SHORT REPORT

Evaluation of an enzymatic Chlamydia trachomatis point-of-care rapid assay in Rwanda: the BioChekSwab Rapid Test

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ABSTRACT

Objectives We evaluated the performance of an enzymatic point-of-care rapid test for Chlamydia trachomatis (CT) (the BioChekSwab CT Rapid Test, EnZtek Diagnostics, Rio Vista, California, USA), which detects CT’s Peptidase 123CBV enzyme and provides a result 15 min after specimen collection.

Methods Two endocervical swabs, including one BioChekSwab, per person were obtained from 137 women who participated in a reproductive health study in Rwanda. The BioChekSwab was processed according to the manufacturer’s instructions. A substrate was squirted over the swab by the study physician immediately after collection, and another reagent was released over the swab tip at arrival in the laboratory. The test was considered positive if a blue colour developed within 2 min. The other regular flocked endocervical swab was processed at the Institute of Tropical Medicine (ITM), Belgium, using a testing algorithm: Abbott RealTime CT/Neisseria gonorrhoeae (NG) assay with the confirmation of positive results by an in-house real-time PCR assay.

Results Of the 137 women, nine were CT positive by the testing algorithm. All nine positive results were missed by the BioChekSwab assay and four false-positive results were obtained. The sensitivity was therefore 0% (95% CI 0% to 33.6%) and the specificity was 96.9% (95% CI 92.2% to 99.1%).

Conclusions The BioChekSwab Rapid Test, although ISO13485 certified and Conformité Européenne (CE) labelled, lacked any sensitivity in our setting.

INTRODUCTION

Chlamydia trachomatis (CT) infection is the most prevalent bacterial sexually transmitted infection worldwide. Most women with CT have minimal or no symptoms, but if left untreated, the infection can result in complications such as pelvic inflammatory disease, ectopic pregnancy and infertility. Given the asymptomatic nature of most CT infections in women, the availability of a point-of-care rapid test could potentially improve CT case finding and reduce the risks of sequelae and onward transmission. While highly sensitive and specific nucleic acid amplification testing (NAAT) for CT is commercially available, it is usually only available in specialised laboratories. In a country as Rwanda, such specialised laboratories are scarce and only available in the capital city Kigali.

Rapid tests are commercially available too, but studies have shown suboptimal sensitivity and a high variability in specificity and ease of use of these tests.1-3 Most rapid tests detect CT-specific antigens. However, the BioChekSwab test, also known as the SELFCheck Chlamydia assay (EnZtek Diagnostics Incorporated, Rio Vista, California, USA), uses an enzyme substrate which, when hydrolysed by a CT-specific peptide hydrolase, produces fluorescence that can be read colorimetrically by adding a colour developing agent. One other test that detects the enzyme Peptidase 123A is available (the Handilab-C test, Zonda Inc, Dallas, Texas, USA), and its sensitivity was 12.5% and specificity was 93.5%.1,3

The BioChekSwab test can be stored at room temperature and provides results within 15 min after sample collection. Therefore, this assay would be ideal for use in resource-limited countries, such as Rwanda.

We assessed the sensitivity and specificity of this enzymatic rapid assay in the setting of a clinical trial in Rwanda that only included non-pregnant women aged between 18 and 35 years.

METHODS

The main study4 (the Ring Plus study; ClinicalTrials.gov NCT01796613) was conducted at Rinda Ubuza (RU) Research Center, Kigali, Rwanda, from May 2013 until March 2014 to investigate the safety and acceptability of vaginal rings that protect women from unintended pregnancy. All participants provided written informed consent.

The manufacturer donated 137 BioChekSwabs. Therefore, the BioChekSwab assay was only performed on the first 137 women who, as part of the main study, underwent a pelvic examination at baseline. Endocervical samples, including the BioChekSwab, were collected by the study physician as follows: first, vaginal fluid from the posterior fornix or vaginal wall was collected by one cotton swab and two regular flocked swabs (Copan Flock Technologies Srl, Brescia, Italy) for testing unrelated to the BioChekSwab evaluation. Second, the BioChekSwab was removed from its tube and placed into the endocervical canal. The swab was
rubbed vigorously over the endocervical wall in order to dislodge cells and allow the swab to absorb the fluid. It was then rotated 360° and processed according to the manufacturer’s instructions. Third, another regular flocked swab (Copan Flock Technologies Srl) was placed into the endocervical canal and this swab was used for molecular detection of CT and Neisseria gonorrhoeae (NG).

The BioChekSwab CT Rapid Test detects CT’s Peptidase 12.3CBV enzyme and consists of a tube that contains two types of reagents: Reagent A (peptide 0.04%—buffer pH 8.3, 74.1% and 25.86% solvent) and Reagent B (colour developer 0.62%, HCl 3.7% and distilled water 95.68%). These two reagents are localised at the two opposite ends of the tube: the red end contains Reagent A (clear fluid) and the black end contains Reagent B (yellow fluid). A movie describing how to use the BioChekSwab can be found on https://www.youtube.com/watch?v=a0Hlm4h1d0h4&feature=youtu.be.

After the physician removed the BioChekSwab from the endocervical canal, he immediately placed it into the accompanying tube and released Reagent A over the swab by breaking the ampoule. The tube was placed upright into a tube rack to enable Reagent A to run down the swab and cover the swab tip. The tube was then brought to the onsite laboratory within 30 min, where it was turned upside down. After 10 min, Reagent B was released. The tube was kept upside down to allow the swab tip to absorb Reagent B. The colour of the tip was read between 1 and 2 min: any presence of blue colour was considered a positive result. The times of specimen collection and reagent B release were documented.

The other endocervical flocked swab for molecular detection of CT/NG was eluted by adding 1.2 mL diluted phosphate buffered saline (PBS) directly onto the swab. After vortexing for 15 s, two aliquots of 550 μL were created and stored at −80°C. Samples were shipped on dry ice to the Institute of Tropical Medicine (ITM) for testing using a testing algorithm: Abbott RealTime CT/NG assay according to the manufacturer’s instructions with the confirmation of positive results for CT by an in-house real-time PCR assay. The result of this testing algorithm was considered the gold standard and the laboratory technician was blind to the results obtained by the BioChekSwab CT Rapid Test.

RESULTS

Of the 351 women who attended the baseline visit for the RingPlus study, 137 women had an additional BioChekSwab taken. All 137 women were aged between 18 and 35 years, sexually active, not using any antimicrobial medication or contraception and did not have menstrual bleeding at the time of sample collection.

All nine samples positive by gold standard NAAT were not detected by the BioChekSwab Rapid Assay (table 1). In addition, the BioChekSwab Rapid Assay generated four false-positive results. This translates into a sensitivity of 0% (95% CI 0% to 33.6%), a specificity of 96.9% (95% CI 92.2% to 99.1%), a positive predictive value of 0% (95% CI 0% to 60.2%) and a negative predictive value of 93.2% (95% CI 87.5% to 96.9%). A total of eight samples became positive after the assay cut-off reading time of 2 min, but none of these were positive by gold standard testing.

DISCUSSION

The sensitivity and specificity of the BioChekSwab test were previously determined by the manufacturer in a Hungarian Genitourinary Clinic. In that study with 197 women, the gold standard was an in-house PCR assay (Chly01_Hun performed by GenoID, Budapest, Hungary), and endocervical swabs for both tests were taken by a physician. The CT prevalence by gold standard test was 27%, and the BioChekSwab test was 98.2% sensitive (95% CI 90.1% to 100%) and 97.9% specific (95% CI 94.0% to 99.6%) (unpublished data, EnZtek Diagnostics, 2013). We could not reproduce that result and obtained a sensitivity of 0%. However, the CT prevalence of our study population was much lower at 8%. According to the manufacturer, the discrepancy between the BioChekSwab and PCR results could be explained by the fact that the former detects live organisms only, whereas the latter also detects DNA from dead bacteria. Alternatively, the manufacturer thought that the sample collection could have been suboptimal and the bacterial load on the samples was too low. However, the study physicians always took the BioChekSwab first, followed by the flocked swab for gold standard PCR testing. Furthermore, participants were excluded when they were using antimicrobial medication.

During this study, we decided to perform the secondary step at the laboratory to avoid additional waiting time at the physician office, to avoid that the result was communicated to the patient and to ensure that good documentary practices were in place. Samples were brought to the laboratory with a maximum delay of 30 min but this does not affect the results according to the insert; on the contrary, samples can be stored for 24 h and transported at 2–30°C according to the insert.

The BioChekSwab Rapid Test is a Conformité Européenne (CE) labelled and ISO-13485:2003 certified medical device. These certifications do not represent the test accuracy but only the quality of medical device manufacturing. However, even though there is no proof of test accuracy, patients can buy this certified rapid test on the internet under the name: SELFCheck Female Chlamydia Test. The WHO Sexually Transmitted Diseases Diagnostics Initiative developed the ASSURED criteria for new rapid tests: Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free and Deliverable to end users. In our opinion, the BioChekSwab does not fulfil these criteria and therefore potentially misleads patients if no confirmatory test by a professional laboratory is done. False-positive test results can cause distress and false-negative test results can lead to onward transmission. Therefore, in our opinion, proof of diagnostic accuracy of tests that are available over the counter or on the internet should be mandatory.

Table 1  The results of the BioChekSwab Rapid Assay against the gold standard

<table>
<thead>
<tr>
<th>BioChekSwab</th>
<th>Gold standard</th>
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<tr>
<td></td>
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<td>Negative</td>
<td>Total</td>
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</tr>
<tr>
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</tr>
<tr>
<td>Total</td>
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<td>137</td>
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</table>

False positive and false negative samples are indicated in bold.

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Contributors All authors were involved in the main study that generated the data. For the present short report, IDB and LM wrote the first draft of the manuscript and contributed equally. EC, TC and JvdW revised and edited the text. TC, VC, IDB created the experimental design. LM and VM performed the testing and IDB.
performed the data analysis. All authors revised and approved the present version of the manuscript.

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**Competing interests** None declared.

**Ethics approval** The BioChekSwab substudy was included in the main protocol, which was approved by the Rwandan National Ethics Committee (approval number 481/RNEC/2013) and the ethics committees of the Institute of Tropical Medicine (ITM) in Antwerp, Belgium (approval number 864/13), the University Teaching Hospital in Antwerp, Belgium, (approval number 13/7/85) and the University of Liverpool in Liverpool, UK (approval number RETG000639IREC).

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**Data sharing statement** The database relevant to the study has been made available to all collaborators.

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