Clinical Evaluation of the OneStep Gonorrhea RapiCard InstaTest for Detection of Neisseria gonorrhoeae in Symptomatic Patients from KwaZulu-Natal, South Africa

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We evaluated a point-of-care test for the detection of Neisseria gonorrhoeae in patients attending a public health clinic in KwaZulu-Natal, South Africa. The test showed a low sensitivity against PCR and culture (<40%); however, a higher specificity was observed (>95%). This test is unsuitable as a screening tool for gonorrhea.

Neisseria gonorrhoeae infections represent 106 million of the estimated 498 million new cases of curable sexually transmitted infections (STIs) that occur globally annually (1). N. gonorrhoeae infections are managed using the syndromic approach in many parts of sub-Saharan Africa (2), including South Africa (3). Given the concern regarding increased antimicrobial use in syndromic management, accurate diagnostic tests are urgently needed to ensure directed treatment for detected pathogens only (4). Point-of-care tests (POCTs) for N. gonorrhoeae have sensitivities and specificities ranging from 60% to 98% and 90% to 98%, respectively (5). These POCTs are immunochromatographic and thus require minimal laboratory facilities (6). The OneStep Gonorrhea RapiCard InstaTest utilizes monoclonal and polyclonal antibodies against N. gonorrhoeae antigen, which is secreted into the genital secretions in patients with gonorrhea. The current literature on the test is restricted to the company’s website (http://www.rapidtest.com/Gonorrhoea_176512.pdf C-DOGRIpi). To the best of our knowledge, this is the first study to evaluate this POCT. The objective of this study is to evaluate the diagnostic performance of the OneStep Gonorrhea RapiCard InstaTest compared with that of culture and a strand-displacement amplification (SDA) assay for the detection of N. gonorrhoeae in symptomatic men and women.

Study population. Patients presenting with vaginal discharge syndrome (VDS) or male urethritis syndrome (MUS) at a primary health care clinic in KwaZulu-Natal, South Africa, between 1 and 31 July 2014 were invited to participate in this study. The study was approved by the biomedical ethics committee at the University of KwaZulu-Natal (BE220/13). Informed consent was obtained from patients before they were enrolled in the study. A total of 138 patients were enrolled in the study: 86 women and 52 men. Endocervical and urethral specimens were collected by a professional nurse, and patients were treated syndromically for VDS and MUS.

Specimen collection, transport, and processing. From each participant, 3 sterile Dacron swabs were collected to conduct the respective tests/assays. Upon collection, the swab for the rapid test was immersed and rotated against the wall of a tube that contained an aliquot of extraction buffer, provided with the kit, and then discarded. The sample was hand mixed by gentle agitation. The sample was then placed on ice for transport to the laboratory for further processing.

The second swab collected for culture was rolled onto a New York City (NYC) agar plate at bedside and transported in a candle extinction jar to the laboratory. The third swab collected for the SDA assay was a dry swab that was placed in a transport container and transported at room temperature to the laboratory. The rapid test and culture were performed on the same day as specimen collection. The swabs collected for the SDA were stored at 4°C and batched and processed within 5 to 7 days of specimen collection. The methodologies of each of the detection tests are briefly described below.

(i) OneStep Gonorrhea RapiCard InstaTest. The swabs were tested in accordance with the manufacturer’s instructions. The N. gonorrhoeae antigen was extracted from the specimen by inserting the swab into a tube containing the extraction buffer. A second diluent was then added to the tube and gently mixed. The extracted antigen was added to the sample window containing the gonorrhoea-coated antibodies, and the results were read after 15 min.

(ii) Culture method. The specimens were inoculated onto NYC agar plates at bedside (Oxoid, United Kingdom) and incubated for 48 h at 37°C in 5% CO2. Morphologically suggestive colonies of N. gonorrhoeae were further processed for confirmation by means of Gram staining, catalase, oxidase, and glucose utilization tests.

(iii) SDA for N. gonorrhoeae only. Strand-displacement amplification (SDA) was performed on all specimens according to...
the manufacturer’s instructions. The assay was used to detect *N. gonorrhoeae* only. The processed sample was added to the priming microwell, which contained the amplification primers and other reagents necessary for amplification. After incubation, the reaction mixture was transferred to the amplification microwell, which contained two enzymes (a DNA polymerase and a restriction endonuclease) necessary for SDA. The results were reported through an algorithm as positive, negative, indeterminate, or equivocal.

**Data analysis.** Data analyses were performed in Stata version 13. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the rapid test compared to each reference test were calculated. Two-sided 95% confidence intervals were computed for all proportions, unless otherwise stated.

The prevalence of *N. gonorrhoeae* was significantly higher in men than in women (50% versus 11.6%; *P* < 0.001). Vaginal discharge (VD) is a nonspecific symptom, and its presence does not always indicate the presence of an STI (7). In addition, *Bacterial vaginosis* and *Trichomonas vaginalis* are more frequent causes than *N. gonorrhoeae* of VD in our setting (8). In male patients from 23 to 32 years old, however, the presence of urethritis is most frequently attributable to an STI, and the most frequent cause is *N. gonorrhoeae*, followed by *Chlamydia trachomatis* (9). The prevalences of *N. gonorrhoeae*, as detected by SDA and culture, were 31.3% and 27.5%, respectively. The diagnostic performances of the rapid test compared to SDA and culture were as follows: 33.3% sensitivity, 97.9% specificity, 87.5% PPV, and 77.0% NPV versus 32.4% sensitivity, 96.0% specificity, 75.0% PPV, 79.5% NPV, respectively (Table 1). A higher specificity was observed for the rapid test versus culture (96%) and SDA (97.9%).

The sensitivity of the OneStep Gonorrhea RapiCard InstaTest in female patients was significantly lower than that in male patients (Table 1). Vaginal secretions may have a dilutional effect on the specimen, thereby decreasing the concentration of microbes and hence the sensitivity of the POCT (10). However, it is possible that the test was affected by other inhibitory substances such as antibiotics. With respect to the antibiotics, the manufacturer claims that the test is unaffected by any medication being taken, and this may hold true, since we did not observe any discordancy between the POCT and reference tests for patients that were on antibiotics. The poor performance of the POCT may be related to microbial load. The kit package insert reports that for optimal results, a microbial load of $10^5$ bacterial cells is required. A microbial load of $10^5$ bacterial cells is not practical in any clinical setting and will lead to many false-negative results. Patients present with various bacterial loads, depending on host and bacterial factors.

While the sensitivity of the test may be superior in male patients, it is still too low for reliable use in the clinical setting. Our findings emphasize the need for more sensitive gender-specific

### Table 1

<table>
<thead>
<tr>
<th>Assay/culture diagnostic results</th>
<th>OneStep Gonorrhea RapiCard InstaTest diagnostic result</th>
<th>Sensitivity (% [95% CI])</th>
<th>Specificity (% [95% CI])</th>
<th>PPV (% [95% CI])</th>
<th>NPV (% [95% CI])</th>
</tr>
</thead>
<tbody>
<tr>
<td>SDA assay</td>
<td>Positive 14, Negative 28, Total 42</td>
<td>33.3 (20.4–49.4)</td>
<td>97.9 (91.9–99.5)</td>
<td>87.5 (57.0–97.4)</td>
<td>77.0 (68.6–83.7)</td>
</tr>
<tr>
<td>Culture</td>
<td>Positive 12, Negative 25, Total 37</td>
<td>32.4 (18.9–49.7)</td>
<td>96.0 (89.8–98.5)</td>
<td>75 (45.7–91.4)</td>
<td>79.5 (71.3–85.8)</td>
</tr>
<tr>
<td>Women</td>
<td>Positive 1, Negative 72, Total 73</td>
<td>7.1 (0.7–44.1)</td>
<td>100 (95.0–100)</td>
<td>100 (2.5–100)</td>
<td>84.7 (75.2–91.0)</td>
</tr>
<tr>
<td>Culture</td>
<td>Positive 0, Negative 10, Total 10</td>
<td>0 (0–30.8)</td>
<td>98.7 (90.9–99.8)</td>
<td>0 (0–0.975)</td>
<td>88.2 (79.3–93.6)</td>
</tr>
<tr>
<td>Men</td>
<td>Positive 15, Negative 24, Total 39</td>
<td>46.4 (28.2–65.7)</td>
<td>91.7 (69.8–98.1)</td>
<td>86.7 (54.6–97.2)</td>
<td>59.5 (42.4–74.5)</td>
</tr>
<tr>
<td>Culture</td>
<td>Positive 12, Negative 22, Total 24</td>
<td>44.4 (26.2–64.3)</td>
<td>88.0 (66.7–96.4)</td>
<td>80 (48.8–94.4)</td>
<td>59.5 (42.4–74.5)</td>
</tr>
</tbody>
</table>

* CI, confidence interval.

* One sided, 97.5% CI.
POCTs to be developed that are collected through noninvasive procedures for use in a developing country setting.

A limitation of this study was that no preliminary studies were conducted for comparison using pure cultures of *N. gonorrhoeae* or other *Neisseria* isolates to provide an estimate of the analytical sensitivity and specificity of this POCT. To this end, we plan to conduct a laboratory-based evaluation of this POCT using a panel of *Neisseria* isolates to provide data on sensitivity and specificity claims.

**ACKNOWLEDGMENTS**

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**REFERENCES**