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## **MHRA 04122**

***Chlamydia trachomatis***

**NAATs:**

**Review of evaluation  
literature**



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# ***Chlamydia trachomatis*** **Nucleic Acid Amplification Tests (NAATs): Review of evaluation literature**

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# Literature Review

## Summary

In this review we aim to provide a summary of the literature describing evaluations of commercially available *C. trachomatis* Nucleic Acid Amplification Tests (NAATs) and to determine the need for further evaluations.

Chlamydia NAATs are considered to be more sensitive than cell culture and other non-culture methods for the diagnosis of *Chlamydia trachomatis*. The method of discrepant analysis and type of reference standard used to determine specimen status has been shown to influence chlamydia NAAT sensitivity and specificity. In general, the sensitivities for NAATs were found to range from 80 to 100%, which is above the range obtained using culture. The specificities ranged from 93 to 100%, with many evaluations giving a specificity above 99%.

Further evaluations would benefit from all NAATs being tested on the same specimen panel and compared with the same reference standard.

**Keywords:** *Chlamydia trachomatis*, Nucleic Acid Amplification Tests, NAAT, literature review, evaluation, assessment.

## Background

Laboratory diagnosis of *Chlamydia trachomatis* was originally conducted by growth and isolation in cell culture. Culture has a specificity near 100% and a sensitivity ranging from 40 to 80% depending on the expertise of the laboratory (1). Non-culture methods such as enzyme immunoassay (EIA) and direct fluorescent-antibody (DFA) are easier to perform, although studies have shown no improvement in sensitivity when compared to culture (2). Nucleic Acid Amplification Tests (NAATs) have been shown to be more sensitive than cell culture and other non-culture methods (3).

Currently, there are mainly three commercially available NAAT platforms for *C. trachomatis* used in UK laboratories. These are the Polymerase Chain Reaction (PCR), for which Roche has two diagnostic kits, the manual Roche Amplicor PCR and the semi-automated Roche COBAS® AMPLICOR PCR; the Gen-Probe APTIMA®-Combo 2, a second-generation assay using Transcription Mediated Amplification (TMA) technology; and the Becton Dickinson ProbeTec® ET assay which uses Strand Displacement Amplification (SDA). Another assay, the RealArt/Rotor-Gene assay from Artus linked to Corbett Instrumentation has become available, but no independent publication was available during the time period of this review.

For this literature review, papers published between 1997 and 2003 and relevant to the performance of the three NAATs were analysed. From each paper the sensitivity and specificity data, as well as the testing and analysis strategy have been summarised in tables. These evaluations provide a comparison with at least two of the NAATs tested on a group of specimens (Table 1), or as an evaluation of one NAAT (Tables 2-4). The specimen panels for the evaluations consisted of urine specimens and/or cervical/urethral swabs from males and/or females.

## Discrepant analysis strategies

Different types of reference/ 'gold' standard and discrepant analysis algorithms have been used to define the sensitivity and specificity of each NAAT. The original 'gold' standard for determining the performance of each new NAAT involved comparison of the results obtained against culture, which has 100% specificity but is considered less sensitive than the NAATs.

Discrepant analysis has been used by many studies to decide the status of specimens that are NAAT test positive but culture negative by testing these samples with a third test (often DFA or another PCR using Major Outer Membrane Protein, MOMP, primers).

However, discrepant analysis has been considered by several researchers to be inadequate for evaluating new tests since analysis can be biased in the favour of the new test (4-7). In this instance only results which weaken the performance of the new test are evaluated by the third test and therefore any changes in status can only favour the new test (6). The third test often has not been fully validated or approved and is a dependent or sister test of the new test under evaluation (5).

Several evaluations have sought to improve upon this and develop new reference standards, often by comparing a new NAAT test to the results obtained by another established NAAT or by using a combination of methods. The discordant analysis strategy used in each evaluation to decide sample status and the tests sensitivity and specificity is described in each of the attached tables.

### COBAS® AMPLICOR PCR

The published data indicates that the COBAS® AMPLICOR PCR has a sensitivity between 82.5 and 98.0% and a specificity between 99.1 and 99.8% (8-13). The COBAS® AMPLICOR PCR was the only NAAT to consistently give specificities above 99%. These overall figures are irrespective of specimen type, sex and discrepant analysis strategy.

Van Doornum et al (8) tested the COBAS® AMPLICOR PCR on paired swab and urine specimens and found a sensitivity of 82.5% when using female urine, 92.8% with male urine, 96.8% with female swabs and 98.0% with males swabs. This indicates better performance when using swabs.

Goessens et al (9) examined male and female urine and used two different reference standards, culture alone and a new 'gold' standard of determining truly infected status whereby two or more kits giving a positive result indicated positive status. The results showed a slightly lower sensitivity with the new 'gold' standard compared to culture reference standard, with the COBAS® AMPLICOR PCR sensitivity changing from 95.5% to 92.7%. The specificity was 99.4% using the new 'gold' standard.

In a third study by Pasternack et al (10), the COBAS® AMPLICOR PCR gave a sensitivity of 94.0% and a specificity of 99.2% using female urine. When testing swabs and using a new standard for determining sensitivity, Shattock et al (11) found a sensitivity of 87.5% with COBAS® AMPLICOR PCR, lower than some of the urine studies.

### **Manual Roche Amplicor PCR**

From the literature review, Roche Amplicor PCR had an overall sensitivity of between 66.7 and 100% and a specificity between 97 and 100% (10;11;14-20). The Amplicor PCR showed the widest sensitivity range of the NAATs reviewed. Jensen et al (16) compared the detection of *C.trachomatis* in urine and swab samples by Roche Amplicor PCR and found a lower sensitivity with urine, 66.7% and 71.9% sensitivity with female and male urine and 93.3% and 87.5% with female and male swabs respectively. However, this study used a relatively low number of positive samples for determining sensitivity.

Black et al (14) compared Abbott LCx and Amplicor PCR using female urine and swab specimens and also found the swabs to have higher sensitivities (85.7 & 89.9%) than the urine samples (75.7 & 79.2%) using the one test reference standard for the Roche Amplicor PCR. Using the two-test reference standard the swabs still gave a higher sensitivity (75.8 & 84.0%) than the urine (74.9 & 79.5%) but there was less of a difference between the two sample types for the Roche Amplicor PCR.

A study using urine specimens, which had three types of reference standard, found sensitivities for the Roche Amplicor PCR of between 81.8 and 93.5%, higher than the previous studies (17). This study showed lower sensitivities when using culture alone as the reference standard or in combination with one NAAT than with the reference standards that used one or more NAATs. This is different to the study using COBAS® AMPLICOR PCR where the culture alone reference standard gave a higher sensitivity (9).

A further study using urine specimens found Amplicor PCR to have a sensitivity of 100% (10). When Dyck et al (15) used swab samples to compare three NAATs they found a sensitivity of 98.7% for the Roche Amplicor PCR and used a reference standard where any two tests positive indicated a true positive.

### **Gen-Probe APTIMA®-Combo 2**

During the period of this review there were just two published studies which evaluated the performance of Gen-Probe APTIMA®-Combo 2, a second generation assay (21;22). There have been a few more studies published using the first generation Amplified CT TMA assay (9;13;19;23-26).

Goessens (9) compared the performance of the Amplified CT TMA assay with COBAS® AMPLICOR PCR and Abbott LCx using urine and found a sensitivity of 85.4% for the assay. Another study of the Amplified CT TMA assay found higher sensitivities of 93.8% with female urine and 100% with female swabs (23).

Gaydos (21) evaluated the APTIMA®-Combo 2 assay and found 94.7% sensitivity for female urine and 94.2% for female swabs, thus showing good agreement between the two specimen types. Moncada (22) evaluated the APTIMA®-Combo 2 assay for testing female urine and endocervical swabs and found a higher sensitivity of 99.4% using a specimen standard, although this reduced to 92.1% sensitivity when using an infected patient standard.

### **Becton Dickinson ProbeTec® ET**

The Becton Dickinson ProbeTec® ET assay was shown in the literature to have a sensitivity between 80.5 and 95.7% and a specificity between 93.8 and 100% (15;19;27-30). The ProbeTec® ET assay had the widest specificity range of all the NAATs reviewed. Lower sensitivities for female urine were found using the ProbeTec® ET assay; female urine gave a sensitivity of 80.5% compared with 92.8% sensitivity for female swabs (29). The same study found that male urine gave a sensitivity of 93.1% and male swabs gave a sensitivity of 92.5%.

A number of studies published for the ProbeTec® ET assay have used swabs specimens. One of these studies showed sensitivities of 91.9% and specificities of 99.8% and 99.5% with vaginal and cervical swabs respectively (28); comparable to the results found in other studies. A study using female urine and cervical swabs and male urine found sensitivities of 95.6% for females and 95.5% for males (30). This study also found the ProbeTec® ET assay to have 100% specificity.

### **Future directions**

This review has shown that some evaluations could benefit from design improvements. In many studies only a small number of *C. trachomatis* positives were included for the determination of sensitivity; often less than 100. Also many studies investigate only one particular type of NAAT. Where more than one NAAT has been evaluated some have compared two types of PCR and have not compared across different technology platforms.

In planning chlamydia NAAT evaluations, sufficient number of specimens should be included to give a confident measure of sensitivity and specificity. Evaluations are also best performed by testing several of the NAAT technology platforms against the same specimen panel and against the same reference standard. Evaluations also need to take account of new assay versions. This includes comparison of the second generation Gen-Probe APTIMA®-Combo 2 with the other platforms, as well as the recently introduced RealArt/Rotor-Gene assay.

**Table 1: Evaluation of more than one NAAT**

Citation: first author & year (ref. number)	Assay(s)	Study population & numbers	Discordant analysis	Sensitivity	Specificity
Van Doornum, G.J. J. 2001 (8)	LCx Cobas <sup>1</sup>	Paired swab & urine samples <sup>2</sup> for 498 men (50 positive) & 503 women (63 positive).	The swab & urine sample from each patient was tested by both LCx & Cobas, giving 4 test results per patient. If a sample gave discordant results on both tests the sample was repeated. Patient considered CT positive if 3 of 4 test results were positive.	<u>LCx:</u> FS = 92.1%, FU = 88.9%, MS = 90.0%, MU = 94.0%. <u>Cobas:</u> FS = 96.8%, FU = 82.5%, MS = 98.0%, MU = 92.8%	<u>LCx:</u> FS = 99.3%, FU = 99.1%, MS = 99.6%, MU = 98.4%. <u>Cobas:</u> FS = 99.1%, FU = 99.8%, MS = 99.1%, MU = 100%
Goessens, W. H.F. 1997 (9)	Cobas <sup>1</sup> LCx Amp CT	1000 FVU <sup>2</sup> samples from 544 males (66 positive) & 456 females (57 positive). (CS or US taken for cell culture).	In 1 <sup>st</sup> discordant analysis each of the 3 NAATs were compared individually to culture to decide status (& in-house PCR used to decide discordant samples). In new gold standard a sample was considered true positive if 2 or more diagnostic assays were positive.	<u>1<sup>st</sup> discordant analysis:</u> LCx = 90.7% Cobas = 95.5% Amp CT = 89.9%. <u>New gold standard:</u> Culture = 57.7% LCx = 83.7% Cobas = 92.7% Amp CT = 85.4%	<u>New gold standard:</u> culture = 99.3% LCx = 99.9% Cobas = 99.4% Amp CT = 99.1%
Pasternack, R. 1997 (10)	Cobas <sup>1</sup> LCx Amplicor	442 female urine <sup>2</sup> (50 positives)	A sample considered CT positive if: 1. Culture positive 2. PCR & LCR positive 3. Positive by 1 of the PCRs & positive by MOMP PCR.	Cobas = 94.0% LCx = 94.0% Amplicor PCR = 100% Culture = 88.0%.	Cobas = 99.2% LCx = 100% Amplicor PCR = 99.7% Culture = 100%.
Shattock, M.S. 1998 (11)	Cobas LCx Amplicor	245 swabs <sup>3</sup> (23 positive) – 120 males (11 positive) & 125 females (12 positive).	IF & MOMP PCR used on discrepant samples. Sample positive if: 1. Culture positive 2. Culture negative but IF positive 3. Positive in 2 or more other assays.	Cobas = 87.5% LCx = 82% Amplicor PCR = 82% Culture = 78%.	Cobas = 99.5% LCx = 100% Amplicor PCR = 99% Culture = 100%.
Semeniuk, H. 2002 (13)	Amp CT Cobas	504 female CS & FVU (28 positives).	True CT infection defined as any patient that was positive on both CS NAAT tests. Repeat testing was performed to resolve any discrepant results	<u>Amp CT:</u> CS = 100% FVU = 80.8% <u>Cobas:</u> CS = 100% FVU = 88.5%	<u>Amp CT:</u> CS = 99.2% FVU = 100% <u>Cobas:</u> CS = 98.5% FVU = 99.4%

Abbreviations: FVU = first void urine, U = urine, US = urethral swab, CS = cervical swab, VS = vaginal swab, FS = female swab, MS = male swab, MU = male urine, FU = female urine, CVS = conjunctival swab, CT = *Chlamydia trachomatis*, BDPT = Becton Dickinson ProbeTec ET, amp CT = *Amplified CT* TMA assay, IF = immunofluorescence, DFA = Direct fluorescence assay.

<sup>1</sup>Inhibition controls used, <sup>2</sup>Specimens stored & processed according to manufacturers' instructions, <sup>3</sup>Swabs transported in 2-sucrose phosphate, sample for LCR was resuspended in LCR diluent,

<sup>4</sup>Dry swabs were resuspended in PBS during processing, <sup>5</sup>Cobas was used to test the last 61 patients & inhibition control was used during testing, <sup>6</sup>Swabs collected in 2-sucrose phosphate.

**Table 1: Evaluation of more than one NAAT**

Citation: first author & year (ref. number)	Assay(s)	Study population & numbers	Discordant analysis	Sensitivity	Specificity
Black,C.M. 2002 (14)	Amplicor LCR	3,551 female urine & CS <sup>2</sup> specimens in total	Compared tests using following reference standards to classify sample as infected: a) 3 different single cervical test reference standards (positive by cervical culture, PCR, or LCR). b) 3 different 2 test reference standards (positive by cervical or urethral culture, cervical or urine LCR, cervical or urine PCR).	<u>Single standard:</u> CS LCR = 95.8 & 96.9% CS PCR = 85.7 & 89.9% U LCR = 82.6 & 83.6% U PCR = 75.7 & 79.2%. <u>2-test standard:</u> CS LCR = 85.5 & 91.4% CS PCR = 75.8 & 84.0% U LCR = 80.8 & 83.4% U PCR = 74.9 & 79.5%.	<u>Single standard:</u> CS LCR = 97.5 & 98.1% CS PCR = 98.2 & 99.5% U LCR = 96.6 & 97.2% U PCR = 97.0 & 98.2%. <u>2-test standard:</u> CS LCR = 98.1 & 99.0% CS PCR = 98.7 & 99.7% U LCR = 97.8 & 98.9% U PCR = 98.0 & 99.4%.
Van Dyck,E. 2001(15)	SDA Amplicor LCR	733 female swabs <sup>4</sup> in total (75 positive) – 396 tested by amplicor, SDA & LCR & 337 tested by amplicor & SDA (with LCR only being tested on discordant results).	Specimens were considered CT true positive if they were positive by any 2 tests. (for the 337 samples any discordant results were considered as true positive if they were LCR positive).	Results for all 733 specimens: PCR = 98.7% SDA = 94.7%. Results for first 396 specimens: PCR = 98.0% SDA = 94.0% LCR = 90.0%	Results for all 733 specimens: PCR = 98.0% SDA = 100%. Results for first 396 specimens PCR = 98.0% SDA = 100% LCR = 98.6%
Johnson,R.E. 2000 (17)	LCx Amplicor	3,639 male urine <sup>2</sup> samples from 5 test centres (swabs taken for culture before urine sample).	Three reference standards were used to determine CT status: (1) Single test – culture alone or LCR alone or PCR alone. (2) Multi-test without discrepant analysis – both remaining tests positive or either remaining test positive. (3) Multi-test with discrepant analysis – i) culture positive or PCR & LCR positive ii) any combination 2/3 tests.	The sensitivity ranges for the 3 standards were: <u>PCR</u> = (1) 85.4 - 84.6%, (2) 81.8 - 90.4%, (3) 85.4 - 93.5% <u>LCx</u> = (1) 84.4 - 85.5,(2) 80.4- 89.3%,(3) 81.7 - 92.7% <u>Culture</u> = (1) 66.2 - 67.7%,(2) 62.9 - 70.8%,(3) 63.9 - 76.0%	The specificity ranges for the 3 standards were: <u>PCR</u> = (1) 96.4 - 98.3%,(2) 95.7 - 99.6%,(3) 99.2 - 99.6% <u>LCx</u> = (1) 96.2 - 98.2%,(2) 95.5 - 99.6%,(3) 99.0 - 99.6% <u>Culture</u> = (1) 98.6 - 98.7%,(2) 97.9 - 99.6%,(3) 99.4 - 99.6%
Templeton,K. 2001 (19).	Amplicor AmpCT SDA	346 male US & urine (all with clinical diagnosis of urethritis).	A result was considered true positive if 2 or more diagnostic assays were positive.	PCR = 98% TMA = 86% SDA = 93%	PCR = 99% TMA = 98% SDA = 97%

Abbreviations: FVU = first void urine, U = urine, US = urethral swab, CS = cervical swab, VS = vaginal swab, FS = female swab, MS = male swab, MU = male urine, FU = female urine, CVS = conjunctival swab, CT = *Chlamydia trachomatis*, BDPT = Becton Dickinson ProbeTec ET, amp CT = *Amplified CT* TMA assay, IF = immunofluorescence, DFA = Direct fluorescence assay.

<sup>1</sup>Inhibition controls used, <sup>2</sup>Specimens stored & processed according to manufacturers' instructions, <sup>3</sup>Swabs transported in 2-sucrose phosphate, sample for LCR was resuspended in LCR diluent, <sup>4</sup>Dry swabs were resuspended in PBS during processing, <sup>5</sup>Cobas was used to test the last 61 patients & inhibition control was used during testing, <sup>6</sup>Swabs collected in 2-sucrose phosphate.

**Table 1: Evaluation of more than one NAAT**

Citation: first author & year (ref. number)	Assay(s)	Study population & numbers	Discordant analysis	Sensitivity	Specificity
Gaydos, C.A. 1998 (20)	Amplicor LCx	408 female CS & FVU.	2 standards were used. For discordant specimens an OMP-1 PCR was used. The patient reference standard – at least specimens positive by 2 tests (PCR on CS & PCR and LCR on FVU). The specimen reference standard – compared on PCR & LCR urine where 2 of 3 tests (including OMP-1) indicated positive.	<u>Patient reference standard:</u> PCR CS = 81.3% PCR FVU = 93.8% LCR FVU = 89.0% <u>Specimen reference standard:</u> PCR FVU = 94.8% LCR FVU = 96.6%	<u>Patient reference standard:</u> PCR CS = 99.7% PCR FVU = 99.1% LCR FVU = 99.1% <u>Specimen reference standard:</u> PCR FVU = 97.7% LCR FVU = 98.9%
Moncada, J. 2004 (22)	Aptima Combo 2 PCR (Cobas & Amplicor) LCx	1411 female FVU & CS <sup>2</sup>	2 reference standards were used. The specimen standard determined true positives as CS samples that were positive by 2 of 3 NAATs. The infected patient standard determined infected as 2 or more positive results by 2 NAATs with either CS or FVU. Discordant analysis tested false positives by another TMA assay.	<u>Aptima Combo 2:</u> Specimen standard = 99.4% Infected standard = 92.1% <u>PCR:</u> Specimen standard = 95.6% Infected standard = 87.1% <u>LCx:</u> Specimen standard = 95.6% Infected standard = 86.6%	<u>Aptima Combo 2:</u> Specimen standard = 97.4% Infected standard = 97.7% <u>PCR:</u> Specimen standard = 99.3% Infected standard = 99.3% <u>LCx:</u> Specimen standard = 99.4% Infected standard = 99.4%

Abbreviations: FVU = first void urine, U = urine, US = urethral swab, CS = cervical swab, VS = vaginal swab, FS = female swab, MS = male swab, MU = male urine, FU = female urine, CVS = conjunctival swab, CT = *Chlamydia trachomatis*, BDPT = Becton Dickinson ProbeTec ET, amp CT = *Amplified CT* TMA assay, IF = immunofluorescence, DFA = Direct fluorescence assay.

<sup>1</sup>Inhibition controls used, <sup>2</sup>Specimens stored & processed according to manufacturers' instructions, <sup>3</sup>Swabs transported in 2-sucrose phosphate, sample for LCR was resuspended in LCR diluent,

<sup>4</sup>Dry swabs were resuspended in PBS during processing, <sup>5</sup>Cobas was used to test the last 61 patients & inhibition control was used during testing, <sup>6</sup>Swabs collected in 2-sucrose phosphate.

**Table 2: PCR evaluations**

Citation: first author & year (ref. number)	Assay(s)	Study population & numbers	Discordant analysis	Sensitivity	Specificity
Livengood,C. H. 2001 (12).	Cobas <sup>1</sup> Amplicor Culture	654 CS	True positives were culture positive samples & concordant samples amongst all assays. Discordant samples were resolved by taking the majority result for the sample after repeat testing.	Cobas = 93.3% Amplicor = 91.7%	Cobas = 99.7% Amplicor = 99.7%
Jensen,I.P. 2003 (16)	Amplicor <sup>5</sup> EIA DFA	410 urine & CS/US <sup>2</sup> total (47 positive) – 243 males (32 positive) & 167 females (15 positive).	True positives were: 1. Culture positive 2. Pos by PCR or EIA & confirmed by DFA or MOMP PCR.	PCR urine: Females = 66.7% Males = 71.9% PCR swab: Females = 93.3% Males = 87.5%	PCR urine: Females = 100% Males = 100% PCR swab: Females = 100% Males = 100%
Chernesky,M .A. 1997 (18)	Amplicor LCx DFA Culture	287 male FVU (35 positive)	Used an expanded reference standard to resolve false positives where negative by culture or DFA and positive by LCR or PCR were tested by a second PCR or LCR directed against MOMP.	PCR = 100% LCx = 94.3%	PCR = 98.0% LCx = 99.6%

Abbreviations: FVU = first void urine, U = urine, US = urethral swab, CS = cervical swab, VS = vaginal swab, FS = female swab, MS = male swab, MU = male urine, FU = female urine, CVS = conjunctival swab, CT = *Chlamydia trachomatis*, BDPT = Becton Dickinson ProbeTec ET, amp CT = *Amplified CT* TMA assay, IF = immunofluorescence, DFA = Direct fluorescence assay.

<sup>1</sup>Inhibition controls used, <sup>2</sup>Specimens stored & processed according to manufacturers' instructions, <sup>3</sup>Swabs transported in 2-sucrose phosphate, sample for LCR was resuspended in LCR diluent,

<sup>4</sup>Dry swabs were resuspended in PBS during processing, <sup>5</sup>Cobas was used to test the last 61 patients & inhibition control was used during testing, <sup>6</sup>Swabs collected in 2-sucrose phosphate.

**Table 3: TMA evaluations**

Citation: first author & year (ref. number)	Assay(s)	Study population & numbers	Discordant analysis	Sensitivity	Specificity
Gaydos, C.A. 2003 (21)	Aptima combo 2	1,389 swab (207 infected). 1,391 FVU (208 infected).	Patient infected status determined in testing algorithm using TMA, LCx & Amplicor PCR. Sample classified with patient infected status if at least 2 positive results were obtained by any combination of assay or specimen type. Sample negative if all assays negative. Sample inconclusive is positive by 1 assay on 1 specimen type.	Swabs = 94.2% Urine = 94.7%	Swabs = 97.6% Urine = 98.9%
Crotchfelt, K. A. 1998 (23)	Amp CT	female = 479 swabs (FS) & 480 urine (FU). Male = 464 urine (MU).	Discrepant analysis tested culture negative, Amp CT positive specimens by DFA to resolve. DFA negatives were tested by 16S rRNA TMA assay to resolve.	FS = 100% FU = 93.8% Matched U & CS = 95.7% MU = 95.6%.	FS = 99.25% FU = 100% Matched U & CS = 99.8% MU = 98.7%.
Pasternack, R. 1999 (24)	Amp CT Cobas.	338 male & 320 female FVU (74 CT positive).	If TMA & Cobas were discrepant, specimens retested by both & by alternative TMA assay & MOMP PCR. CT positive if 1) pos by both TMA & PCR or 2) pos by TMA or PCR & either alternative TMA or MOMP PCR.	No sensitivity & specificity calculations. In total 74 CT positive samples, 74 detected by TMA & 72 by PCR (2 samples determined TMA positive & PCR negative by discrepant analysis. 584 CT negative, 6 initially pos by PCR but negative on retesting.	
Pasternack, R. 1997 (25)	Amp CT amplicor	561 female urine (& swab for culture). 70 CT positive after discordant analysis.	Sample CT positive if: 1. Culture positive 2. Positive by 2 NAATs.	TMA = 91.4% (98.6% after discordant tests). PCR = 97.1% (100% after discordant tests).	TMA = 99.6% PCR = 99.8%
Stary, A. 1998. (26)	Amp CT LCx	308 female VS, CS & FVU (25 infected) and 240 male US & FVU (44 infected).	An expanded gold standard was used to determine infected patient status where a positive result in at least 1 sample site by culture or by both TMA & LCR, or by either TMA or LCR and confirmed by DFA or TMA by another target.	<u>TMA:</u> Female CS = 88.0% Female VS = 92.0% Female FVU = 76.0% Male US = 93.2% Male FVU = 88.6%	<u>TMA:</u> Female CS = 99.6% Female VS = 99.6% Female FVU = 99.3% Male US = 99.0% Male FVU = 99.0%

Abbreviations: FVU = first void urine, U = urine, US = urethral swab, CS = cervical swab, VS = vaginal swab, FS = female swab, MS = male swab, MU = male urine, FU = female urine, CVS = conjunctival swab, CT = *Chlamydia trachomatis*, BDPT = Becton Dickinson ProbeTec ET, amp CT = *Amplified CT* TMA assay, IF = immunofluorescence, DFA = Direct fluorescence assay.

<sup>1</sup>Inhibition controls used, <sup>2</sup>Specimens stored & processed according to manufacturers' instructions, <sup>3</sup>Swabs transported in 2-sucrose phosphate, sample for LCR was resuspended in LCR diluent,

<sup>4</sup>Dry swabs were resuspended in PBS during processing, <sup>5</sup>Cobas was used to test the last 61 patients & inhibition control was used during testing, <sup>6</sup>Swabs collected in 2-sucrose phosphate.

**Table 4: SDA evaluations**

Citation: first author & year (ref. number)	Assay(s)	Study population & numbers	Discordant analysis	Sensitivity	Specificity
Bang,D. 2003 (27)	BDPT Cobas	492 US, CS & CVS <sup>6</sup> (47 positive samples)	All samples tested by both methods. Cobas results were used as the reference standard.	SDA = 95.7%	SDA = 99.5%
Cosentino,L. A. 2003 (28)	BDPT	455 cervical (CS) & vaginal swabs (VS) (37 true positives)	Samples tested by BDPT & Amplior PCR. If results were discordant tested by LCR. Sample considered true positive if positive by 2 molecular tests.	CS = 89.2% PCR VS = 91.9% SDA CS = 91.9% SDA	CS = 99.5% PCR VS = 99.8% SDA CS = 99.5% SDA
Van der pol,B. 2001 (29)	BDPT	1,419 (125 positive) FS 1,336 (123 positive) FU 678 (146 positive) MS 675 (145 positive) MU	Samples tested by BDPT, LCR & culture (DFA used in discordant analysis). Sample had positive infection status if : 1. culture pos 2. swab or urine LCR pos & DFA pos 3. LCR pos by swab & urine.	BDPT vs positive infection status: FS = 92.8% FU = 80.5% MS = 92.5% MU = 93.1%	BDPT vs positive infection status: FS = 98.7% FU = 98.4% MS = 96.4% MU = 93.8%
McCartney,R .A. 2001 (30)	SDA LCx (PCR)	291 female FVU & CS & 715 male FVU	Specimen positive if positive by 2 out of 3 assays.	SDA: Females = 95.6% Males = 95.5% LCx: Females = 100% Males = 97%	SDA: Females = 100% Males = 100% LCx: Females = 100% Males = 100%

Abbreviations: FVU = first void urine, U = urine, US = urethral swab, CS = cervical swab, VS = vaginal swab, FS = female swab, MS = male swab, MU = male urine, FU = female urine, CVS = conjunctival swab, CT = *Chlamydia trachomatis*, BDPT = Becton Dickinson ProbeTec ET, amp CT = *Amplified CT* TMA assay, IF = immunofluorescence, DFA = Direct fluorescence assay.

<sup>1</sup>Inhibition controls used, <sup>2</sup>Specimens stored & processed according to manufacturers' instructions, <sup>3</sup>Swabs transported in 2-sucrose phosphate, sample for LCR was resuspended in LCR diluent,

<sup>4</sup>Dry swabs were resuspended in PBS during processing, <sup>5</sup>Cobas was used to test the last 61 patients & inhibition control was used during testing, <sup>6</sup>Swabs collected in 2-sucrose phosphate.

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