A laboratory-based evaluation of the BioStar Optical ImmunoAssay point-of-care test for diagnosing *Neisseria gonorrhoeae* infection

Amanda Samarawickrama,¹ Sarah Alexander² and Catherine Ison²

¹Department of Sexual Health and HIV, Caldecot Centre, King’s College Hospital, Denmark Hill, London SE5 9RS, UK
²Sexually Transmitted Bacteria Reference Laboratory, Health Protection Agency, 61 Colindale Avenue, London NW9 5HT, UK

The development of gonococcal point-of-care tests (POCTs) has been challenging due to the relatively monomorphic nature of the *Neisseria* genus. The BioStar Optical ImmunoAssay (OIA) POCT for diagnosing *Neisseria gonorrhoeae* infection detects a specific epitope on the L7/L12 ribosomal protein, which reduces cross-reactivity with other neisseriae, and produces a highly specific test. A laboratory-based evaluation of this POCT was performed to determine its analytical sensitivity and specificity. A panel of *N. gonorrhoeae* (*n*=158) and non-gonococcal *Neisseria* (*n*=62) isolates were examined. The OIA GC POCT positively reacted with 99.4% of *N. gonorrhoeae* isolates and produced no reaction with 88.7% of non-gonococcal *Neisseria* isolates. It cross-reacted with six strains of *N. meningitidis* and one non-speciated *Neisseria* sp., but failed to produce a positive result with one isolate of *N. gonorrhoeae*. The OIA GC POCT required a bacterial suspension of ~6.4×10⁵ c.f.u. *N. gonorrhoeae* ml⁻¹ and ~6.2×10⁵ c.f.u. *N. meningitidis* ml⁻¹ to produce a reactive result. The OIA POCT detected the majority of *N. gonorrhoeae* (99.4%) isolates examined.

INTRODUCTION

Gonorrhoea is the second most common bacterial sexually transmitted infection in the UK (Health Protection Agency, 2010). In diagnosing gonorrhoea, culture remains the gold standard, but it does not provide an immediate result (Ison, 1990; Sherrard, 1996). With advances in diagnostic technology, rapid point-of-care tests (POCTs) have been developed. Their main advantage is early detection of infection, enabling immediate treatment, thereby reducing the risk of onward transmission and the development of complications. They are useful in patients at high risk of sexually transmitted infections who are unlikely to return for their test results where a single dose or immediate prescription of antibiotics could be administered, and in resource-poor settings utilizing syndromic management, and have the potential to be developed for home-testing use (Peeling, 2006). They do not require additional equipment or laboratory facilities. The BioStar Optical ImmunoAssay (OIA) gonorrhoea (GC) POCT (Inverness Medical – BioStar) is similar. This POCT detects an epitope specific to *N. gonorrhoeae* within the L7/ L12 ribosomal protein marker, and, according to the manufacturer’s instructions, this reduces the possibility of with genes from commensal and other pathogenic neisseriae, POCTs for gonorrhoea have the potential to produce high rates of false-positive results (Ison, 2006). Other disadvantages include cost and the need for numerous processing steps, making them impractical in a busy sexual health clinic (Peeling, 2006; Vickerman et al., 2005). Although the tests claim high rates of sensitivity and specificity, many have not been independently evaluated or validated against culture. In practice, sensitivity has been lower than with culture and nucleic acid amplification tests, and can vary with site or the presence of symptoms (Benzaken et al., 2006; Alary et al., 2006). However, mathematical modelling shows that POCTs for gonorrhoea can be beneficial and cost-effective in high-prevalence populations, especially when the sensitivity of the POCT is higher than the rate of patients returning for results and treatment (Vickerman et al., 2003, 2006; Gift et al., 1999).

POCTs for use by health-care workers to diagnose gonorrhoea comprise immunochromatographic strip tests which produce a visual result within 30 min. They do not require additional equipment or laboratory facilities. The BioStar Optical ImmunoAssay (OIA) gonorrhoea (GC) POCT (Inverness Medical – BioStar) is similar. This POCT detects an epitope specific to *N. gonorrhoeae* within the L7/ L12 ribosomal protein marker, and, according to the manufacturer’s instructions, this reduces the possibility of

Abbreviations: OIA, Optical ImmunoAssay; POCT, point-of-care test.
cross-reactivity with other neisseriae, and produces a highly specific test (Inverness Medical – BioStar, 2006). This POCT test is approved for use to examine both cervical swabs in women and urine specimens in men. The aim of this study was to perform a laboratory-based evaluation of the OIA GC POCT to determine its analytical sensitivity and specificity.

METHODS

Specimens examined. The OIA GC POCT was tested against a panel of 220 gonococcal and non-gonococcal Neisseria isolates: N. gonorrhoeae (158), Neisseria sp. (27), Neisseria cinerea (18), Neisseria lactamica (7), Neisseria meningitidis (6), Neisseria polysaccharea (3) and Neisseria sicca/subflava (1). All isolates had previously been identified at the Sexually Transmitted Bacteria Reference Laboratory using a range of biochemical, immunological and molecular tests.

Specimen preparation and OIA POCT assay procedure. Bacterial suspensions of each of the above Neisseria species were prepared by taking a sample of the isolate from a blinded culture plate and producing a suspension in phosphate buffer solution to a final McFarland’s density of 0.5 (confirmed by a densitometer). A 30 μl volume of this suspension was then added to a 0.3 ml volume of the extraction reagent. The OIA GC POCT was challenged following the endocervical swab protocol according to the manufacturer’s instructions. Both a positive and negative control were included with each batch of testing to ensure that correct procedural technique, antigen extraction, reagent integrity and correct interpretation of end points were obtained.

Blinding of samples. To reduce operator bias, the identity of all bacterial cultures was blinded prior to testing. Cultures generating discordant results were retested a further two times and the majority result was deemed the correct result.

Organism load analysis. Any non-gonococcal Neisseria suspensions which produced false-positive results using the OIA GC POCT were subject to an organism load analysis. Each strain was compared to a gonococcal reference strain from the World Health Organization. A serial dilution of each standardized bacterial suspension was conducted (ranging from 10^{-1} to 10^{-7}), and each dilution was (i) used to challenge the OIA GC POCT and (ii) plated out on to GC Agar Base (Becton Dickinson) supplemented with 1 % Vitox (Oxoid) for colony counts to be undertaken.

Data analysis. The results of the OIA GC POCT were compared with the known identities of the Neisseria isolates. For the organism load analysis, the lowest concentration that gave viable colonies was used as the limit of detection and the test sensitivity for each strain was calculated.

RESULTS

The OIA GC POCT was evaluated against a panel of 220 Neisseria isolates, of which 72 % were N. gonorrhoeae and the remainder a variety of non-gonococcal Neisseria species. The OIA GC POCT reacted positively with 157/158 (99.4 %) N. gonorrhoeae isolates and produced no reaction with 55/62 (88.7 %) non-gonococcal Neisseria isolates (Table 1). There were seven false-positive results in total. The first was from an unspeciated Neisseria sp. isolate. Interestingly, this isolate was also confirmed as a non-pathogenic Neisseria species using biochemical and immunological methods, but did, however, also produce a false-positive result when using a commercial N. gonorrhoeae nucleic acid amplification test. The remaining six false-positive results were generated when the OIA GC POCT was challenged with N. meningitidis isolates. An organism load analysis determined that in order to produce a reactive result with a 30 μl volume of each bacterial suspension, the OIA GC POCT required a minimum concentration of ~6.4 × 10^6 c.f.u. N. gonorrhoeae ml⁻¹ and ~6.2 × 10^6 c.f.u. N. meningitidis ml⁻¹.

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<thead>
<tr>
<th>BioStar OIA GC POCT</th>
<th>Bacteriological culture identification</th>
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<tr>
<td></td>
<td>N. gonorrhoeae</td>
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</tr>
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DISCUSSION

In this study, we evaluated the analytical sensitivity and specificity of the BioStar OIA GC POCT. The results were promising, with the OIA GC POCT reacting positively with 99.4 % of N. gonorrhoeae isolates and producing no reaction with 88.7 % of non-gonococcal Neisseria isolates. Although its analytical sensitivity was high, the analytical specificity of the OIA GC POCT was lower in this study than that previously stated by the manufacturer. This is because in our study false-positive results were obtained when challenging the OIA GC POCT with isolates of N. meningitidis. Interestingly, according to the kit insert, the manufacturer tested a panel of 15 strains of N. meningitidis at a final concentration of 1 × 10^8 cells ml⁻¹ in the absence and presence of N. gonorrhoeae cells at a concentration of 8 × 10^6 cells ml⁻¹, but did not produce any false-positive or false-negative results. This concentration is actually much higher than the limit of detection we calculated from the organism load analysis where six different N. meningitidis isolates examined produced positive results using the OIA GC POCT. Whilst this is a significant finding, the true clinical relevance of this cross-reactivity with N. meningitidis has yet to be determined as N. meningitidis is rarely found in the genital tract, and, even when present, is unlikely to be present in enough numbers to cross-react with the OIA GC POCT.

Although we have performed this interesting laboratory analysis, we have not yet tested the OIA GC POCT against clinically obtained samples from male and female patients. The manufacturer of the OIA GC POCT has tested it on a total of 904 samples from 400 and 504 symptomatic male and female patients, respectively. By comparing the results
to those obtained by culture, they calculated the sensitivity of the OIA GC POCT to be 94.0% and 70.7%, and the specificity as 97.2% and 99.4% for male urine and female endocervical samples, respectively (Inverness Medical – BioStar, 2006). However, in an independent study examining endocervical swabs from female sex workers where the gold standard was culture, the sensitivity and specificity of the OIA GC POCT were determined to be 60.0% and 89.9%, respectively (Benzaken et al., 2006). Clearly more independent clinical study data examining the performance of the OIA GC POCT are warranted, particularly in light of the findings from this study where cross-reactivity with N. meningitidis has been reported.

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REFERENCES


