DIAGNOSTICS

Should *Chlamydia trachomatis* confirmation make you *cross*? Performance of collection kits tested across three nucleic acid amplification test platforms

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Objective: To investigate the feasibility of confirming initially reactive nucleic acid amplification assays for *Chlamydia trachomatis* (CT) by cross testing on a second molecular platform. The three platforms investigated were Aptima Combo 2 assay (AC2), Cobas Amplicor CT test (PCR) and ProbeTec ET CT assay (SDA).

Methods: Serial dilutions of a CT culture were prepared in 0.9% saline; used to prepare simulated swab samples for all three platforms, and tested as in the manufacturer's instructions. For the cross testing investigation, 1 ml of the simulated swab samples prepared in each of the three collection kits was transferred into the appropriate collection kit for the second platform.

Results: AC2 demonstrated a higher analytical sensitivity than the SDA and PCR assays. Upon cross testing AC2 again demonstrated a superior performance to the SDA and PCR assays even when testing swab samples originally prepared in the SDA and PCR transport medium. The SDA assay was inhibited by the addition of transport medium from both the AC2 and PCR assays.

Conclusion: The analytical sensitivity of the three assays is not identical, therefore confirming initially reactive samples on a second platform may prove to be difficult. However, the higher sensitivity of the AC2 assay could allow its use as a confirmatory assay for reactive swab samples collected in the SDA and PCR transport medium.

The application of nucleic acid amplification tests (NAATs) offers an unprecedented sensitivity for the detection of infection with *Chlamydia trachomatis* (CT). The current UK Department of Health recommendation is that NAATs are utilised for the detection of CT and further indicates that initially reactive samples are confirmed by repeating the assay.[1 2] It has been suggested, however, that it would be preferable to use an alternative amplification method for confirming initially reactive samples.[3] The repeat alternative testing of urine samples is readily achievable by testing any residual urine sample, but it may be more difficult with swab samples because of component differences in the specimen collection kits. For the confirmation to be considered valid the second confirmatory platform should also have a sensitivity that matches the initial test. Three commercially available NAATs that are currently available for the detection of CT are the Aptima Combo 2 assay (Gen-Probe Incorporated, San Diego, Ca 92121) (AC2) which is a second generation transcription mediated amplification assay, the Cobas Amplicor CT test (Roche Molecular Systems, Inc, Branchburg, NJ, USA) (PCR), which is based on the polymerase chain reaction and the ProbeTec ET CT assay (Becton-Dickinson, MD, USA) (SDA) based upon the strand displacement amplification assay. Each of these assays is supplied with a manufacturer recommended swab collection kit.

In order to investigate whether it is feasible to test, using a second platform, a swab sample that has been collected in a particular transport medium we have compared the analytical sensitivity of these three assays and carried out the cross testing of a series of simulated CT positive swab samples.

METHOD

Preparation of simulated swab samples

Cultures of a wild strain of CT were prepared in McCoy cell cultures contained in shell vials. Serial dilutions (10–2 to 10–9) of the stock culture, judged by immunofluorescence to contain 6×10^5/ml chlamydial elementary bodies were prepared in sterile 0.9% saline and stored at −70℃ in 1 ml aliquots. Simulated swab samples were prepared by thawing an aliquot of each dilution at room temperature and spiking (in triplicate) an Aptima unisex swab collection tube (containing 2.9 ml of a 3% lithium lauryl sulphate solution), a Remel M4-RT collection tube for PCR (Remel, KS, USA) (containing 3.0 ml of Modified Hanks balanced salt solution supplemented with bovine serum albumin, gelatin, sucrose and glutamic acid), and a BD ProbeTec ET transport medium tube (containing 2.0 ml of a potassium phosphate, DMSO, glycerol, Polysorbate 20 and 0.03% Proclin solution) with 200 µl of each serial dilution. The sampling swab supplied with each collection kit was broken off into the spiked collection tubes. The AC2, PCR and SDA simulated swab samples were then stored and tested within the manufacturers' specifications. The AC2 samples were held overnight at ambient temperature while the PCR samples were stored at 4℃ overnight before testing. The AC2 and PCR testing was carried out by the clinical microbiology department, University Hospital Aintree, according to the manufacturer's instructions. The SDA samples were transported overnight at ambient temperature to the clinical microbiology department, Portsmouth, before testing according to the manufacturer's instructions.

One first dilution series of AC2 simulated swab samples was also examined using the monospecific APTIMA CT kit (Gen-Probe, San Diego, CA, USA).

Abbreviations: AC2, Aptima Combo 2; CT, *Chlamydia trachomatis*; MOTA, method other than acceleration; NAATs, nucleic acid amplification tests; PCR, polymerase chain reaction; RLU, relative light unit; SDA, strand displacement amplification assay

Table 1 Comparative results on three different occasions for the AC2, SDA, and PCR assays on the serial simulated swab samples and the monospecific ACT results

	Run 1				Run 2			Run 3		
Dilution	AC2 1	PCR 1	SDA 1	ACT	AC2 2	PCR2	SDA2	AC2 3	PCR3	SDA3
10–3	Pos (1367)	Pos (>3.9)	Pos (6471)	Pos (6550)	Pos (1049)	Pos (2.450)	Pos (39031)	Pos (1185)	Pos (3.537)	Pos (35885)
10–4	Pos (1099)	Neg	Neg	Pos (6445)	Pos (931)	Neg	Pos (21400)	Pos (751)	Neg	Neg
10–5	Pos (1020)	Neg	Neg	Pos (6567)	Pos (610)	Neg	Neg	Pos (589)	Neg	Neg
10–6	Pos (650)	Neg	Neg	Pos (6497)	Pos (188)	Neg	Neg	Pos (122)	Neg	Neg
10–7	Neg	Neg	Neg	Neg	Pos (162)	Neg	Neg	Neg	Neg	Neg
10–8	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
10–9	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

Data in parentheses indicate the relative light unit (RLU) value for AC2 and ACT (positive cut-off value >100), optical density reading for PCR (positive cut-off value >0.8) and method other than acceleration (MOTA) values for SDA (positive cut-off value >2000).

Cross testing of simulated swab samples

For the cross testing investigation, simulated swab samples were prepared by thawing an aliquot of the 10–2, 10–3, and 10–4 serial dilutions at room temperature and spiking an Aptima unisex swab collection tube, a Remel M4-RT collection tube, and a BD ProbeTec ET transport medium tube with 200 µl of each serial dilution. The sampling swab supplied with each collection kit was broken off into the spiked collection tubes. Samples for cross testing were prepared by inoculating fresh Aptima unisex swab collection tubes, Remel M4-RT collection tubes and BD ProbeTec ET transport medium tubes with 1000 µl of each simulated swab sample. The AC2 samples were held overnight at ambient temperature while the PCR samples were stored at 4°C overnight before testing. The AC2 and PCR testing was carried out by the clinical microbiology department, University Hospital Aintree, according to the manufacturer's instructions. The SDA samples were transported overnight at ambient temperature to the clinical microbiology department, Portsmouth, before testing according to the manufacturer's instructions.

RESULTS

Determination of analytical sensitivity (table 1)

All three assays were consistently positive at a dilution of 10–3. The AC2 demonstrated the greatest analytical sensitivity being consistently positive at a dilution of 10–6. The SDA assay was positive once at a dilution of 10–4 while the PCR assay remained consistently negative at 10–4. The SDA and PCR assays did not demonstrate the presence of inhibitors in any of the negative results.

Cross testing of simulated swab samples

The results of the cross testing of the simulated swab samples are displayed in table 2.

Upon cross testing of the simulated swab samples the AC2 assay was able to confirm all of the positive results generated by the PCR and SDA assays. The AC2 also gave a positive signal further down the dilution series than the SDA assay, even with the simulated swabs prepared in the SDA medium (10–4 TMA compared with 10–3 for SDA). The AC2 also gave strongly reactive results for swabs prepared in M4RT medium when the PCR was failing or only weakly positive. At the volume used the addition of both the TMA and M4RT medium into the SDA assay resulted in the generation of inhibitory results.

DISCUSSION

In this study we have clearly demonstrated that the AC2 has a superior analytical sensitivity for simulated swab samples over the PCR and SDA assays. This finding mirrors those of other studies that have demonstrated a superior sensitivity of the AC2 in detecting CT in clinical samples.[4 5] It is clear that the less sensitive PCR and SDA assays should not be used to confirm positive AC2 results, however the superior analytical sensitivity of the AC2 assay potentially allows its use to confirm reactive samples generated by the other assays. It is acknowledged that this study was carried out on simulated swab samples and as such the findings may not transfer to the clinical situation where samples could have a higher chlamydial load and that the study is limited in that cross testing was only carried out on one dilution series. However, as the AC2 succeeded in confirming positive results beyond

Table 2 Results of cross testing of simulated swab samples

		Second platform cross testing result by		
Seed	Simulated swab (initial results)	AC2 (1 ml of sample in 2.9 ml of TMA medium)	PCR (1 ml of sample in 3.0 ml of M4RT medium)	SDA (1 ml of sample in 2.0 ml of SDA medium)
10–2	0.2 ml of dilution in 2.9 ml TMA medium	pos (1275)	pos (>3.9)	Inhibitory
	0.2 ml of dilution in 3.0 ml M4RT medium	pos (1184)	pos (>3.9)	pos (19313)
	0.2 ml of dilution in 2.0 ml SDA medium	pos (1209)	pos (>3.9)	pos (27493)
10–3	0.2 ml of dilution in 2.9 ml TMA medium [pos, pos, pos]	pos (1018)	neg (0.003)	Inhibitory
	0.2 ml of dilution in 3.0 ml M4RT medium [pos, pos, pos]	pos (1150)	neg (0.002)	Inhibitory
	0.2 ml of dilution in 2.0 ml SDA medium [pos, pos, pos]	pos (1063)	neg (0.001)	pos (25620)
10–4	0.2 ml of dilution in 2.9 ml TMA medium [pos, pos, pos]	pos (739)	pos (0.937)	inhibitory
	0.2 ml of dilution in 3.0 ml M4RT medium [neg, neg, neg]	pos (196)	neg (0.001)	inhibitory
	0.2 ml of dilution in 2.0 ml SDA medium [neg, pos, neg]	pos (866)	neg (0.002)	neg (109)

Data in parentheses indicate the RLU value for AC2 (positive cut-off value <100), OD for PCR (positive cut-off value>0.8), and MOTA values for SDA (positive cut-off value >2000)). Data in brackets indicate the results obtained for that dilution previously displayed in table 1.

Key messages

- The cross platform confirmation of initially reactive *C trachomatis* samples is difficult to perform owing to component differences in specimen collection kits and a demonstrated variation in analytical sensitivity of three commercially available CT assays
- The superior analytical sensitivity of the AC2 assay suggests that this platform could potentially be used for the confirmation of initially reactive PCR and SDA samples
- The Aptima platform supports the detection of two distinct molecular targets allowing the confirmation of initially reactive samples on one assay to be confirmed by the other

the detection limits of the other assays, we consider that the AC2 could potentially be used to confirm initially reactive swab samples collected in both the M4-RT and the SDA media.

The addition of both the M4-RT and AC2 media into the SDA medium resulted in the generation of inhibitory results. It is therefore unlikely that the SDA assay could be used to confirm the results from the AC2 and PCR without additional sample preparation.

A more stringent solution to the problem of confirming initially reactive NAAT results would be to use the same molecular platform but to detect different molecular targets. The Aptima platform supports the detection of two different targets, with the AC2 replicating a region of the 23S rRNA while the ACT targets a specific region of the 16S rRNA of CT. The performance of the monospecific Aptima CT assay in this study matched the performance of the AC2. Although this study was limited it mirrors other reports where the monospecific assay was demonstrated to be of equal sensitivity and specificity to the AC2.[6]

In this study we have not examined issues of specimen stability, the Aptima platform unlike the SDA and PCR, detects a ribosomal RNA target that is stabilised upon addition to the Aptima transport medium. The stability of the RNA in the samples collected in the SDA and PCR medium is unknown and as such further work is required to determine if a delay in transferring the sample into the Aptima medium could have a detrimental effect on assay performance.

In conclusion, the performance of the AC2 suggests that it can be used as a confirmatory assay for initially reactive SDA and PCR swab samples. In addition the monospecific Aptima CT assay can easily be utilised to confirm initially reactive AC2 results, a strategy that has been previously reported by Golden *et al* for the confirmation of reactive AC2 *Neisseria gonorrhoeae* samples.[7]

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CONTRIBUTORS

The study was designed by HM and SS; sample preparation, AC2, and PCR testing was carried out by SS and AB and SDA was carried out by the Portsmouth Microbiology Laboratory; preparation of the manuscript was carried out by SS.

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