

UPDATE

Chlamydia trachomatis diagnostics

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Nucleic acid amplification (NAA) assays for the diagnosis of *Chlamydia trachomatis* infections started to appear in the peer reviewed literature about 12 years ago and during that period we have seen an incredible effort put into the development and evaluation of commercially developed NAA kits to diagnose and treat infections.

Before the nucleic acid amplification (NAA) decade laboratories were doing a commendable job using non-NAA assays, especially if you consider that before 1980 very few diagnostic laboratories were in the chlamydial diagnostics business, because of the cell culture and microscopy capabilities required. Some laboratories were performing serology for *C trachomatis*, which provided questionable clinically useful information.¹ The major diagnostic breakthrough was the commercialisation of antigen detection tests.^{2,3} The fact that false positives were generated led to the use of confirmatory testing⁴ and, in retrospect, probably identified as many true positives as culture because, in many cases, culturing was not done well in routine diagnostic laboratories. We now seem to be at a crossroads, where the exceptionally better sensitivity of NAA tests threatens the usefulness of non-NAA assays. These antigen detection tests are still being used in some settings and deserve a re-examination of their role. Recent restructuring of an older enzyme immunoassay (EIA) to have an amplified signal, using recycling enzymes, has produced a more promising EIA called IDEIA PCE.⁵ This assay now deserves further critical evaluation of its use. Also during the antigen detection era of the 1980s we saw the commercialisation of nucleic acid hybridisation (NAH) testing, when the PACE 2 assay

from GenProbe became available.⁶ This test also contributed substantially towards our understanding of the natural history of *C trachomatis* infections, but it too is being threatened with replacement by NAA assays. Why not re-examine the NAH concept before discarding the technology? Digene Corporation has constructed its chlamydia hybrid capture NAH test to incorporate signal amplification and recent evaluations have shown good sensitivity and specificity performance.^{7,8}

At the present time four companies have US FDA approved NAA assays for *C trachomatis*, usually combined with the capacity to diagnose *Neisseria gonorrhoeae* infections from the same specimen (table 1). These tests have received stringent approval for use on cervical and urethral swabs and first catch (void) urine, and are being sold worldwide. Many peer reviewed publications have appeared comparing the performance of these assays with culture,⁹ antigen detection,¹¹ and with each other.¹²⁻¹³ The most useful comparisons have used multiple specimens and tests to allow comparisons of the combination of specimen type and test to an expanded reference standard (the infected patient).^{14,15} We now are understanding that multiple testing may yield more accurate results. More studies of this type should enable us to create investigation algorithms which are more cost and patient beneficial. Costs have been addressed for testing larger numbers of specimens through pooling and reflex testing the individual specimens that were placed into a positive pool. This appears to be a cost beneficial approach when prevalence rates are low and large numbers of specimens are being processed daily.^{16,17}

Analysis of the many publications addressing comparative performance qualities of the commercial NAA tests reveals that there are not many differences in sensitivity and specificity on the

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Table 1 Selective characteristics of commercial nucleic acid amplification assays for the diagnosis of *Chlamydia trachomatis* in cervical or urethral swabs and urine

Molecular method*	Company	Assay	Maximum specimens per run	Time (hours)		Amplification control
				Hands-on	Total	
LCR	Abbott	LCx	40†	1 to 1.5	5.5 to 6.25	No
PCR	Roche	Amplicor	92	3 to 3.75	6.75 to 8	Optional
		Cobas Amplicor	44‡	2 to 2.5	5 to 6	Optional
SDA	Becton Dickinson	ProbeTec ET	46	1 to 1.25	3.1 to 3.8	Yes
TMA	Gen-Probe	Amp CT	98	2.5	5.2	No
		APTIMA Combo 2	98	1.6 to 2.25	5.3 to 6	No

*Ligase chain reaction (LCR), polymerase chain reaction (PCR), strand displacement amplification (SDA), transcription mediated amplification (TMA).

†A full amplification run of 40 specimens will require 2 detection runs on the LCx analyser.

‡An amplification run of 44 would require 2 sets of rings to be placed into the instrument at staggered time intervals.

specimens for which they are approved.¹⁸ From the limited number of studies published, all of the NAA tests seem to be impacted by inhibitors in certain types of clinical specimens. We know that these inhibitors are not always the same and vary in prevalence according to the amplification technology. We have learned that these amplification inhibitors may account for sensitivities below 100% and that some of them disappear by heat, freezing and thawing, dilution, and time.¹⁹ More studies are needed examining the impact of these manoeuvres on diagnosing and treating infections. Critical evaluation of the reporting of inhibitors of NAA tests requires the use of a *C trachomatis* spike into the clinical specimen before and after extraction and testing. The spike must also be at a strength near the cut-off of each test because if it is too strong no inhibition will ever be seen and if it is too weak it may be lost in the processing and too many specimens will appear to be inhibitory. At the present time we do not have an appreciation for the impact of the extraction process on the relative proportions of inhibitors and indigenous *C trachomatis* in a specimen going into the test.

The impact of specimen adequacy on the performance of NAA tests has been reported.²⁰ Examination of the specimen by the laboratory before testing it by NAA is a debatable procedure, as inadequate specimens require corrective follow up, requiring patient revisits and a new collection; this can be difficult and unproductive. A simple rapid test for specimen adequacy in the hands of the specimen collector might be an initiative worth developing and evaluating.

Examination of the procedures involved in the commercially available NAA tests are summarised in table 1 and reveal that all of the assays, as they are constructed, suffer from limited throughput. Technician hands-on time varies from approximately 1 hour for the Abbott LCx, and the BD ProbeTec systems, to 3–4 hours for the Roche Amplicor. The total time to get the answer is 5–8 hours for all of the tests, except for the ProbeTec which is 3–4 hours. GenProbe's Aptima Combo and TMA tests and the Abbott LCx do not have an internal control to monitor for amplification inhibitors, whereas Roche's Amplicor has one that is optional. The BD ProbeTec does have an internal control but it is not specific for measuring inhibition of the amplification of *C trachomatis* DNA (unpublished data).

Choosing one of these assays, which are all priced about the same, tends to be made on customer confidence in the company involved, as performances are similar. Lack of throughput is still a problem for high volume laboratories. The use of pooling and automated pipetting at the front end of these assays has helped,²¹ but industry needs to provide capacity for processing larger numbers of specimens while preserving non-contaminating integrity.

Because *C trachomatis* infections of the lower genital tract are commonly asymptomatic they often do not get diagnosed and treated. The result of this inefficiency in infection management has been the substantial increase in upper tract infections, especially in younger, sexually active women, leading to alarming increases in rates of pelvic inflammatory disease and the sequelae of infertility, ectopic pregnancy, and chronic pelvic pain. We now have excellent diagnostics that can be used on non-invasive specimens, such as urine,¹² and swabs from the vagina,²² vulva,²³ and introitus.²⁴ We need more studies on the usefulness of these specimens which can be self collected. We need to determine the role of inhibitors of NAA tests for these newer specimens, which in some studies appear to be more sensitive than urine.

Cost-benefit analysis studies have shown that focused screening for *C trachomatis* by testing non-invasive samples from sexually active women can be effective.^{25–26} We need more studies examining the feasibility and benefits to women of screening men.

Last, but not least, the commercialisation of diagnostics for *C trachomatis* and *N gonorrhoeae* infections has, for the most part, concentrated on the diagnosis and treatment in patients from developed countries. It is now well recognised that the burden of *C trachomatis* infections is probably even greater in the developing world.²⁷ *C trachomatis* infections have been implicated to amplify the HIV epidemic in certain settings.²⁸ The new focus should be to improve existing technology; and to reformat the best of the technologies into simple, inexpensive point of care tests so that more of the world's population can be screened and treated, and the "silent" epidemic can be brought under control worldwide.

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