reference laboratory of the Institute of Tropical Medicine in Antwerp, Belgium.

All women were asked to self-sample two polyester swabs during the main visit. This was optional but none of the women refused. Each swab head was stored in a 2 ml cryovial containing 1 ml RNA*later* (ThermoFisher Scientific, Paisley, UK) at -80°C during study implementation. Urine samples from women who underwent pregnancy and/or UTI testing (N=641) were centrifuged at 3,000g and the pellet was stored at -80°C in 2 ml cryovials. After all main visits had been completed, swab heads in RNA*later* were defrosted and shipped to Belgium at room temperature. Urine pellets were transported to Belgium on dry ice. DNA was extracted from vaginal swab heads and urine pellets using the Abbott m2000sp automated extraction platform (Abbott Laboratories, Chicago, USA) and an elution volume of 200 µL, incorporating an extra lysis step.30

Validated in-house polymerase chain reaction (PCR) assays were performed targeting the following organisms: TV, *Mycoplasma genitalium* (MG), *Candida albicans*, *Lactobacillus* genus, *G. vaginalis*, and *A. vaginae* on vaginal swabs, and *E. coli* on urine samples. The real time TV PCR targeted a fragment of 92 base pairs of a TV-specific repeat gene. In brief, the 25 µL PCR mixture contained 12·5 µL of Platinum Q, PCR SuperMix UDG (Invitrogen, California, USA), 0·9 µL of 25 µM Primers TV001 (5’AAAGATGGGTGTTTTAAGCTAGATAAGG-3’) and of 25 µM Primers TV002 (5’ TCTGTGCCGTCTTCAAGTATGC-3’), 0·25 µL Probe TV003 (5’ /56 FAM/AGTTCATGT/ZEN/CCTCTCCAAGCGTAACT/3IABkFQ/-3’), 10 µL DNA extract and 0·45 µL RNAse-free water. Primers and probes were described by Pillay et al.31 The amplification comprised an initial heating of 50°C for 2 minutes and of 95°C for 3 minutes followed by 45 cycles of 95°C for 20 seconds and 60°C for 60 seconds. MG was detected by applying a previously published in house real time PCR assay.32 The concentrations of *Lactobacillus* genus, *G. vaginalis* and *A. vaginae* were determined by quantitative (q)PCRs as previously described.33 The concentrations of *C. albicans* were determined by qPCR with primers previously published by Cools et al (CA\_rRNA R 5’-TTGAAGATATACGTGGTGG-3’ and CA\_rRNA F 5’-TTTGCTTGAAAGACGGTA-3’).34 The 25µL PCR mixture contained 12·5µL Rotor-Gene SYBR Green (Qiagen, Hilden, Germany), 2·5µL of 10 µM of each primer, 2·5µL RNAse-free water and 5µL of DNA extract. The concentrations of *E. coli* were determined by qPCR with primers previously published by Chern et al. (EC23S857 assay, *E. coli*\_rRNA R 5’-TGTCTCCCGTGATAACtTTCTC-3, *E. coli*\_rRNA F 5’-GGTAGAGCACTGTTTtGGCA-3’, *E. coli*\_rRNA probe 5’-TCATCCCGACTTACCAACCCG-3’).35 The 25µL PCR mixture contained 12·5µL Platinum Q PCR SuperMix-UDG (2x) (Invitrogen, California, USA, 0·9µL of 25µM of each primer, 0·7µL of 5µM probe and 10µL of DNA extract. The amplification protocols of *C. albicans* and *E. coli* were the same as previously described for *Lactobacillus* genus.33 All primers and probes were synthesized by Integrated DNA Technologies (IDT, Illinois, USA), and all amplification reactions were performed using the Corbett Life Science Rotor-GeneTM 6000 (Qiagen, Venlo, the Netherlands).

The qPCRs for TV and MG were qualitative. The other qPCRs were quantitative and run in duplicate. Organism concentrations were expressed as genome equivalents per ml (geq/ml; the mean of duplicates) and log10-transformed. The qualitative PCR results for TV and MG were considered gold standards. A gold standard BV diagnosis was made when the vaginal qPCR score was below -2 (see next paragraph). The *C. albicans* qPCR was not considered a true gold standard for VVC because VVC can be caused by multiple yeasts/fungi. We have, however, referred to it as a gold standard test in this paper for convenience and readability, and would also like to point out the following: 1) it is estimated that 70-95% of VVC cases are caused by *C. albicans*;36 and 2) *Candida* species are known to be proinflammatory; asymptomatic ‘carriage’ is therefore only likely when present in low concentrations. In the WISH study, *C. albicans* was only detected by qPCR in 8·6% of the women, and in 68% of those women, it was present at concentrations ≥105 geq/ml. *C. albicans* therefore does not seem to qualify as a ‘harmless commensal’ in the vagina. However, it is currently not known above which concentration threshold on average *C. albicans* (plus other yeasts/fungi) causes symptoms and/or complications, and more research is needed to develop an optimal gold standard test. Similarly, the *E. coli* qPCR was not considered a true gold standard for UTI because UTIs can be caused by multiple organisms (see above). Because UTIs are not included in the WHO syndromic guidelines, and we did not have optimal gold standard test results, we opted to compare the urinalysis dipstick and *E. coli* qPCR results in this Appendix (Appendix, table 5), but to not formally evaluate the urinalysis dipstick performance.

*Vaginal qPCR score to identify true BV cases*

As described above, from a clinical perspective, the gold standard BV diagnosis is a Nugent score of 7-10.12 We followed Kenyan, Rwandan and South African women over two menstrual cycles and assessed their vaginal microbiota by Gram stain Nugent scoring and by conducting qPCRs of relevant vaginal bacteria on vaginal swabs.37 About a third of the 387 women (35·7%) had a Nugent score of 7-10 (BV). Individual and combinations of qPCR assay results were compared to a Nugent score of 7-10, and the highest diagnostic accuracy was achieved with what we subsequently termed the vaginal qPCR score [log10 geq/ml (*Lactobacillus* genus) - log10 geq/ml (*G. vaginalis* + *A. vaginae*)] below -2. The sensitivity was 93·4% and specificity 83·6%. We wanted to keep the number of research procedures in the WISH study to a minimum in order to mimic a real life clinic setting, and we therefore elected not to conduct Gram stain Nugent scoring. Instead, we collected two extra vaginal swabs to allow for all required gold standard testing after study completion, and used the vaginal qPCR score as the gold standard for BV. Please note that the use of such a continuous score acknowledges that the vaginal microbiota exist as a continuum from completely lactobacilli-dominated (a typical concentration in the order of 106 lactobacilli per vaginal swab, which would translate into a vaginal qPCR score of 6) to completely dysbiotic (0 lactobacilli but high concentrations of other bacteria, which would translate into a vaginal qPCR score below 0) and everything in between. Some women may not reach the BV threshold of -2, but could still have too many inflammatory bacteria compared to immuno-tolerant lactobacilli, and even develop symptoms and/or complications because of this. We have termed this ‘mild dysbiosis’. It is currently not known above which concentration threshold on average vaginal bacterial dysbiosis causes symptoms and/or complications.

**Additional information about the WHO and WISH algorithms**

The WHO and WISH algorithms are described in detail in the text and figure 1 of the manuscript. We offer additional clarifications here. WHO published two vaginal discharge syndrome (VDS) algorithms: one that incorporates speculum examinations and one that does not.38 We used the algorithm without speculum examination but with differentiation between not VVC-like (treated for CT, NG, TV, and BV because prevalences in our study population were expected to be high) and VVC-like (also treated for VVC) based on structural reporting instead of speculum examination findings. The WHO and WISH lower abdominal pain (LAP) algorithms aim to identify patients with pelvic inflammatory disease, which can be life-threatening: if LAP is reported, a bimanual exam is done, and pelvic inflammatory disease is diagnosed if there is adnexal or cervical motion tenderness during the bimanual exam. The WHO algorithm does not specifically mention pain during sex as a reason to do a bimanual exam, but the WISH study team decided that pain during sex may also be indicative of pelvic inflammatory disease. We therefore included it in the WISH algorithm. This resulted in only three additional patients being diagnosed with pelvic inflammatory disease compared to the WHO algorithm (32 and 29 cases, respectively; see Manuscript, table 3). Finally, the genital ulcer disease (GUD) algorithm included genital warts in the WISH study but not in the WHO guidelines. However, no genital warts were diagnosed during the study.

As is described in the manuscript, study physicians performed speculum/bimanual examinations on 399/705 (56·6%) participants, which was more than we had anticipated. The data show that they did examinations in all cases of LAP and pain during sex as required, but also in almost all cases of participant-reported VDS that were not VVC-like (which was only recommended in cases of substantial VDS), and all cases of GUD/buboes (whereas genital inspection may have sufficed). Furthermore, we question the added value of speculum/bimanual examinations other than for diagnosing pelvic inflammatory disease: participant and physician judgments on whether VDS was VVC-like or not were not accurate (Manuscript, table 5).

**Treatment, partner notification, and referral**

Urogenital infections were managed in accordance with the Republic of Rwanda National Guidelines for Prevention and Management of HIV, STIs & Other Blood Borne Infections (2013).39 Only medically qualified physicians were allowed to dispense treatments (commonly used drugs were stocked in the study clinic) or prescriptions for treatments, and they were instructed to refer complications to a qualified specialist in a referral hospital in Kigali. First and second choice treatment recommendations at the time of the study for the most common infections are listed in the table below. In some cases, etiologic diagnoses became available after the participant had already left the study clinic (e.g. after completion of the gold standard testing). In the case of curable STIs (NG, CT, TV, and syphilis), women were contacted and asked to attend the study clinic as soon as possible for treatment and partner notification. Pregnant women were referred to antenatal care, HIV-positive women to HIV care, and all women were informed where they could obtain contraceptive methods and screening for cervical cancer.

|  |  |  |  |
| --- | --- | --- | --- |
| **Infection** | **First choice treatment** | **Second choice treatment** | **Window periods for partner notification** |
| CT | Doxycycline 100mg twice per day for 7 days | Erythromycin 1g twice per day for 7 days | 4 weeks if symptomatic; 6 months if asymptomatic or PID. |
| NG | Ciprofloxacin 1g single oral dose | Ceftriaxone 250mg in one single IM dose [After discussion with MoH: 500mg IM allowed in case of suspected resistance] | 3 months, or 6 months if PID. |
| Syphilis (primary) | Benzathine benzyl penicillin 2·4 million IU IM single dose | Erythromycin 1g orally 2x per day x 14 days OR Doxycycline 100mg orally twice per day x 14 days | 3 months |
| TV | Metronidazole 2g single oral dose OR 400 or 500mg orally twice per day for 7 days | Tinidazole 2g single oral dose OR 500 mg twice per day for 7 days | 4 weeks |
| BV | Metronidazole 400 or 500mg orally twice per day for 7 days OR 2g single oral dose. | Tinidazole 2g single oral dose once a day for 2 days or 1g per day for 5 days. Alternative: Clindamycin 300mg orally twice per day for 7 days | NA |
| VVC | Fluconazole 150mg single oral dose | Clotrimazole 200mg pessaries every night for 3 nights | NA |
| UTI | Ciprofloxacin 500mg orally twice per day x 7 days. | Ceftriaxone IM 125mg twice per day for 5 days. | NA |

BV=bacterial vaginosis. CT=*Chlamydia trachomatis.* IM=intramuscular. IU=international units. MoH=Ministry of Health. NA=not applicable. NG=*Neisseria gonorrhoeae.* PID=pelvic inflammatory disease. TV=*Trichomonas vaginalis*. UTI=urinary tract infection. VVC=vulvovaginal candidiasis.

Partner notification can cause social harms and was therefore only done in the case of laboratory-confirmed curable STIs or pelvic inflammatory disease and when the participant consented to a certain partner to be notified. Women with HIV were referred to health centres that provided comprehensive HIV care, including partner notification for HIV. The window periods in the table above were used to identify partners requiring notification. Partners were not tested but received treatment for the infection that was diagnosed in the index case with one exception: partners of women with pelvic inflammatory disease were tested for CT and NG and only treated for these infections if one or both tests were positive.

Women were offered partner notification choices for each partner as follows:

1. She could give a partner notification card to the partner. This card listed the address, contact information, and opening hours of the studyclinic, and a request to come to the clinic as soon as possible for ‘medical follow-up’.

2. She could allow study staff to notify the partner by telephone, mail or home visit. This could be done anonymously if preferred by the participant.

As described in the manuscript, treatments and referrals were delivered as required with few treatment failures, but the uptake of partner notification was suboptimal: 782 identified partners of 201 women (28·5%) required partner notification but only 61 (7·8%) of them were treated at the study clinic (Appendix, table 6). The main reasons were that many women did not consent to notifying some or all partners, or insisted on notifying partners themselves but likely did not follow through (we only have anecdotal evidence for the latter). These are well-known hurdles to partner notification, especially in understaffed and underresourced clinics. Partner notification results might be improved by improving index case privacy and confidentiality (e.g. mobile phone or internet-based notification with the option to notify anonymously),40 or by improving convenience (e.g. providing the index case with multiple treatment courses, and asking her/him to deliver these to relevant partners).41 We did not investigate these options in the WISH study.

**Steps undertaken to optimise the BV algorithm**

We first determined the optimal vaginal pH cut-off for diagnosing BV in both symptomatic (defined as structurally reporting genital itching/burning, any unusual vaginal discharge, lower abdominal pain and/or pain during sex) and asymptomatic women. This was pH 5·5, with similar performance in both groups (data not shown; all data indicated as not shown in this appendix will be published separately). The negative predictive value (NPV) was 92%, but the positive predictive value (PPV) was only 34%, in both groups. A confirmatory test in those with pH≥5·5 regardless of symptoms therefore seemed required to improve the PPV. We achieved the best balance between reducing BV false-positives and numbers of women requiring testing by determining vaginal pH in all women (as had previously been done), but adding a confirmatory test (the vaginal qPCR score) when pH≥5·5. This resulted in a sensitivity of 73·6% and specificity of 100% (Manuscript, table 4), and would require 275/705 (39·0%) confirmatory tests (223 if women already being treated with metronidazole for TV are subtracted). In the performance calculations of this new optimal BV algorithm, we used the vaginal qPCR score as the confirmatory test (see explanation above). However, this score may not be practical in real life because it requires three separate qPCR assays. We therefore assessed the performance of *Lactobacillus* genus concentration on its own as a confirmatory test. We found that a *Lactobacillus* concentration of <105 geq/ml only slightly reduced algorithm performance (Appendix, table 4). Finally, we determined the performance of *Lactobacillus* genus concentration done on all women (compared to the vaginal qPCR score on all women as the gold standard) and achieved a sensitivity of 78·4% and specificity of 95·0% (Appendix, table 4). Unfortunately, we did not have Nugent scores available to us (see explanation in ‘vaginal qPCR score to identify true BV cases’ above), but it is important to note that it would also be possible to screen all women for vaginal pH and use Gram stain Nugent scoring as the confirmatory test (see ‘selection of POCTs for evaluation in WISH’ for a more detailed discussion about the advantages and disadvantes of Gram stain Nugent scoring).

**Steps undertaken to optimise the VVC algorithm**

We did not conduct any tests for VVC in the WISH study but evaluated several potential ways to improve the VVC algorithm with the *C. albicans* qPCR data generated with stored swabs after completion of the WISH study. Clinicians often claim that they can recognise VVC during a speculum exam but our data show many false positives and low PPV both in the presence and absence of a speculum exam (PPV was 17·5% and 14·3%, respectively; data not shown). We also found that neither participant-reported symptoms nor clinician-observed signs were correlated with the presence of VVC or any other infection (Manuscript, table 5). We then investigated whether the presence of clinical signs and/or self-reported symptoms among women who tested qPCR positive for *C. albicans* depended on the *C. albicans* concentration. However, the median concentrations in women reporting different symptoms or exhibiting different signs were similar (ranging from 5·3 to 5·8 log10 geq/ml) with overlapping interquartile ranges (data not shown). We concluded that VVC cannot be accurately diagnosed based on symptoms and signs. Next, we assessed the relationship between vaginal pH and VVC but found a wide pH range in women with a positive qPCR for *C. albicans* with and without symptoms (data not shown). We did observe, however, that pregnant women were more likely to have VVC than BV (19·4% and 6·5%, respectively). This led to the following optimal VVC algorithm: women would only be tested for VVC if they had VVC-like symptoms and had tested negative for CT, NG, TV, and BV (using the optimal algorithm) or were pregnant (regardless of symptoms). The sensitivity, specificity, positive predictive value and negative predictive value were 59·3%, 100%, 100% and 96·3%, respectively. This is, however, a complex algorithm ‘by exclusion’ and better POCTs are therefore desirable as discussed in the main manuscript.

**Comparison of urinalysis dipstick and urine *E. coli* qPCR results**

As was mentioned in the manuscript, 161/705 (22·8%) of WISH participants were treated for an UTI because urinalysis detected any nitrite and/or leukocytes in their urine. However, only 41/161 (25·5%) of these women had a urine *E. coli* concentration of ≥105 geq/ml by qPCR, and an additional 12/161 (7·5%) had a urine *E. coli* concentration of >0 and <105 geq/ml (Appendix, table 5). We used a cut-off of ≥105 geq/ml because many clinical guidelines use a cut-off of ≥105 colony forming units in culture per ml urine for UTI diagnosis.42 As mentioned earlier, it has been estimated that about 70% of UTIs are caused by *E. coli*,28 but this 30% gap cannot fully explain the positive urinalysis results in the 108/161 (67·1%) of women without any *E. coli* in their urine. Appendix table 5 also shows that 19·8% of women without symptoms had some *E. coli* in their urine by qPCR, and that the correlation between urinalysis results and *E. coli* qPCR results in symptomatic women was generally poor (Pearson’s correlations range from -0·0423 to 0·4562; we did not have urinalysis results for asymptomatic women).

**Feasibility and acceptability**

As part of our feasibility and acceptability procedures, physicians who were not part of the day-to-day Rwandan study implementation team observed study staff and participants during monitoring visits to Rwanda. The observers were Dutch physicians with STI management and gynaecology experience. Participants to be observed were selected as follows: they had scheduled visits while the observers were in Rwanda and roughly equal proportions had a positive versus a negative CT/NG risk score (this was done because the CT/NG testing procedures took 90 min). In addition, care was taken to observe both study physicians and both study nurses roughly an equal number of times. Participants were observed from when they entered the study clinic until they left, and all of the procedures that they underwent were timed (Appendix, table 9).

In addition, we conducted client satisfaction surveys with 107 participants (Appendix, table 10). We aimed to interview about 100 women because we estimated that we would have reached data saturation of the open-ended survey questions by then. We deliberately started the client satisfaction interviews after study staff had settled into a comfortable routine with the study procedures, about six weeks into study enrolment. We selected about five participants per week, and they were selected based on the availability of a study clinician to conduct their interview: the interviewer had to be a study clinician who had not personally implemented study procedures with the interviewee.

One of the external observers also conducted interviews with all eight Rwandan study team members soon after data collection had been completed, but the information obtained during these interviews only confirmed the findings described in this manuscript based on other data sources and did not add any new insights. These data are therefore not shown.

***Appendix Table 1:* Spontaneously versus structurally reported symptoms**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Urogenital symptoms**  **n (% of 705) in each column1** | **Spontaneous**  **total** | **Structural**  **total** | **Spontaneous,**  **not structural** | **Structural,**  **not spontaneous** |
| Any reported | 575 (81·6) | 604 (85·7)2 | 0 | 29 (4·1) |
| Any unusual VDS | 247 (35·0) | 386 (54·8) | 2 (0·3) | 141 (20·0) |
| Unusual VDS, curdlike | 176 (25·0) | 265 (37·6) | 3 (0·4) | 92 (13·0) |
| Unusual VDS, offensive smell | 52 (7·4) | 119 (16·9) | 1 (0·1) | 68 (9·6) |
| Unusual VDS, other | 213 (3·0) | 264 (3·7) | 11 (1·6) | 16 (2·3) |
| Any genital itching and/or burning | 384 (54·5) | 470 (66·7) | 1 (0·1) | 87 (12·3) |
| Genital itching | 344 (48·8) | 409 (58·0) | 5 (0·7) | 70 (9·9) |
| Genital burning | 65 (9·2) | 212 (30·1) | 0 | 147 (20·9) |
| Any LAP and/or pain during sex | 167 (23·7) | 308 (43·7) | 0 | 141 (20·0) |
| LAP | 144 (20·4) | 245 (34·8) | 0 | 101 (14·3) |
| Pain during sex | 33 (4·7) | 142 (20·1) | 0 | 109 (15·5) |
| Any UTI symptoms | 179 (25·4) | 348 (49·4) | 1 (0·1) | 170 (24·1) |
| Burning when passing urine | 133 (18·9) | 262 (37·2) | 6 (0·9) | 135 (19·1) |
| Frequent urination/urge | 54 (7·7) | 176 (25·0) | 0 | 122 (17·3) |
| Blood in urine | 1 (0·1) | 5 (0·7) | 0 | 4 (0·6) |
| Other: smelly odour in urine | 2 (0·3) | 0 | 2 (0·3) | 0 |
| Ulcers/blisters/sores genital/anal | 10 (1·42) | 41 (5·8) | 0 | 31 (4·4) |
| Swelling/bubo inguinal area | 0 | 1 (0·1) | 0 | 1 (0·1) |
| Warts genital/anal | 0 | 0 | 0 | 0 |
| Postcoital/intermenstrual bleeding | 1 (0·1) | 14 (2·0) | 0 | 13 (1·8) |

LAP=lower abdominal pain. UTI=urinary tract infection. VDS=vaginal discharge syndrome.

1. May total to more than 100% because the participant could report multiple symptoms.
2. We did not assess participant-reported severity of the symptoms but 43 of the 604 participants with structurally reported symptoms (7·1%) had been seeking medical care for their symptoms and 103 (17·1%) had used traditional medications.
3. Five participants described the VDS as having a yellow color; two as “pus-like”; and 14 as unusual without specifying further.
4. 18 participants described the VDS as having a yellow color; three as “pus-like”; two as “chocolate-like”; and three as unusual without specifying further.

***Appendix Table 2:* Gold standard infections correlation matrix**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Outcome  n (r)1  (N=690) | HIV2 | CT | NG | TV | BV | VVC | Syphilis3 | MG |
| HIV2 | 162 | 7  (-0·08) | 24  (0·16) | 39  (0·12) | 31  (0·01) | 16  (0·03) | 14  (0·18) | 11  (0·09) |
| CT | 7  (-0·08) | 58 | 10  (0·12) | 13  (0·05) | 14  (0·05) | 3  (-0·04) | 0  (-0·05) | 4  (0·05) |
| NG | 24  (0·16) | 10  (0·12) | 50 | 15  (0·11) | 8  (-0·01) | 4  (0) | 5  (0·11) | 4  (0·06) |
| TV | 39  (0·12) | 13  (0·05) | 15  (0·11) | 111 | 27  (0·07) | 7  (-0·04) | 7  (0·08) | 7  (0·06) |
| BV | 31  (0·01) | 14  (0·05) | 8  (-0·01) | 27  (0·07) | 125 | 9  (-0·03) | 5  (0·03) | 8  (0·07) |
| VVC | 16  (0·03) | 3  (-0·04) | 4  (0) | 7  (-0·04) | 9  (-0·03) | 59 | 1  (-0·02) | 3  (0·02) |
| Syphilis3 | 14  (0·18) | 0  (-0·05) | 5  (0·11) | 7  (0·08) | 5  (0·03) | 1  (-0·02) | 21 | 0  (-0·04) |
| MG | 11  (0·09) | 4  (0·05) | 4  (0·06) | 7  (0·06) | 8  (0·07) | 3  (0·02) | 0  (-0·04) | 26 |

BV=bacterial vaginosis. CT=*Chlamydia trachomatis.* MG=*Mycoplasma genitalium*.NG=*Neisseria gonorrhoeae.* TV=*Trichomonas vaginalis.* VVC=vulvovaginal candidiasis.

1. Pearson correlation coefficient
2. Either newly diagnosed, known infection confirmed, or known infection not tested in the WISH study but reported by participant.
3. Syphilis by WISH procedures: women who were positive for the syphilis risk score were tested by Syphilis Determine assay (with confirmation of active infection by rapid plasma reagin if needed). Women negative for the risk score were considered negative for syphilis.

***Appendix Table 3:* Risk scoring results**

|  |  |
| --- | --- |
| CT/NG risk score | n (% of 705) |
| Currently pregnant | 62 (8·8) |
| Exchanged sex for money or goods in the past 12 months1 | 250 (35·5) |
| New sex partner in the past three months1 | 224 (31·8) |
| Abnormal cervicovaginal discharge during speculum and/or cervical motion/adnexal tenderness during bimanual examination (if speculum/bimanual examination not done, the answer was no)2 | 80 (11·4) |
| Final CT/NG risk score positive3 | 396 (56·2) |
| Syphilis risk score | **n (% of 705)** |
| Currently pregnant | 62 (8·8) |
| Exchanged sex for money or goods in the past 12 months1 | 250 (35·5) |
| New sex partner in the past three months1 | 224 (31·8) |
| Genital ulcers/blisters/sores visible during speculum examination (if not done, the answer was no)4 | 40 (5·7) |
| Final syphilis risk score positive3 | 378 (53·6) |

CT=*Chlamydia trachomatis*. NG=*Neisseria gonorrhoeae*.

1. 157 women reported both sex work and a new sex partner, 93 women reported sex work only, and 67 women reported a new sex partner only. Therefore, the risk in our population was predominanty via sex work.
2. 75 of these 80 women reported VDS or LAP during structural questioning. However, 376 and 239 women in total reported VDS and/or LAP, respectively; the risk score would not have been feasible if we had included all women reporting VDS and/or LAP symptoms.
3. The risk score was positive if at least one of the four criteria was positive.
4. 16 of these 40 women reported GUD during structural questioning. In total, 25 women reported GUD but did not actually have any upon speculum examination, and 24 women did not report any but turned out to have them during a speculum examination that was done for another reason. Therefore, GUD self-reporting is unreliable.

***Appendix Table 4*: Performance of WISH and optimal BV/VVC algorithms compared to gold standard testing among women who sought care**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Women who sought care (N=141)4 | GS1 | | WISH (CT, NG, TV)1 and optimal (BV, VVC)2 algorithms in women who sought care3 | | | | | | | | | |
| **Neg**  **n** | **Pos**  **n** | **n POC**5  **tested** | **n GS**5  **tested** | **TP**  **n** | **FP**  **n** | **FN**  **n** | **TN**  **n** | **Sens**  **% (95% CI)** | **Spec**  **% (95% CI)** | **PPV**  **% (95% CI)** | **NPV**  **% (95% CI)** |
| CT | 131 | 10 | 74 | 0 | 6 | 0 | 4 | 131 | 60·0  (28·1-85·2) | 100  (100-100) | 100  (100-100) | 97·0  (92·3-98·9) |
| NG | 124 | 17 | 74 | 0 | 10 | 0 | 7 | 124 | 58·8  (34·4-79·6) | 100  (100-100) | 100  (100-100) | 94·7  (89·1-97·5) |
| CT and/or NG | 118 | 23 | 74 | 0 | 14 | 0 | 9 | 118 | 60·9  (39·6-78·7) | 100  (100-100) | 100  (100-100) | 92·9  (86·8-96·3) |
| TV | 127 | 12 | 139 | 0 | 7 | 0 | 5 | 127 | 58·3  (29·5-82·4) | 100  (100-100) | 100  (100-100) | 96·2  (91·1-98·4) |
| BV | 110 | 29 | 139 | 56 | 21 | 0 | 8 | 119 | 72·4  (53·2-85·8) | 100  (100-100) | 100  (100-100) | 93·2  (86·9-96·6) |
| BV and/or TV | 103 | 36 | 139 | 49 | 23 | 0 | 13 | 103 | 63·9  (46·9-78·0) | 100  (100-100) | 100  (100-100) | 88·8  (81·5-93·4) |
| VVC | 121 | 18 | 139 | 72 | 11 | 0 | 7 | 121 | 61·1  (37·0-80·8) | 100  (100-100) | 100  (100-100) | 94·5  (88·9-97·4) |
| All women (N=690) | **GS** | | **Optimal BV/VVC algorithms but using *Lactobacillus* qPCR as confirmatory test6** | | | | | | | | | |
| BV | 565 | 125 | 690 | 275 | 72 | 19 | 53 | 546 | 57·6  (48·7-66·0) | 96·6  (94·8-97·8) | 79·1  (69·5-86·3) | 91·2  (88·6-93·2) |
| BV and/or TV | 481 | 209 | 690 | 223 | 136 | 25 | 73 | 456 | 65·1  (58·3-71·3) | 94·8  (92·4-96·5) | 84·5  (78·0-89·3) | 86·2  (83·0-88·9) |
| VVC | 631 | 59 | 690 | 281 | 37 | 0 | 22 | 631 | 62·7  (49·7-74·1) | 100  (100-100) | 100  (100-100) | 96·6  (94·9-97·8) |
| Women who sought care (N=139)4 |  | |  | | | | | | | | | |
| BV | 110 | 29 | 139 | 56 | 18 | 1 | 11 | 109 | 62·1  (43·1-77·9) | 99·1  (93·7-99·9) | 94·7  (69·1-99·3) | 90·8  (84·1-94·9) |
| BV and/or TV | 103 | 36 | 139 | 49 | 21 | 1 | 15 | 102 | 58·3  (41·5-73·4) | 99·0  (93·3-99·9) | 95·5  (72·6-99·4) | 87·2  (79·7-92·2) |
| VVC | 121 | 18 | 139 | 72 | 12 | 0 | 6 | 121 | 66·7  (42·0-84·7) | 100  (100-100) | 100  (100-100) | 95·3  (89·8-97·9) |
| All women (N=690) | ***Lactobacillus* qPCR on all women to diagnose BV (with <105 geq/ml treated for BV)** | | | | | | | | | | | |
| BV | 565 | 125 | 690 | 0 | 98 | 28 | 27 | 537 | 78·4  (70·3-84·8) | 95·0  (92·9-96·6) | 77·8  (69·6-84·2) | 95·2  (93·1-96·7) |

BV=bacterial vaginosis. CI=confidence interval. CT=*Chlamydia trachomatis*. FN=false negative. FP=false positive. GS=gold standard. NG=*Neisseria gonorrhoeae*. Neg=negative. NPV=negative predictive value. POC=point of care. Pos=positive. PPV=positive predictive value. Sens=sensitivity. Spec=specificity. TN=true negative. TP=true positive. TV=*Trichomonas vaginalis*. VDS=vaginal discharge syndrome. VVC=vulvovaginal candidiasis.

1. See manuscript table 1 for definitions. Performance statistics were also calculated for CT, NG combined, and BV and TV combined, because the former are assessed by one assay and the latter require the same treatment.
2. All women would have a vaginal pH determined and those with pH ≥5·5 would also have a vaginal qPCR score done (see methods). Only women with pH ≥5·5 and a positive vaginal qPCR score would be treated for BV. Women would only be tested for VVC if they had VVC-like symptoms and had tested negative for CT, NG, TV, and BV (by optimal algorithm), or were pregnant (regardless of symptoms).
3. Defined as women who had visited a clinic and/or women who had taken traditional medications for symptoms reported to be current or recent (last two weeks).
4. Performance measures are compared to gold standard testing. For TV, BV, and VVC, N=139 due to 2 invalid PCR results.
5. These are the numbers that would require POCT and gold standard testing if each respective algorithm were to be implemented in a real-life situation.
6. Same as 2, but instead of using the vaginal qPCR score as the confirmatory test after vaginal pH ≥5·5, we used the *Lactobacillus* qPCR only (with <105 geq/ml treated for BV). This same definition of BV was used in the VVC algorithm. The vaginal qPCR score on everyone was used as the gold standard BV result.

***Appendix Table 5:* Urinalysis compared to *Escherichia coli* qPCR test results**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| All women with urine samples (N=641)1 | Total n | E*. coli* qPCR2 | | | OR(95% CI)4  Chi-squared p |
| **0 geq/ml**  **n (%)3** | **>0 to <105 geq/ml**  **n (%)3** | **≥105 geq/ml**  **n (%)3** |
| With UTI symptoms | 363 | 262 (72·2) | 32 (8·8) | 69 (19·0) | 2·67 (1·60-4·45)  p<0·0001 |
| Without symptoms | 278 | 223 (80·2) | 33 (11·9) | 22 (7·9) |
| Women reporting UTI symptoms only (N=363)1 | **Total n** | **E*. coli* qPCR2** | | | **Correlation r5**  **(95% CI)** |
| **0 geq/ml**  **n (%)3** | **>0 to <105 geq/ml**  **n (%)3** | **≥105 geq/ml**  **n (%)3** |
| ≥1+ leukocytes and/or nitrite-positive6 | 161 | 108 (67·1) | 12 (7·5) | 41 (25·5) | NA |
| Nitrite negative, 0 leukocytes | 202 | 154 (76·2) | 20 (9·9) | 28 (13·9) | -0·042  (-0·149-0·066) |
| Nitrite negative, 1+ leukocytes | 64 | 52 (81·3) | 6 (9·4) | 6 (9·4) |
| Nitrite negative, 2+ leukocytes | 46 | 36 (78·3) | 5 (10·9) | 5 (10·9) |
| Nitrite negative, 3+ leukocytes | 21 | 17 (80·9) | 1 (4·8) | 3 (14·3) |
| Nitrite positive, 0 leukocytes | 1 | 1 (100) | 0 | 0 | 0·186  (-0·187-0·512) |
| Nitrite positive, 1+ leukocytes | 6 | 0 | 0 | 6 (100) |
| Nitrite positive, 2+ leukocytes | 14 | 2 (14·3) | 0 | 12 (85·7) |
| Nitrite positive, 3+ leukocytes | 9 | 0 | 0 | 9 (100) |

CI=confidence interval. Geq=genome-equivalent units. ml=milliliter. NA=not applicable. OR=odds ratio. qPCR=quantitative polymerase chain reaction. UTI=urinary tract infection.

1. 363/641 women underwent urinalysis testing after reporting UTI-related symptoms. Urine was collected from the other 278/641 women for pregnancy testing, but these women did not undergo urinalysis testing because they did not report symptoms. All 641 urine samples were tested for *E. coli* concentration by qPCR.
2. *E. coli* qPCR concentration levels, devided into three categories: 0 geq/ml, >0 and <105 geq/ml, and **≥**105 geq/ml.
3. Listed percentages are row percentages.
4. Chi-squared test for trend. The OR compares *E. coli* qPCR ≥105 geq/ml with 0 geq/ml in women with and without UTI symptoms.
5. Pearson’s correlation of leukocyte level as an ordinal variable with *E. coli* concentration by qPCR in log10 geq/ml as a continuous variable. The overall correlation of the 8 nitrite/leukocyte level rows as one ordinal variable with *E. coli* concentration is r=0·4562 (95% CI 0·371-0·534).
6. This is the algorithm that was used in the WISH study. All 161 women who were positive for this algorithm were treated for a UTI.

***Appendix Table 6:* Clinical findings and actions by study clinicians**

|  |  |
| --- | --- |
| **Speculum/bimanual exam results at main visits (study physicians)** | **n (% of 399)** |
| Any abnormalities observed during speculum exam | 216 (54·1) |
| Abnormalities1:  - Enlarged/tender inguinal lymph nodes  - Abnormal (genital) odour  - Warts or condylomata (any location genitalia)  - Ulcers/blisters/sores suggestive of STI in vulva  - Vulvitis  - Any other lesion on vulva  - Vaginal mass (polyp, myoma, etc.)  - Ulcers/blisters/sores suggestive of STI in vagina  - Vaginitis  - Any other lesion on vaginal epithelium  - Cervicitis  - Any other lesion on cervical epithelium  - Abnormal vaginal or cervical discharge/pus  - Other3 | 0  34 (8·5)  5 (1·3)2  22 (5·5)  21 (5·3)  0  4 (1·0)  3 (0·8)  39 (9·8)  0  42 (10·5)  1 (0·2)  138 (34·6)  14 (3·2) |
| Any abnormalities observed during bimanual exam | 41 (10·3) |
| Abnormalities1:  - Any uterine or adnexal mass  - Any uterine, adnexal or cervical motion tenderness  - Other4 | 4 (9·8)  31 (4·4)  12 (29·3) |
| **Syndromic diagnoses within WISH algorithms at main visits (study physicians)** | **n (% of 705)** |
| None  VVC  VDS – not VVC, tested negative for BV/TV/CT/NG  LAP (with or without VDS) - no tenderness during bimanual  LAP (with or without VDS) - tenderness during bimanual (=PID)  Suspected PID for other reasons5  GUD with or without inguinal buboes - tested negative for syphilis  Inguinal buboes without GUD  Genital warts/condylomata  UTI symptoms testing negative for UTI  Other: cervical tumor/mass | 262 (37·2)  238 (33·8)  12 (1·7)  204 (28·9)  29 (4·1)  3 (0·4)  16 (2·3)  0  3 (0·4)  134 (19·0)  1 (0·1) |
| **Delivery of positive POCT results (study physicians or nurses)6** | **n (% of 705)** |
| Had no positive results  Received all her positive results at main visit; no pending results when leaving  Received all her positive results at main visit; all pending results negative and received by phone/texts  Received all her positive results at main visit; all pending results negative and received at additional visit  A pending result came back positive and received result and/or treatment at additional visit  A pending result came back positive and received result by phone/text1  Received all her positive results at an additional visit  Received all positive results by phone/text | 164 (23·3)  505 (71·6)  26 (3·7)  1 (0·1)  7 (1·0)  1 (0·1)  1 (0·1)  0 |
| **Treatment failures (study physicians)7** | **n (% of 705)** |
| Had at least one suspected ongoing untreated infection | 4 (0·6) |
| Had at least one suspected re-infection after having received appropriate treatment | 2 (0·3) |
| Had at least one suspected treatment failure | 4 (0·6) |
| **Active referrals made (study physicians)** | **n (% of 705)** |
| No referrals needed  Because of new HIV diagnosis  Because of new pregnancy  For further gynecological evaluation/treatment  Because of wish to start/change family planning method  Other: For other medical specialist diagnosis & treatment  Other: For starting antiretroviral therapy of previously known HIV infection  At least one referral offered but declined | 626 (88·8)  34 (4·8)  25 (3·6)  18 (2·6)  0  1 (0·1)  4 (0·6)  2 (0·3) |
| **Partner notification and treatment (study physicians or nurses)** | **n (% of 705)** |
| Had at least one partner requiring notification during the study8 | 201 (28·5) |
| Total number of partners requiring notification during the study | 782 |
| Median number of partners requiring notification per woman with an infection [IQR] | 2 [1-3] |
| Total numbers of partners that the women consented to being notified | 238 |
| Number of women who agreed to:   * + All of the identified partners being notified   + Some of the identified partners being notified   + None of the identified partners being notified | 111 (15·7)  59 (8·4)  31 (4·4) |
| Number of women who chose to:   * + Notify all partners herself   + Have RU staff notify all partners (in agreed-upon manner)   + Notify some partners herself and some by RU staff   + Other: Notification by RU staff with both partners present | 150 (21·3)  13 (1·8)  2 (0·3)  2 (0·3) |
| Total number of eligible partners treated (% out of the total number of partners identified) | 61 (7·8) |

BV=bacterial vaginosis. CT=*Chlamydia trachomatis*. GUD=genital ulcer disease. LAP=lower abdominal pain. NG=*Neisseria gonorrhoeae*. PID=pelvic inflammatory disease. POCT=point-of-care test. TV=*Trichomonas vaginalis*. UTI=urinary tract infection. VDS=vaginal discharge syndrome. VVC=vulvovaginal candidiasis.

1. May total to more than 100% because one woman could have multiple signs/diagnoses.
2. Two of these cases were a small number of warts that the study physician found difficult to reliably differentiate from ulcers. These participants were not treated for warts.
3. Includes menstrual blood from cervical os (8), Bartholin’s cyst (1), dry skin at external genitalia (1), uterine prolapse (1), acne due to shaving (1), depigmentation of the vulva (1), and satellite lesions suggestive of VVC (1).
4. Includes hard, indured cervix (4), large volume of uterus consistent with early pregnancy(2), abnormal pain during exam (5), and absence of cervix consistent with hysterectomy (1).
5. Two participants underwent a bimanual exam for reasons other than LAP, had tenderness, and were treated for PID. One participant reported severe LAP in the past two weeks but not during the main visit, and did not have tenderness during the bimanual exam. However, her CT and NG results both came back positive, and the study physician decided to treat her for PID. Her LAP disappeared after completion of the treatment.
6. GeneXpert CT/NG counts as one result and was considered positive if the test was positive for at least one organism.
7. Treatment was in accordance with the Rwanda national treatment guidelines at the time of the study (see above). Drugs dispensed included metronidazole (BV, TV, PID), tinidazole (BV), fluconazole (VVC), clotrimazole (VVC), ciprofloxacin (NG, PID, UTI), ceftriaxone (NG), doxycycline (CT), erythromycin (CT, PID), benzyl penicillin (syphilis), acyclovir (HSV-2), pivmecillinam (UTI), and amoxicillin/clavulanic acid (UTI).
8. Based on both POCT and gold standard testing results.

***Appendix Table 7:* WISH services offered and accepted**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Services | Offered  n (% 0f 705) | Accepted  (% offered) | Reasons for declining1 | Sample taken  (% offered) | Received result same day | Received tx or referral same day |
| HIV test | 7002 (99·3%) | Yes:  593 (84·7%)  No:  107 (15·3%) | - Known HIV+: 106  - Other, was tested few months ago: 1  - Missing: 0 | - EDTA blood: 591 (99·7%)  - Fingerstick: 2 (0·3%)  - Missing: 0 | - Yes: 590  - No, other time: 3  - Not at all: 0  - Missing: 0  **55 positive** | - Yes, newly diagnosed: 31  - Yes, known infection3: 3  - No, other time: 0  - Not at all4: 21  - Missing: 0 |
| Pregnancy test | 7025 (99·6%) | Yes:  583 (83·0%)  No:  119 (17·0%) | - Known pregnant: 26  - Uses reliable contraception: 52  - Other6: 38  - Missing6: 3 | * + Urine: 581 (99·7%)   + Missing: 2 (0·3%) | **-** Yes (offered): 579  **-** Yes (mistake)7: 2  - No, other time: 1  - Not at all: 0  - Missing: 3  **33 positive** | - Yes: 24  - No, other time: 0  - Not at all8: 9  - Missing: 0 |
| UTI test | 352 (49·9%) | All | NA | * + Urine: 351 (99·7%)   + Missing9: 1 (0·3%) | - Yes (offered): 351  - Yes (mistake)10: 12  - No, other time: 0  - Not at all: 0  - Missing9: 1  **161 positive** | - Yes: 160  - No, other time: 1  - Not at all: 0  - Missing: 0 |
| Vaginal pH for BV | 705 (100%) | All | NA | - pH swab: 705 (100%)  - NA: 0  - Missing: 0 | - Yes: 705  - No, other time: 0  - Not at all: 0  - NA: 0  - Missing: 0  **466 positive** | - Yes: 464  - No, other time: 1  - Not at all: 0  - Missing11: 1 |
| TV test | 705 (100%) | All | NA | * + Kit swab: 705 (100%)   + NA: 0   + Missing: 0 | - Yes: 703  - No, other time: 2  - Not at all: 0  - Missing: 0  **92 positive** | - Yes: 92  - No, other time: 0  - Not at all: 0  - Missing: 0 |
| CT/NG test12 | 396 (56·2%) | All | NA | - Kit swab: 396 (100%)  - Urine13: 1 (0·3%)  - Missing: 0 | - Yes: 354  - No, other time: 42  - Not at all: 0  - Missing: 0  **75 positive14** | - Yes: 66  - No, other time: 9  - Not at all: 0  - Missing: 0 |
| Syphilis test | 378 (53·6%) | All | NA | - EDTA blood: 377 (99·7%)  - Fingerstick15: 2 (0·5%)  - Missing: 0 | - Yes (offered): 375  - Yes (mistake)16: 1  - No, other time: 2  - Not at all: 0  - Missing17: 1  **21 positive** | - Yes: 21  - No, other time: 0  - Not at all: 0  - Missing: 0 |
| Services | **Offered**  **n (% 705)** | **Accepted**  **(% offered)** | **Reasons for**  **declining1** | | **Done the same day?** | |
| Speculum/ bimanual examination | 397 (56·3%) | Yes:  395 (99·5%)  No18:  2 (0·5%) | - Refused out of fear: 1  - Does not tolerate speculum: 1 | | - Yes: 391  - Yes, but not offered19: 8  - No, other time: 0  - Missing20: 4 | |
| Counselling | 705 (100%) | All | NA | | All | |
| Male condoms (MCs) | 705 (100%) | Yes:  386 (54·8%)  No:  319 (45·2%) | - Never uses MCs: 247  - Still has MCs at home: 35  - Partner(s) refuse(s) MCs: 20  - Partner(s) bring(s) MCs: 10  - Wants to get pregnant: 1  - Missing: 9 | | All | |
| Willing to wait for CT/NG results (women whose risk score was positive and who did not opt-out of testing) | | | *n=396*  - Yes, wanted to wait for the results: 344 (86·9%)  - No, wanted to come back for results later: 5 (1·3%)  - No, wanted to receive results by text/phone/letter: 41 (10·4%)  - Missing: 6 (1·5%)  - Never received results: 0 | | | |

ART=antiretroviral treatment. BV=bacterial vaginosis. CT=*Chlamydia trachomatis*. NA=not applicable. NG=*Neisseria gonorrhoeae*. PID=patient identification number. TV=*Trichomonas vaginalis*. UTI=urinary tract infection.

1. May total to more than 100% because multiple answers possible.
2. Five women were not offered HIV testing because they were known HIV-positive.
3. These participants were known HIV-positive but had not yet been referred for ART.
4. These participants were known HIV-positive and were in care, with some already receiving ART and others not yet. At the time of the WISH study, the Rwandan government was still rolling out the latest WHO recommendations of starting all HIV-positive people on ART regardless of CD4 count. These women opted in for HIV testing because they wanted to be retested.
5. Three women were not offered a pregnancy test because they were visibly pregnant.
6. Other reasons included: is sterilised or menopausal (12), states that last sexual encounter was a long time ago (7), had menses recently (4), believes that she cannot get pregnant due to having given birth recently (1), recently had a pregnancy test (1). Two women who opted out refused to give a reason. All three women with missing reasons used an IUD.
7. Two women who had opted out of a pregnancy test were mistakenly tested anyway.
8. Seven women were already in antenatal care. Two women did not want to be referred.
9. One woman was offered a urinalysis test but the urine sample was not taken.
10. 12 women had a urine sample taken for a UTI test despite not being offered a UTI test. Of these, 5 were mistakenly ordered from the laboratory, and 7 were not ordered but accidentally performed (because the urine was available for pregnancy testing). 2 of the 12 women should have been offered a UTI test because they reported relevant symptoms.
11. Treatment information for one woman is missing.
12. This does not include samples taken from women who had a negative risk score for testing later on in the study. That was done to enable test performance calculations but was not part of the WISH clinical algorithms.
13. One woman had both a vaginal swab and a urine sample taken because swab testing came back invalid twice.
14. 75 women had a positive CT and/or NG result: 43 positive for CT and 38 for NG (6 were positive for both CT and NG).
15. One woman had both EDTA blood and a fingerstick sample taken for syphilis testing.
16. One woman had a syphilis test performed despite not being offered one; the laboratory performed the test by mistake on blood available for an HIV test.
17. One woman had a positive risk score and was offered syphilis testing. However, the test was not requested from the lab.
18. One woman accepted the examination initially, which was postponed because of time contraints. However, the woman opted out of the exam at the additional visit because of fear.
19. Two of these eight women requested an exam without having relevant symptoms. The other six women were symptomatic and it is likely that the study physician neglected to document that an exam was offered.
20. Four women were offered an examination but not results are reported. One of these only reported "frequent urination or urgent need to urinate" and the physician therefore likely erroneously reported that an examination was offered.

***Appendix Table 8:* Counselling topics chosen by participants**

|  |  |  |
| --- | --- | --- |
| Counsellors and counselling topics | Main visit  n (% of 705) | Additional visit  n (% of 4) |
| General counselling performed by:  - Nurse/counsellor  - Physician | 704 (99·9)  1 (0·1) | 0  4 (100) |
| Topics that were discussed during general counselling1:   * HIV basic facts * STIs basic facts * HIV & STI treatment * HIV & STI prevention * HIV & STIs: Condom use demonstration * BV and VVC basic facts * BV and VVC treatment * BV and VVC prevention * UTIs: what it is, consequences if not treated, prevention * Family planning * Domestic violence: including referrals * Other, specify: hepatitis * Other, specify: condylomata | 56 (7·9)  130 (18·4)  330 (46·8)  652 (92·5)  44 (6·2)  594 (84·3)  553 (78·4)  694 (98·4)  244 (34·6)  295 (41·8)  116 (16·5)  1 (0·1)  1 (0·1) | 1 (25·0)  4 (100)  4 (100)  3 (75·0)  1 (25·0)  1 (25·0)  0  0  1 (25·0)  0  1 (25·0)  0  0 |
| HIV post-test counselling performed:  - Nurse/counsellor  - Physician4 | *(N=5882)*  30 (5·1)  558 (94·9) | *(N=33)*  0  3 (100) |
| Topics that were discussed during HIV post-test counselling:   * Negative result for HIV test * Positive or equivocal result for HIV test | *(N=5882)*  534 (90·8)  54 (9·2) | *(N=33)*  3 (100)  0 |

BV=bacterial vaginosis. STI=sexually transmitted infection. UTI=urinary tract infection.

1. May total to more than 100% because women could choose multiple topics.
2. Numbers are lower than 705 due to women opting out of HIV testing.
3. No participants were tested for HIV during additional visits; these counselling sessions were provided to women who came with their partners for partner treatment and testing.
4. The study physicians often performed the HIV post-test counselling at the same time as giving women their other test results. This fitted better into the clinic flow and enabled the nurse/counsellors to see new patients.

***Appendix Table 9*: Timing of procedures at Main Visits**

|  |  |  |
| --- | --- | --- |
| Procedures | n | Duration  in median min (IQR) |
| Duration of procedures at reception | 14 | 5 (3-12) |
| Duration of informed consent procedures and obtaining contact details | 20 | 25·5 (21-32·5) |
| Duration of face-to-face interview | 20 | 23 (21·5-27) |
| Duration of counselling | 20 | 7 (6-9·5) |
| Duration of blood collection | 19 | 4 (3-5) |
| Duration of vaginal swab collection | 20 | 6 (5·5-7·5) |
| Duration of urine collection | 18 | 2 (2-3) |
| Duration of speculum examination | 7 | 4 (3-8) |
| Duration of bimanual examination | 6 | 2 (1-2) |
| Duration between delivering the last sample to the lab and being called for results | 20 | 95·5 (14-104) |
| Duration of diagnosing and counselling by physician | 20 | 6·5 (5-12·5) |
| Duration of treatment and partner notification procedures by physician | 18 | 3·5 (2-6) |
| Overall trajectories | **n** | **Duration** |
| Total duration spent at RU with nurse/counsellor | 20 | 82 (73-93) |
| Total duration spent at RU with physician | 20 | 14 (10-24·5) |
| Total duration spent on laboratory testing | 21 | 104 (26-115) |
| Total duration spent at RU | 20 | 222·5 (138-237·5) |
| Total duration spent at RU without research procedures1 | 20 | 182·5 (111-216) |
| Total duration without research procedures with CT/NG testing+results2 | 13 | 212 (190-219) |
| Total duration without research procedures and without CT/NG testing+results3 | 7 | 98 (78-123) |

CT=*Chlamydia trachomatis*. IQR=inter-quartile range. NG=*Neisseria gonorrhoeae*. RU=Rinda Ubuzima research clinic.

1. Duration excludes time for informed consent procedures and client satisfaction survey.
2. The participant was positive for the CT/NG risk score and elected to wait for the results.
3. The participant was either negative for the CT/NG risk score or chose not to wait for the results.

***Appendix Table 10*: Client satisfaction survey results**

|  |  |
| --- | --- |
| Questions asked in face-to-face interview | n (% of 107) |
| Agreed with the following statements:  - “I felt welcome at RU”  - “The study staff were friendly”  - “The instructions I received along the way were clear”  - “The medical services I received were of good quality”  - “The medical services I received were useful”  - “The counselling/information I received was of good quality”  - “The counselling/information I received was useful” | 107 (100)  107 (100)  107 (100)  107 (100)  107 (100)  107 (100)  107 (100) |
| Time spent at RU, estimated by the participant: median minutes (IQR) | 209 (150-251) |
| Feelings about the clinic visit duration:  - Thought it was fine  - Was bothered by it but not much  - Thought it was very long, but worth it due to all the services received  - Thought it was much too long and would not do it again | 62 (57·9)  4 (3·8)  41 (38·3)  0 |
| Comparison of experience at RU during study visit, compared to other places where HIV/STI/women’s issues-related services are given:  - Liked RU better  - All services are similar  - Liked the other services better  - Has never been to other places | 104 (97·2)  0  0  3 (2·8) |
| Reasons for preferring RU over other clinics1:  - Friendly staff / attention was paid to participants2  - High number of tests performed / more useful or better-quality tests than elsewhere3  - Thought that the counselling and information obtained were useful  - Quick and well-organized services  - Services free-of-charge  - Unclear / very general reasons given | *(N = 104)*  58 (55·8)  56 (53·8)  28 (26·9)  28 (26·9)  24 (23·0)  7 (6·7) |
| Is willing to be tested in future, even when asymptomatic | 100 (93·5) |
| Is willing to pay for services such as those offered at RU | *(N=106)*  95 (89·6) |

IQR=interquartile range. RU=Rinda Ubuzima research clinic. STIs=sexually transmitted infections.

1. May total to more than 100% because the participant could give multiple answers. The question was open-ended and categories were created during the data analysis stage.

2. Includes answers such as “the staff is friendly”, and comments about refreshments being offered to participants.

3. Includes quotes suggesting that more testing was done in WISH than at local clinics, such as “you test all the diseases”, “you take many samples”, “you test before treatment”, or “you examine deeply”.

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