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Review of use of a new rapid real-time PCR, the Cepheid GeneXpert® (Xpert) CT/NG assay, for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*: results for patients while in a clinical setting

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Abstract

Rapid diagnostics for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* are desirable so that patients can be treated while they are still in the clinic or doctor's office. The Cepheid GeneXpert® (Xpert) CT/NG assay was US FDA-cleared in December 2012. The assay is a rapid real-time PCR nucleic acid amplified test. The cartridge-based assay detects DNA of *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. It is FDA-cleared for use in female endocervical swabs, patient-collected vaginal swabs and for female and male urine specimens from symptomatic and asymptomatic patients. It has demonstrated near-perfect sensitivity and specificity in urogenital specimens. The Xpert is a modular platform for testing samples directly from patients, which requires no hands-on manipulation from specimen loading until results are available. Results are provided in approximately 90 minutes. It has been graded by the FDA as moderately complex for Clinical Laboratory Improvement Amendments. Several publications have reported its promising use in clinical settings.

Keywords

Cepheid GeneXpert®; *Chlamydia trachomatis*; *Neisseria gonorrhoeae*; ocular samples; point of care; rapid real-time PCR; rectal samples

Expert commentary

New diagnostics for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are needed in order that infected patients can be treated while they are still in the clinic or doctor's office. Such a 'near-patient' or point-of-care assay is the Cepheid GeneXpert®

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(Xpert) CT/NG assay that was US FDA cleared in December 2012. The assay is a 90-min, real-time PCR nucleic acid amplified test. Although some experts would not call this assay a point-of-care test, it certainly could be called a 'near-patient' assay. In the report of the clinical trial data, the Xpert assay was compared with results to results from Aptima Combo2 (Gen-Probe Hologic) and Amplified DNA Assay on the BD ProbeTec assay with XTRTM Technology (Becton Dickinson) for over 1700 females and nearly 1400 males [1]. A patient-infected status was determined for each patient sample type, based on the results of the two comparison tests. Sensitivity and specificity estimates were based on the patient-infected status as the 'gold standard'. The PCR gene targets are two highly conserved, noncontiguous NG unique chromosomal targets for NG, while for CT, the target is a chromosomal CT gene target. Results of the Xpert assay also include a specimen adequacy control result and a sample processing control, which is an amplification control. The platform equipment ranges from module units that test one assay, two assays, four assays and up to 48 assays at one time.

Chlamydia

The Xpert results for chlamydia demonstrated sensitivities for endocervical, vaginal and urine samples of 97.4, 98.7 and 97.6%, respectively, in females and in male urine, the sensitivity was 97.5%. Specificity estimates were 99.6, 99.4, 99.8% in females, and 99.9% in male urine, respectively (TABLE 1) [1].

Gonorrhea

Results for the gonorrhea assay demonstrated sensitivities for endocervical, vaginal and urine samples of 100.0, 100.0 and 95.6%, respectively, in females, with specificities of 100, 99.9 and 99.9%. In male urine, the sensitivity for gonorrhea was 98.0% and the specificity was 99.9% (TABLE 2) [1]. For both CT and NG, there were no statistical differences in sensitivities for symptomatic and asymptomatic patients.

The GenXpert CT/NG assay has been carefully studied for specificity for NG [2], which is of concern because some nucleic acid amplification test assays demonstrated cross-reactivity with nonpathogenic *Neisseria* sp. The GeneXpert CT/NG was evaluated with 372 characterized gonorrhea and chlamydial bacterial strains for both sensitivity and specificity [2]. Sensitivity of 10 genome copies/reaction was obtained for both organisms. The assay was analytically highly sensitive and specific for gonorrhea. Of all the *Neisseria* sp. and respiratory commensal strains tested (n = 236), only four *Neisseria mucosa* and two *Neisseria subflava* isolates were positive for one of the two gonococcal targets. However, since the assay requires both targets to be positive, the assay correctly reported all six strains as negative for NG [2]. Thus, there were no false positives detected. Yet, perfect sensitivity was also demonstrated; of 111 gonorrhea strains tested, all were correctly identified as NG. Additionally, for CT, of 25 strains tested, representing all the known serovars, including the new variant strain of CT. The new variant strain of CT has a deletion of nucleotides in the plasmid gene and is commonly seen in Sweden. Since the Xpert target is a chromosomal gene, this plasmid gene deletion will not affect the assay sensitivity. All strains of lymphogranuloma venereum were also correctly identified as CT [2].

The Cepheid GenXpert assay has also been evaluated in ocular samples compared with the Abbott m2000 Real-time PCR CT assay and the Roche Amplicor CT assay [3], as well as for performance in field settings in Tanzania [4] and has been found to perform in an excellent manner. For the ocular samples tested for comparison to m2000 and Amplicor, the sensitivity and specificity of the GenXpert assay were both 100% [3].

One study reported Cepheid assay performance using 409 remnant rectal samples that were tested by the GenProbe Aptima Combo2 [5]. Results were compared with the original Combo2 assay and were diluted before testing by Cepheid. Using Aptima as the reference standard, the sensitivity, specificity and positive and negative predictive values of GeneXpert for the detection of CT and NG were 86, 99.2, 92.5 and 98.4% and 91.1, 100, and 98.6% respectively.

Five-year view

The GeneXpert System is a rapid, self-contained, fully automated platform [6] for CT/NG testing that demonstrated excellent sensitivities and specificities in urogenital samples from women and men. Additionally, it has the potential to be used for ocular and rectal samples [3–5]. The assay can provide results to guide treatment decisions before patients leave the clinic in many settings. Such same-day treatment may have the ability to significantly improve chlamydia and gonorrhea control efforts.

This assay has the potential to change the diagnostic protocols used in clinics, emergency departments and doctors' offices in the next few years. There are expectations that it will eventually achieve CLIA waiver, which will make it even more useful, since the assay may be able to then be performed outside the laboratory by healthcare workers. As well, the Xpert may be able to be offered in minute clinics in pharmacies and urgent care clinics. Having the patient being able to receive results of diagnostic testing and treatment if needed for chlamydia and gonorrhea has great appeal to both the clinician and the patient. However, since the amount of time a patient remains in an office or clinic can vary widely, a 90-min assay requires clinic flow adjustment, and especially if the number of patient tests is more than the number of test modules available on the platform at a given time. This assay meets many of the requirements outlined in a model which demonstrated that treating patients before they leave the clinic has the potential to prevent more pelvic inflammatory disease and also be cost effective [7].

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Papers of special note have been highlighted as:

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manuscript is important since it demonstrated the excellent specificity of the Cepheid assay for gonorrhea isolates. It did not react with over 200 nongonococcal *Neisseria* sp. and other commensal organisms.

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Key issues

- Some of the key issues for use of the Cepheid assay revolve around whether patients will wait the 90 min that is required by the assay time. This issue may be a barrier to wide adoption of the assay as a 'near-patient' or point-of-care (POC) test in doctors' offices and clinics. Potential ways to overcome such a barrier is to adjust clinic flow policies to begin the test procedure when the patient arrives to the clinic, although even with altering clinic flow, patients may not want to wait 90 min.
- Another barrier to uptake of such an assay is cost. Pricing will need to be competitive with other nucleic acid amplified test assays, as well as other rapid tests.
- At this time, the Centers for Disease Control and Prevention does not recommend the use of POC tests for chlamydia and gonorrhea, but as better tests evolve, this may change in future recommendations [8]. The literature has demonstrated generally poor performance of the use of POC tests for chlamydia in the clinic and field, thus far [9, 10].

Table 1

Xpert *Chlamydia trachomatis/Neisseria gonorrhoeae* sensitivity and specificity versus patient-infected status for *Chlamydia trachomatis*.

Sex	Specimen	Sensitivity (%)	Specificity (%)
Female	Cervical	97.4	99.6
	Vaginal	98.7	99.4
	Urine	97.6	99.9
Male	Urine	97.5	99.9

Data taken from [1].

Table 2

Xpert *Chlamydia trachomatis/Neisseria gonorrhoea* sensitivity and specificity versus patient-infected status for *Neisseria gonorrhoeae*.

Sex	Specimen	Sensitivity (%)	Specificity (%)
Female	Cervical	100	100
	Vaginal	100	99.9
	Urine	95.6	99.9
Male	Urine	98.0	99.9

Data taken from [1].