Performance of cobas® 4800 and m2000 real-time™ assays for detection of Chlamydia trachomatis and Neisseria gonorrhoeae in rectal and self-collected vaginal specimen

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ARTICLE INFO

Article history:
Received 8 March 2013
Received in revised form 10 June 2013
Accepted 14 June 2013
Available online 23 July 2013

Keywords:
Sexually transmitted infections
Chlamydia trachomatis
Neisseria gonorrhoeae
Diagnostic screening
PCR
Rectal swabs
Self-collected vaginal swabs

ABSTRACT

A prospective, multicenter trial was designed to compare the performance characteristics of the cobas® 4800 (Roche Diagnostics, Indianapolis, IN, USA) and m2000 real-time™ (Abbott Molecular Inc., Des Plaines, IL, USA) assays for detection of Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) in rectal and self-collected vaginal swabs. Rectal (n = 234) or self-collected vaginal swabs (n = 687) were obtained from consenting individuals visiting their general practitioners, dermatologists, gynecologists, sexually transmitted disease clinics, or family planning centers from May 2010 to February 2011. High concordance (≥96%) were observed between the cobas® 4800 and m2000 real-time™ assays for CT/NG detection in both rectal and self-collected vaginal swabs. The performance profiles confirm the usefulness of both kinds of swab types for CT and NG detection using described nucleic acid amplification tests assays. Based on this study, rectal and self-collected vaginal swabs offer a noninvasive alternative, which may improve screening for CT and NG infections.

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1. Introduction

Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) belong to the most common pathogens causing sexually transmitted infections (STIs). Infections are asymptomatic in 50% of men and 70% of women (Taylor et al., 2012). However, undetected and, therefore, untreated STIs may lead to cervicitis, pelvic inflammatory disease, ectopic pregnancy, neonatal conjunctivitis, and infertility in women and to urethritis in men (Cates and Wasserheit, 1991). Therefore, timely, sensitive, and accurate detection of CT and NG is critical to avoid chronic and potentially life-threatening complications.

Today, nucleic acid amplification tests (NAATs) are widely used for high-throughput screening of CT and NG. Previous studies have demonstrated enhanced sensitivity and specificity of NAATs compared to previous existing diagnostic test (Crotchfelt et al., 1998; Schachter et al., 1994) in detecting CT and NG in samples originating from the vagina, endocervix, and urethra (Barbosa et al., 2010; Cheng et al., 2011; Fang et al., 2008; Ford et al., 2004; Gaydos et al., 2010; LaMontagne et al., 2004; Mangold et al., 2007; Ostergaard et al., 2000).

Although rectal testing is less common, previous research suggests that many infections at this site go undetected if not properly screened (Kent et al., 2005). Studies report that anal intercourse has doubled over the past decade in the Netherlands, with an incidence reaching 15% in men and women from 12 to 25 years of age (Brugman et al., 1995; de Graaf et al., 2005; Vanwesenbeeck et al., 2006). This observation coincides with an increase in CT and NG infection rates (van den Broek et al., 2008). Moreover, up to 85% of CT and NG rectal infections are asymptomatic among men who have sex with men stressing the importance of regular screening of rectum samples (Kent et al., 2005).

Only few studies report on the validity of using rectal swab specimens for detection of CT and NG (Alexander et al., 2007, 2008; Ivens et al., 2007; Schachter et al., 2008; van der Helm et al., 2009), and manufacturers have not included it as a validated specimen type in their assay protocol. To improve screening of STI, the availability of reliable noninvasive collected specimens such as rectal swabs is important. Therefore, it is necessary to provide data to support the use
of specific NAATs for rectal swab specimens. To our knowledge, performance data are nearly lacking for both cobas® 4800 (Roche Diagnostics, Indianapolis, IN, USA) and m2000 real-time™ (Abbott Molecular Inc, Des Plaines, IL, USA) assays using rectal swabs as testing specimen. In addition, self-collected vaginal swabs provide an opportunity to enhance CT and NG detection in non-clinical settings. These non-invasive swabs could function as acceptable alternatives for endocervical specimens obtained by clinicians and improve screening rates (Graseck et al., 2011). Although there are several commercial assays available for NAA Ts, validated assays for self-collected vaginal swabs are limited (Gaydos et al., 2010; Schachter et al., 2005; Van Der Pol et al., 2012, 2013). Also, evaluations characterizing assay performance (specificity and sensitivity) of CT and NG detection in self-collected vaginal swabs are limited in the published literature (Fang et al., 2008; Gaydos et al., 2010; Masek et al., 2009; Schachter et al., 2003, 2005; Van Der Pol et al., 2013).

The aim of this study was to assess the clinical performance of both rectal and self-collected vaginal swabs for the detection of CT and NG using the Roche cobas®4800 CT/NG assay compared to the performance of these sample types using the Abbott m2000 real-time™ CT/NG assay in a prospective multicentre study.

2. Materials and methods

2.1. Specimen collection

Between May 2010 and February 2011, 687 self-collected vaginal or 234 rectal swabs were obtained from 921 individuals in duplicate from different specimen collection sites, including general practitioners, dermatologists, gynecologists, sexually transmitted disease clinics, or family planning centers in 2 Dutch cities (Groningen and Tilburg). All samples obtained were analyzed for the presence of CT and/or NG using the assays described below.

2.2. Ethics statement

All precautions were taken to de-link testing information used in this study from the identification of the patient submitting the test specimen[s]. All collected specimens used in this study got an alphanumeric identification code, which could not be linked to the identification of the patient’s name or medical history. Prior to specimen collection, protocols for sample collection procedures were approved by the medical ethical board, and informed consent was obtained from all participants.

2.2.1. Cobas® 4800 CT/NG assay

The cobas® 4800 CT/NG assay is a qualitative, multiplex in vitro NAAT designed to detect CT and/or NG from vaginal swabs and urine specimens collected from symptomatic or asymptomatic subjects. The cobas® 4800 CT/NG assay integrates automated nucleic acid isolation and polymerase chain reaction (PCR) setup and real-time PCR.

An internal control (IC) was added to all samples prior to automated sample preparation to monitor the efficiency of extraction and amplification. The IC is a combination of 2 noninfectious recombinant DNA plasmids that contained primer binding regions identical to either CT or NG genomic target sequences. DNA was automatically extracted using magnetic bead technology by the use of the cobas x 480 instrument. This automated PCR setup followed by real-time PCR amplification and detection using the cobas x 480 Analyzer. The assay detects a dual target by using primers that detect the cryptic plasmid DNA of CT and another primer set that defines a sequence of chromosomal DNA within the gene encoding the major outer membrane protein A of CT. This design allows detection of all CT strains including those having deletions in the cryptic plasmid (like the Swedish new variant [nvCT]) or those which do not carry the cryptic plasmid. The NG primers are directed against the highly conserved direct repeat region DR-9. Another set of primers is directed at a sequence variant from this region, thereby enhancing the sensitivity and eliminating cross-reactivity with other Neisseria and related bacterial species. All samples were processed according to the manufacturer’s instructions. Performance of the test in a large US clinical trial has been published (Taylor et al., 2012; Van Der Pol et al., 2012).

2.2.2. m2000 real-time™ CT/NG assay

The m2000 real-time™ CT/NG assay is an in vitro PCR assay for the detection of CT and/or NG DNA in endocervical, urethral, clinician- and patient-collected vaginal swabs and urine specimens of symptomatic patients and clinician- and patient-collected vaginal swab and urine specimens in asymptomatic patients. The Abbott multi-Collect Specimen Collection Kit was used to collect the samples. The assay detects both wild-type CT and nvCT strains using 2 primer sets targeting 2 regions of the cryptic plasmid. One primer set is specific for a highly conserved 122-bp sequence within the plasmid and the second set detects a 140-bp fragment located outside the (sometimes deleted) region in the cryptic plasmid. The NG PCR primers specifically detect a region of the Opa gene conserved in all gonococcal strains. All samples were tested using the m2000SP sample preparation robot and the m2000RT real-time PCR system according to the manufacturer’s instructions. The performance of the assay in a large US clinical trial has been published (Gaydos et al., 2010).

2.3. Target specific inhibition assay for rectal swabs

As rectal swabs are not a validated specimen type for most NAATs, including the cobas® 4800 or the m2000 real-time™ CT/NG assays, possible target-specific inhibition was investigated to rule out the possibility of false negatives and further confirm the binding specificity in rectal swab specimens. Negative rectal swab samples (n = 178) were combined with low quantities of DNA from pure cultures of CT and NG, and cycle thresholds (Ct) were subsequently measured on the cobas® 4800 system. As a reference, 10 blank samples phosphate buffered saline (PBS) were also spiked with pure CT and NG DNA and analyzed. A set of 4 runs was conducted to confirm findings.

2.4. Discrepancy analysis for rectal and vaginal swabs

Discrepancy analysis was performed with an independent PCR CT/NG test, i.e., the Presto CT/NG Real time PCR assay kit (GMT, Etten-Leur, The Netherlands). For CT and NG discrepancy analysis, DNA was extracted in duplicate from original clinical samples using the NucliSENS easyMAG (bioMérieux, Marcy l’Étoile, France). One of the duplicates was treated with proteinase K for 1 h at 56 °C prior to extraction to check for insufficient lysis. Real-time PCR targeting the cryptic plasmid for CT and targeting the Opa gene for NG was performed using LightCycler® 480 Probes Master (Roche Applied Science) and the LightCycler 480 real time PCR system System (Roche Applied Science).

2.5. True-positive and true-negative samples

All samples included were tested for CT and NG detection using cobas® 4800 and m2000 real-time assays. Samples were defined as true positive for either CT or NG if positive assay results were confirmed positive in at least 2 of the 3 assays used (cobas® 4800, m2000 real-time™, or Presto kit). Samples identified as invalid (n = 23) due to, e.g., internal control inhibition or material quality problems, were excluded from further analysis (2.5% of total included isolates).

2.6. Statistical analysis and result interpretation

Statistical analyses were performed for each type of specimen. The concordance percentage was calculated by dividing the number of
times the methods were in agreement by the number of analyses done. High concordance rates (>90%) indicate high consistency between results from both assays. The agreement (Cohen’s κ coefficient) of the cobas® 4800 assay to the m2000 real-time™ assay was calculated. Excellent agreement was defined as κ > 0.75 (Landis and Koch, 1977). After discrepancies were resolved, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Sensitivity and specificity of each specimen type were calculated against the true-positive and true-negative samples.

3. Results

3.1. Target-specific inhibition analysis of rectal swabs

Possible target-specific inhibition in this specimen type was investigated by performing a target-specific inhibition analysis. All spiked samples yielded mean Ct values with a maximum deviance of 1.4 Ct values compared to the mean Ct values obtained by the reference DNA (spike) added in blank samples (Fig. 1). These comparable values indicate that none of the rectal samples demonstrated target-specific inhibition.

3.2. Rectal swabs

Of the 234 rectal swabs initially collected, 223 rectal swabs were available for analysis after excluding invalid samples. Performance characteristics using rectal swabs are provided in Table 1. Sensitivity and specificity for the detection of CT were 87.1% and 100%, respectively, for the cobas® 4800 and were comparable to those obtained by the m2000 real-time™ (87.1% and 99.5%, respectively). For the detection of NG, the sensitivity was 100% for the m2000 real-time™ compared to 75% for the cobas® 4800, whereas the specificity was identical (100%). High concordance rates for CT and NG detection (>96%) were found comparing results obtained with the cobas® 4800 to the m2000 real-time™ assay. In addition, κ coefficients were all above 0.75, indicating excellent agreement between the 2 assays (Landis and Koch, 1977).

3.2.1. C. trachomatis

A total of 31 samples (13.9% prevalence) were identified as true positives for CT. When comparing the cobas® 4800 with the m2000 real-time™ assay, 23 samples were concordant positive, and 191 were concordant negative for CT (Table 2A). Nine specimens had discrepant results. Four of them were only positive with cobas® 4800 and 5 only positive with m2000 real-time™ assay. All but 1 appeared to be positive in the discrepant test using Presto PCR. One discrepant (Ct value of 39.1 in the m2000 real-time™) could not be confirmed with the Presto PCR and, therefore, assigned as true negative.

3.2.2. N. gonorrhoeae

For NG, 12 true-positive samples were detected among the rectal swabs evaluated (5.4% prevalence). When comparing the cobas® 4800 with the m2000 real-time™, 9 swabs were concordant positive, and 211 were concordant negative (Table 2B). Three specimens had discrepant results, all only positive with m2000 real-time™ assay. All of these discrepant specimens were also positive in the discrepant test using Presto RT-PCR assay.

3.3. Self-collected vaginal swabs

Of the 687 self-collected vaginal swabs, an additional 12 invalid samples were excluded, resulting in 675 samples available for analysis. Performance specification using self-collected vaginal swabs is provided in Table 3. High sensitivity and specificity values were obtained for detection of CT and NG in self-collected vaginal swabs using the cobas® 4800 (sensitivity of 97.1% and 100%, specificity of 99.7% and 99.9%, respectively) and the m2000 real-time™ (sensitivity of 100% for both, specificity of 99.5 and 100%, respectively). A high concordance rate (>99%) was found between results in self-collected vaginal swabs using the cobas® 4800 and m2000 real-time™ assays for detection of CT and NG.

3.3.1. C. trachomatis

A total of 70 self-collected swabs (10.4% prevalence) were true positive for CT. When comparing the cobas® 4800 with the m2000 real-time™ for detection of CT, 68 samples were considered concordant positive and 600 concordant negative (Table 4A). Only 7 samples showed discordant results between the 2 assays. Two discrepant specimens were only cobas® 4800 positive, and 5 discrepants, only m2000 real-time™ positive. Two m2000 real-time™ specimens were confirmed positive by the Presto kit. The other 5 discrepasts were not confirmed and, therefore, assigned as true negative.

3.3.2. N. gonorrhoeae

For NG, 4 true-positive samples (0.6% prevalence) were identified. Four samples were concordant positive, and 670 samples were

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Table 1

<table>
<thead>
<tr>
<th>Rectal swabs (n = 223)</th>
<th>Cobas® 4800</th>
<th>m2000 real-time™</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT</td>
<td>NG</td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>87.1</td>
<td>75.0</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>98.0</td>
<td>98.6</td>
</tr>
<tr>
<td>κ-value</td>
<td>0.92</td>
<td>0.85</td>
</tr>
<tr>
<td>Prevalence</td>
<td>CT = 13.8%</td>
<td>NG = 5.4%</td>
</tr>
<tr>
<td>Concordance</td>
<td>CT = 100%</td>
<td>NG = 99%</td>
</tr>
</tbody>
</table>

Table 2A

<table>
<thead>
<tr>
<th>Concordant/discrepant results of rectal swabs for CT.</th>
<th>m2000 real-time™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobas® 4800</td>
<td>CT</td>
</tr>
<tr>
<td>Positive</td>
<td>23</td>
</tr>
<tr>
<td>Negative</td>
<td>5^</td>
</tr>
<tr>
<td></td>
<td>28</td>
</tr>
</tbody>
</table>

^ Contains 1 true-negative sample.
concordant negative according to both the cobas® 4800 and the m2000 real-time™ (Table 4B). One sample showed a discordant result, only cobas® 4800 positive. However, this was not confirmed by the Presto kit and assigned as true negative.

4. Discussion

In this study, the agreement between the cobas® 4800 and m2000 real-time™ CT/NG assays was very high when rectal and self-collected vaginal swabs were evaluated. Thus, both systems performed equivalent for the detection of CT and NG using rectal or self-collected vaginal swabs. Based on the performance values, both tested specimens offer reliable, alternative, and noninvasive approaches to obtain samples for CT and NG detection using NAATs.

Although NAATs are the preferred approach for detecting CT and NG infections, to date, rectal swabs have not been approved for such use (Alexander et al., 2007; Schachter et al., 2008). Both of the assays performed well using rectal swabs, with comparable performance values reported before in published studies using rectal swabs for CT and NG detection in other NAATs assays (Alexander et al., 2008; Bachmann et al., 2010; Moncada et al., 2009; Schachter et al., 2008). Specifically, sensitivity and specificity of the cobas® 4800 ranged between 75 and 100%, and those obtained by the m2000 real-time™, between 87 and 100%, respectively. In addition, the discrepant samples all had high Ct values suggesting the presence of low bacterial load. As samples were obtained in duplicate, second sample could contain less bacterial load, indicating that low DNA load may account for discrepant results. Based on the novelty of using rectal swabs as a specimen type for CT and NG detection, the sample size available for analysis was small compared with other specimen types. Despite the small sample size, a high concordance rate and comparable assay specifications were observed between the 2 assays. Summarizing, these findings are in agreement with the published studies reporting on these specimen types that previously confirmed rectal swabs as a valid, feasible, and acceptable approach for sample collection (Alexander et al., 2008; Bachmann et al., 2010; Hopkins et al., 2012; Moncada et al., 2009; Schachter et al., 2008; van der Helm et al., 2009).

The primary reason why NAATs have been not approved for rectal swabs for NG detection, in particular, is the likelihood of cross-reaction with non-pathogenic Neisseria species resulting in false-positive results (Tabrizi et al., 2011; Whiley et al., 2006). However, all potential false positives were accounted for in this study by confirming discordant sample results with a third independent RT-PCR analysis. As a result, we anticipated that the occurrence rate of false-positive results would be minimal and, therefore, should not affect the overall interpretation of the study results. In this regard, Tabrizi et al. (2011) recently reported the high specificity of the cobas 4800 test for the detection of NG using a battery of non-gonococcal Neisseria isolates. In addition, as rectal swabs are still not validated clinical specimens for NAATs, possible false-negative results could occur using rectal swabs due to the presence of inhibitory substances. However, the presence of false-negative samples does not appear likely as target inhibition analysis did not reveal interference. These results confirmed true-negative result obtained in rectal swabs. Discordant results between NAATs are likely explained by other factors including the presence of low numbers of bacteria in some stool samples.

We also compared performance of these assays with self-collected vaginal swabs. Assay performance of self-collected swabs also provided excellent performance with a minimal sensitivity and specificity of 97.1 and 99.5%, respectively. These results indicate that good performance can be achieved when self-collected vaginal swabs are used for testing of CT and NG, indicating that self-collected swabs are acceptable for screening using NAATs. This finding is in consistency with previous studies (Fang et al., 2008; Gaydos et al., 2010; Schachter et al., 2003; Shafer et al., 2003).

The prevalence of CT and NG using rectal swabs (13.8 and 5.4%, respectively) was higher than those obtained for self-collected vaginal swabs (10.4 and 0.6% respectively). Comparable CT prevalence rates (~10%) of rectal and self-collected vaginal swabs were also observed in another study (van der Helm et al., 2009). As shown here, NG infections were not as prevalent in the study sample compared with CT infections, a finding that is also common in the literature (Sakem et al., 2011). A weakness of this study is that it is difficult to compare the prevalence of different specimen sources when they are not collected from the same study subject. Differences in prevalence rates can be expected due to the study population (Gaydos et al., 2011; Peters et al., 2011). However, clinical patient information is lacking because collected swabs were tested anonymously. Despite these limitations, rectal specimens ranked highest in prevalence rate for both CT and NG. This finding underlines the importance of including this specimen type for screening and the detection of CT and NG infections using described assays.

One of the strengths of this study is the inclusion of commercially-available assays that are frequently used for CT and NG detection. This level of control validates our study results and further supports the feasibility of rectal swabs and self-collected vaginal swabs for CT and NG detection.

### Table 3
Clinical performance characteristics of 2 assays for detection of CT and NG using self-collected vaginal swabs.

<table>
<thead>
<tr>
<th>Self-collected vaginal swabs (n = 675)</th>
<th>Cobas® 4800</th>
<th>m2000 real-time™</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CT</strong></td>
<td><strong>NG</strong></td>
<td><strong>CT</strong></td>
</tr>
<tr>
<td>Sensitivity (%)</td>
<td>97.1</td>
<td>100</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>99.7</td>
<td>99.9</td>
</tr>
<tr>
<td>PPV (%)</td>
<td>97.1</td>
<td>80.0</td>
</tr>
<tr>
<td>NPV (%)</td>
<td>99.7</td>
<td>100</td>
</tr>
<tr>
<td>( \kappa )-value</td>
<td>0.97</td>
<td>0.89</td>
</tr>
<tr>
<td>Prevalence</td>
<td>CT = 10.4%</td>
<td>NG = 0.6%</td>
</tr>
<tr>
<td>Concordance</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 4A
Concordant/discrepant result of self-collected vaginal swabs for CT.

<table>
<thead>
<tr>
<th>m2000 real-time™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobas® 4800</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

* Contains 5 true-negative samples.

### Table 4B
Concordant/discrepant result of self-collected vaginal swabs for NG.

<table>
<thead>
<tr>
<th>m2000 real-time™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobas® 4800</td>
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<tr>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
</tr>
<tr>
<td>Total</td>
</tr>
</tbody>
</table>

* Contains 1 true-negative sample.
NG detection. Future studies should focus on improving assay specifications in larger cohorts and on identifying more specific NG targets to reduce the level of cross-reaction (Tabrizi et al., 2011).

In conclusion, the cobas® 4800 CT/NG assay was comparable to the m2000 real-time™ CT/NG assay for CT and NG detection in analysing rectal and self-collected vaginal swabs. These specimen types offer a noninvasive, private approach to specimen collection. The implications of approving these specimen types for use in clinical practice could significantly enhance the prevention, detection, and treatment of STI infections.

Acknowledgments

This work was supported by Roche. In collaboration with the ESCMID Study Group on Molecular Diagnostics (ESGMD), Basel, Switzerland.

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