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External Quality Assessment for Detection of *Chlamydia trachomatis*

V. J. Chalker,* H. Vaughan, P. Patel, A. Rossouw, H. Seyedzadeh, K. Gerrard, and V. L. A. James

United Kingdom National External Quality Assessment Scheme for Microbiology, Quality Assurance Laboratory, Health Protection Agency Centre for Infections, London, United Kingdom

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The use of molecular methods for detection of *Chlamydia trachomatis* is increasing in clinical laboratories. External quality assessment enables unbiased monitoring of the performance of laboratories in the detection of specific pathogens. This study details the results of molecular and enzyme immunosorbent assay (EIA) testing for *C. trachomatis* detection in simulated endocervical swab specimens recently distributed internationally by United Kingdom National External Quality Assessment Scheme for Microbiology (UK NEQAS for Microbiology) external quality assessment panels. The frequency of accurate detection of *C. trachomatis* in the panels ranged from 32 to 100%. Participants using molecular methods were significantly more likely to detect *C. trachomatis* in specimens than those using an EIA. Two strains were distributed with the panels: an L2 laboratory-adapted strain and an uncharacterized primary isolate. Further analysis indicated a difference in detection of *C. trachomatis* between specific methods only with the L2 strain at lower concentrations. In addition, eight negative specimens were distributed, and false positives were found to be rare by all methods included in the study.

Chlamydia trachomatis is an important sexually transmitted pathogen that causes upper and lower genital tract infections in males and females, leading to endometritis, infertility, and infections of the neonate. Clinical specimens that are tested for *C. trachomatis* include endocervical swabs, urethral swabs, urine specimens, conjunctival swabs, and vulvovaginal swabs. Traditional testing relied on cell culture and/or detection of *C. trachomatis* by enzyme immunosorbent assays (EIA) or immunofluorescence (IF). Due to the low sensitivity of culture, the new "gold standard," known as the expanded gold standard, requires that a positive result be confirmed by repeat testing with a different assay (25, 28). Several molecular tests or nucleic acid amplification tests (NAAT), including BDProbeTecET, Gen-Probe AMP-CT, Gen-Probe APTIMA Combo 2, Roche AMPLICOR CT/NG, and Roche COBAS AMPLICOR CT/NG, are now commercially available; some are no longer available (Abbott LCx); and new technologies such as molecular beacons are being developed (1). Previous studies have attributed discrepant results with NAAT to inhibitors in the specimens, inappropriate specimen handling, cross-contamination, or poor sensitivity (4, 7, 10, 11, 16, 19, 23, 24). The reporting of false-positive results has medical, social, and psychological effects on the patient and results in increased health care costs and inappropriate treatment (6).

The United Kingdom National External Quality Assessment Scheme for Microbiology (UK NEQAS for Microbiology) has specialized in the production and performance analysis of external quality assessment (EQA) panels for a wide range of bacteria, fungi, parasites, and viruses for more than 30 years. An EQA panel for the detection of *C. trachomatis* by EIA and IF was introduced in 1991, and more-challenging specimens specifically designed to test NAAT methods have been included in the scheme from 2002. In addition to the EIA-IF schemes, a pilot scheme for the molecular detection of *C. trachomatis* is under development, and panels for testing by NAAT methods only have been distributed recently.

Verkooyen et al. (26) described the results obtained from an international EQA scheme consisting of freeze-dried urine specimens. The authors reported difficulty in the detection of *C. trachomatis* at lower concentrations and found no difference between the NAAT methods Roche AMPLICOR CT/NG, Roche COBAS AMPLICOR CT/NG, and Abbott LCx. Accurate detection of *C. trachomatis* was common, ranging from 89 to 100%. A further EQA study of detection of *C. trachomatis* in urine samples, based in Australia, found varying performance depending on the concentration (0 to 100%) and reported that low-level positives could not be detected consistently by a single test (18). In contrast to the results reported by Verkooyen et al. (26), Land et al. (18) found that at lower concentrations of *C. trachomatis*, the Roche AMPLICOR CT/NG assay was more sensitive than the Abbott LCx. However, Goessens et al. (14) examined the detection of *C. trachomatis* in female specimens (urine and swabs) and found no difference among three commercial tests (Abbott LCx, Gen-Probe AMP-CT, and Roche COBAS AMPLICOR CT/NG).

The aim of this retrospective study was to analyze the effective detection of *C. trachomatis* in simulated endocervical swab specimens by clinical laboratories participating in the EIA and molecular EQA schemes distributed internationally by UK NEQAS for Microbiology between January 2002 and June 2004. Negative specimens and those that were designed to challenge participants by including low levels of elementary bodies (EB) per milliliter were included in the study. Two *C. trachomatis* strains were included in the study: one L2 laboratory-adapted strain and one primary clinical isolate. Due to the large number of participants in the study, additional data were

* Corresponding author. Mailing address: UK NEQAS for Microbiology, Quality Assurance Laboratory, Health Protection Agency Centre for Infections, 61 Colindale Ave., London, NW9 5HT, United Kingdom. Phone: 020 89059890. Fax: 020 82051488. E-mail: vicki.chalker@hpa.org.uk.

TABLE 1. Performance of all methods taken together, EIA, and molecular methods

Panel (no. of participants)	Date	Specimen	Result[a] by:						*P* (EIA vs molecular methods)[b]
			All methods		EIA		Molecular methods		
			No. pos/total	% Pos (95% CI)	No. pos/total	% Pos (95% CI)	No. pos/total	% Pos (95% CI)	
L2 strain CT001									
1500 (289)	Jan. 2002	6157	116/267	43.4 (37.4–49.6)	14/162	8.6 (4.8–14.0)	102/105	97.1 (91.9–99.4)	<0.0001
1523 (291)	Mar. 2002	6233	117/279	41.9 (36.1–47.9)	8/159	5.0 (2.2–9.7)	109/120	90.8 (84.2–95.3)	<0.0001
1565 (294)	Jul. 2002	6346	112/292	38.4 (32.9–44.1)	6/156	3.9 (1.4–8.2)	106/136	77.9 (70.0–84.6)	<0.0001
1585 (293)	Oct. 2002	6424	128/290	44.1 (38.5–49.9)	6/152	3.9 (1.5–8.4)	122/138	88.4 (81.9–93.2)	<0.0001
1614 (295)	Mar. 2003	6547	102/284	35.9 (30.5–41.7)	8/150	5.3 (2.3–10.3)	94/134	70.1 (61.6–77.7)	<0.0001
1660 (285)	June 2003	6680	240/287	83.6 (79.1–87.5)	101/146	69.2 (61.0–76.6)	139/141	98.6 (94.9–99.8)	<0.0001
		6681	111/283	39.2 (33.7–45.0)	7/147	4.8 (1.9–9.6)	104/136	76.5 (68.4–83.3)	<0.0001
1689 (280)	Oct. 2003	6788	200/254	78.7 (73.5–83.2)	61/112	54.5 (44.8–63.9)	139/142	97.9 (93.9–99.6)	<0.0001
1733 (280)	Mar. 2004	6962	80/248	32.3 (26.7–38.3)	3/132	2.3 (0.5–6.5)	77/116	66.4 (57.0–74.9)	<0.0001
Clinical isolate 6498									
1734 (91)	Mar. 2004	6963	84/84	100 (96.5–100)	NA	NA	84/84	100 (96.5–100)	NA
		6964	84/84	100 (96.5–100)	NA	NA	84/84	100 (96.5–100)	NA
1785 (103)	June 2004	7112	98/98	100 (96.9–100)	NA	NA	98/98	100 (96.9–100)	NA
		7113	32/98	32.7 (23.5–42.9)	NA	NA	32/98	32.7 (23.5–42.9)	NA
		7115	98/98	100 (96.9–100)	NA	NA	98/98	100 (96.9–100)	NA
Negative									
1500 (289)	Jan. 2002	6158	3/269	1.1 (0.3–3.2)	2/164	1.2 (0.2–4.3)	1/105	1.0 (0.02–5.2)	1.0
1523 (291)	Mar. 2002	6235	4/271	1.5 (0.4–3.7)	3/151	2.0 (0.4–5.7)	1/120	0.8 (0.02–4.6)	0.6
1565 (294)	July 2002	6347	1/300	0.3 (0.01–1.8)	1/164	0.6 (0.02–3.4)	0/136	0 (0–2.2)	1.0
1585 (293)	Oct. 2002	6423	1/290	0.3 (0.01–1.9)	1/152	0.6 (0.02–3.6)	0/138	0 (0–2.2)	1.0
1614 (295)	Mar. 2003	6545	4/291	1.4 (0.4–3.4)	3/151	2.0 (0.4–5.7)	1/140	0.7 (0.02–3.9)	0.6
1660 (285)	June 2003	6682	3/281	1.1 (0.2–3.0)	2/145	1.4 (0.2–4.9)	1/136	0.7 (0.05–4.0)	1.0
1733 (280)	Mar. 2004	6959	3/283	1.1 (0.3–3.1)	1/135	0.7 (0.02–4.1)	2/148	1.4 (0.2–4.8)	1.0
1785 (103)	June 2004	7114	1/98	1.0 (0.03–5.6)	NA	NA	1/98	1.0 (0.03–5.6)	NA

[a] pos, positive and equivocal; total, total number of tests (some participants used more than one method). NA, not applicable.
[b] By Fisher's exact text.

collected that provided insight into the differential performance of the methodologies in use in clinical laboratories. Therefore, the accurate detection of *C. trachomatis* by participants, different methods (EIA or NAAT), and commercial assays was compared with the *C. trachomatis* EQA specimens distributed. A total of eight negative specimens were also distributed, and the number of false positives reported was calculated.

MATERIALS AND METHODS

Specimen preparation. Two strains of *C. trachomatis* were used in the study: a laboratory-adapted L2 strain (kindly donated by the Bristol Health Protection Agency [HPA]) cultured in McCoy cells (1×10^6 EB/ml) and a primary clinical isolate of unknown serotype, strain 6498, cultured in McCoy cells (3.4×10^6 EB/ml) from an endocervical sample from an 18-year-old female attending a clinic (kindly donated by Dudley Road Hospital, Birmingham, United Kingdom). Specific dilution series were made (see Table 1) in Earle's balanced salt solution (L2 strain) with 0.05% Bronidox (an antibacterial agent) or in 5% sucrose (strain 6498), and 0.5 ml was immediately dispensed into 2-ml plastic or glass vials. Specimens of the L2 strain were dispatched in liquid form, and those of 6498 in 5% sucrose were freeze-dried in a Lyoflex automated machine. The vacuum integrity of sealed freeze-dried vials was confirmed by spark testing followed by confirmation of a moisture content of <2% (Mitsubishi CA 100). Sealed freeze-dried vials were then stored at −30°C, and liquid vials were stored at 4°C, until distribution. Freeze-dried specimens were reconstituted in 0.5 ml of molecular-grade RNase-free water immediately prior to analysis in-house.

A sample specimen from each dilution was sterility tested by inoculation of 100 μl of the liquid or pre-freeze-dried specimen into 5 ml of nutrient broth followed by incubation for 48 h at room temperature and +37°C. No specimens yielded microbiological growth after 48 h of incubation. Specimens were monitored for stability at a range of temperatures; liquid specimens were found to be stable at 4°C for at least 2 months, and freeze-dried specimens were found to be stable at 4°C, room temperature, and −30°C for as long as 12 months (data not shown).

Before distribution, the specimens were tested for *C. trachomatis* by a range of laboratories using BDProbeTecET, Gen-Probe AMP-CT, in-house PCR, and Roche AMPLICOR CT/NG (for all panels) and Dade Behring Microtrak II EIA and Dako IDEIA PCE (for panels 1500 to 1733).

To monitor specimen delivery and the effects of transit on the specimen, five specimen sets from each panel were posted within each consignment to five different sites around the United Kingdom and, upon receipt, were returned by post to UK NEQAS for Microbiology for retesting.

Distribution information. Each proficiency panel consisted of four specimens, a reply form, and an instruction sheet. Participants were provided with instructions to reconstitute freeze-dried specimens in 0.5 ml of molecular-grade RNase-free water immediately prior to analysis, add 0.1 ml of the reconstituted specimen to their transport medium, and extract by the usual laboratory routine. For liquid specimens, participants were instructed to examine 0.1 ml of each specimen for *C. trachomatis* by their routine method. Participants were requested to test for the presence of *C. trachomatis* in each specimen and report results within 21 days of posting. Results were received by fax, post, or e-mail or via a World Wide Web online reporting system. Ten panels have been distributed since 2002, and the data presented here include a total of 22 specimens, 14 positive and 8 negative, from these distributions (Table 1). The median concentration of *C. trachomatis* in each specimen, in EB per milliliter, is shown in Table 2. Specimens were distributed to 91 to 295 participating laboratories (Table 1), predominantly in the United Kingdom, but also in other countries including Austria, Belgium, Denmark, Finland, Greece, Hong Kong, Ireland, Israel, Italy, Kuwait, The Netherlands, Norway, Portugal, Slovenia, Sweden, Switzerland, and Zambia.

Data analysis. The number of participants detecting *C. trachomatis* was calculated in total and by method per specimen. The EIA and molecular method groups were compared by using Fisher's exact test. The methods included in the category of molecular tests (Table 1) were as follows: Abbott LCx, BDProbe TecET, Gen-Probe PACE 2, Gen-Probe AMP-CT, in-house PCR (amplifying a 207-bp region of a cryptic plasmid), and Roche AMPLICOR CT/NG. The

TABLE 2. Performance of molecular methods

Specimen	Dilution (EB/ml[a])	Result[b] by:						Risk difference coefficient (*P*) for BDProbeTecET vs Roche AMPLICOR
		Abbott LCx		BDProbeTecET		Roche AMPLICOR/COBASCT/NG		
		No. pos/total	% Pos (95% CI)	No. pos/total	% Pos (95% CI)	No. Pos/total	% Pos (95% CI)	
L2 strain CT001								
6680	1:200 (5,000)	12/12	100 (76.0–100)	59/59	100 (93.9–100)	66/66	100 (94.5–100)	NA
6788	1:500 (2,000)	4/4	100 (51.0–100)	65/65	100 (94.4–100)	66/67	98.5 (92.0–99.7)	−0.15 (0.31)
6157	1:1,000 (1,000)	29/29	100 (88.3–100)	30/32	93.8 (79.9–98.3)	39/39	100 (91.0–100)	0.63 (0.14)
6962	1:1,200 (833)	2/2	100 (34.2–100)	11/35	31.4 (18.6–47.9)	60/71	84.5 (74.4–91.1)	0.53 (0.00)
6424	1:1,500 (667)	28/30	93.3 (78.7–98.2)	40/51	78.4 (65.4–87.5)	52/53	98.1 (90.1–99.7)	0.19 (0.001)
6233	1:2,000 (500)	30/31	96.8 (83.8–99.4)	32/40	80.0 (65.2–89.5)	42/44	95.5 (84.9–98.7)	0.26 (0.001)
6681	1:2,000 (500)	10/12	83.3 (55.2–95.3)	36/57	63.2 (50.2–74.5)	56/63	88.9 (78.8–94.5)	0.15 (0.03)
6346	1:2,500 (400)	27/33	81.8 (65.6–91.4)	26/47	55.3 (41.3–68.6)	51/52	98.1 (89.9–99.7)	0.43 (0.00)
6547	1:3,000 (333)	8/11	72.7 (43.4–90.3)	27/59	45.8 (33.7–58.3)	54/58	93.1 (83.6–97.3)	0.47 (0.00)
Total for L2	NA	150/164	91.5 (86.1–95.3)	326/445	73.3 (68.9–77.3)	486/613	94.7 (92.4–96.5)	NA
Clinical isolate 6498								
6963	1:40 (85,750)	NA	NA	47/47	100 (93.8–100)	35/35	100 (91.8–100)	NA
6964	1:400 (8,575)	NA	NA	47/47	100 (93.8–100)	35/35	100 (91.8–100)	1.4×10^{-24} (1.0)
7112	1:400 (8,575)	NA	NA	54/54	100 (94.6–100)	41/41	100 (92.9–100)	1.3×10^{-15} (1.0)
7113	1:250,000 (14)	NA	NA	18.54	33.3 (21.1–47.5)	13/41	31.7 (18.1–48.1)	−0.16 (0.87)
7115	1:1,200 (2.858)	NA	NA	54/54	100 (94.6–100)	41/41	100 (92.9–100)	4.4×10^{-15} (1.0)
Total for 6498	NA	NA	NA	220/256	85.9 (81.3–89.7)	165/193	85.5 (80.1–89.8)	NA
Negative								
6158	NA	1/29	3.5 (0.1–17.7)	0/32	0 (0–8.9)	0/39	0 (0–7.4)	NA
6235	NA	0/32	0 (0–8.9)	0/39	0 (0–7.4)	1/44	2.3 (0.06–12.0)	NA
6347	NA	0/33	0 (0–8.7)	0/47	0 (0–6.2)	0/52	0 (0–5.6)	NA
6423	NA	0/30	0 (0–9.5)	0/51	0 (0–5.7)	0/53	0 (0–5.7)	NA
6545	NA	0/11	0 (0–23.8)	0/61	0 (0–4.8)	1/62	1.6 (0.04–8.7)	NA
6682	NA	0/12	0 (0–22.1)	1/58	1.7 (0.04–9.2)	0/62	0 (0–4.7)	NA
6959	NA	0/2	0 (0–77.6)	1/66	1.5 (0.04–8.2)	0/72	0 (0–4.1)	NA
7114	NA	NA	NA	0/53	0 (0–5.5)	1/42	2.4 (0.06–12.6)	NA

[a] Estimated EB per milliliter are extrapolated from IF data for a 1:1,000 dilution (4 × 10-μl wells).
[b] NA, not applicable.

methods included in the EIA category (Table 1) were as follows: Abbott Chlamydiazyme IMx, Beckman Coulter Access Chlamydia, bioMérieux VIDAS, Bio-Rad Pathfinder EIA, Murex Diagnostics Inc. BioStar Chlamydia OIA, Dade Behring MicroTrak Chlamydia EIA, Dako IDIEA, Dako IDEIA PCE, Launch Diagnostics Phadebact Chlamydia EIA, and Unipath Clearview Chlamydia. Result collation is dependent on the information provided by participants, and the majority of users of Roche methods stated "Roche" only and did not specify Roche AMPLICOR CT/NG or Roche COBAS AMPLICOR CT/NG. For the purposes of this study, the two methods have been collated into a single group, termed Roche AMPLICOR/COBAS CT/NG. The detection of *C. trachomatis* by users of the Roche AMPLICOR/COBAS CT/NG method group was compared to detection by the BDProbeTecET method by using risk difference analysis (STATA statistical analysis package) with differences calculated at each level of dilution. Similar analysis was performed to compare the detection of the L2 strain by the Abbott LCx method to that by the Roche AMPLICOR/COBAS CT/NG method group and the BDProbeTecET method in specimens distributed when the Abbott LCx method was still available.

RESULTS

Results are summarized in Tables 1 and 2. Overall detection of *C. trachomatis* by participants ranged from 32 to 100%.

For the L2 strain, the rate of detection of *C. trachomatis* by participants ranged from 32.3 to 83.6% (Table 1). The rate of *C. trachomatis* detection by users of molecular methods ranged from 66.4 to 98.6% and was significantly higher than that with EIA methods, which ranged from 2.3 to 69.2% ($P = 0.00$ by Fisher's exact test [Table 1]).

When all data for the L2 strain were compared, no difference was noted between the Abbott LCx, the BDProbeTecET (risk difference coefficient, 7.93×10^{-14}; $P = 1.0$), and the Roche AMPLICOR/COBAS CT/NG method group (risk difference coefficient, 2.89×10^{-14}; $P = 1.0$). There was no significant difference between molecular methods at dilutions of 1:200 through 1:2,000. However, detection of *C. trachomatis* by all methods showed significant differences at dilutions greater than 1:2,000 (risk difference coefficients, −1.81 at 1:2,500 [$P = 0.007$] and −0.27 at 1:3,000 [$P = 0.04$]), indicating that all methods are less likely to detect *C. trachomatis* at lower concentrations of the pathogen. The Abbott LCx accurately detected *C. trachomatis* in 72.7 to 100% of specimens, the BDProbeTecET method detected it in 31.4 to 100% of specimens, and the Roche AMPLICOR/COBAS CT/NG method group detected it in 84.5 to 100% of specimens. Significant differences were found in the likelihood that each method would report a positive result when risk difference analysis was used to compare the Abbott LCx, the BDProbeTecET method, and the Roche AMPLICOR/COBAS CT/NG method group, with differences calculated at each level of dilution. The BDProbeTecET method was less likely to give a positive result at dilutions of 1:1,200 and higher (risk difference coefficients, −0.69 at 1:1,200 [$P = 0.00$], −0.14 at 1:1,500 [$P = 0.04$], −0.2 at 1:2,000 [$P = 0.11$], −0.17 at 1:2,000 [$P = 0.02$], −0.26 at 1:2,500 [$P = 0.007$], and −0.27 at 1:3,000 [$P = 0.071$]), and the Abbott LCx was less likely to give a positive result at dilutions of 1:2,500 and higher (risk difference coefficients, 0.16 at 1:2,500 [$P = 0.02$] and 0.2 at 1:3,000 [$P = 0.14$]).

To enable comparison of results for the L2 strain and strain 6498 (for which no Abbott LCx data were available), further analysis was performed to compare all L2 strain data for the BDProbeTecET and the Roche AMPLICOR/COBAS CT/NG method group. No difference was found when all data were compared (risk difference coefficient, 2.03×10^{-14}, $P = 1.0$). Significant differences were found in the likelihood that each method would report a positive result when risk difference analysis was used to compare the BDProbeTecET method and the Roche AMPLICOR/COBAS CT/NG method group, with differences calculated at each level of dilution (Table 2). The BDProbeTecET method was less likely to give a positive result than the Roche AMPLICOR/COBAS CT/NG method group at dilutions of 1:1,200 to 1:3,000 (Table 2) for the L2 strain.

The rate of detection of strain 6498 ranged from 32.7 to 100% for the specimens distributed (Table 1). Only molecular tests were performed on these specimens. No difference was noted between the BDProbeTecET and the Roche AMPLICOR/COBAS CT/NG method group when all data for the specimens containing strain 6498 were compared (risk difference coefficient, -5.02×10^{-24}, $P = 1.0$). In addition, comparison of *C. trachomatis* detection by the two methods between the 1:40 dilution and other dilutions gave a significant difference at the 1:250,000 dilution only (risk difference coefficient at 1:250,000, −0.67; $P = 0.00$), indicating that both methods are less likely to detect *C. trachomatis* at lower concentrations of the bacterium. The BDProbeTecET method accurately detected *C. trachomatis* in 33.3 to 100% of specimens, and the Roche AMPLICOR/COBAS CT/NG method group detected it in 31.7 to 100%. Unlike the findings for the L2 strain, no significant difference was found in the likelihood that each method would report a positive result when risk difference analysis was used to compare the BDProbeTecET method and the Roche AMPLICOR/COBAS CT/NG method group with differences calculated at each level of dilution (Table 2). The two methods were equally likely to give a positive result with strain 6498.

There was no significant difference in the detection of the two different *C. trachomatis* strains by users of all molecular tests ($P = 0.76$ by Fisher's exact test; for strain L2, 992 of 1,168 tests [84.9%] were positive [95% confidence interval {95% CI}, 82.8 to 86.9%]; for strain 6498, 396 of 462 tests [85.7%] were positive [95% CI, 82.2 to 88.8%]). Similarly, comparison of the detection of the two *C. trachomatis* strains by users of the BDProbeTecET method showed no difference ($P = 0.18$ by Fisher's exact test), and no difference was noted in the detection of the two strains by the Roche AMPLICOR/COBAS CT/NG method group tests ($P < 0.42$ by Fisher's exact test). However, comparison of BDProbeTecET with the Roche AMPLICOR/COBAS CT/NG method group showed that the latter was more likely to detect *C. trachomatis* in specimens containing the L2 strain ($P < 0.008$ by Fisher's exact test), whereas no difference was seen in the detection of strain 6498 ($P = 1.0$ by Fisher's exact test).

For the eight negative specimens that were distributed, 0.3 to 1.5% of results were positive. A total of 20 positive results and 2,080 negative results were received. The positive results were received from 18 different participants using a range of methods (bioMérieux VIDAS [$n = 4$], Murex Diagnostics [$n = 1$], Dako IDIEA [$n = 1$], Launch Diagnostics Phadebact Chlamydia EIA [$n = 3$], Unipath Clearview Chlamydia [$n = 4$], Abbott LCx [$n = 1$], BDProbeTecET [$n = 3$], Gen-Probe AMP-CT [$n = 1$], and Roche AMPLICOR CT/NG [$n = 2$]). A single participant reported three negative specimens as positive, whereas all other false positives were received from separate participants.

DISCUSSION

EQA enables monitoring of the performance of laboratories with defined specimens from a position external to the host organization and therefore in an unbiased fashion. Such monitoring enables the detection of specific pathogens to be compared among laboratories both nationally and internationally depending on the participant base, highlighting areas for improvement and promoting continuing maintenance and improvements in clinical diagnostic standards. Several aspects of routine procedures can be tested by EQA, such as issues of specimen handling, test performance, result reporting, and general laboratory performance over time. However, some aspects, such as the individual routines specific to laboratories (18), routine reporting using internal standards, and individual laboratory workers, cannot be monitored. Nonetheless, EQA provides valuable insight into the standard of clinical diagnostic testing by laboratories. In addition to monitoring standards, the process of EQA collates information that can be utilized to provide insight into the performance of specific laboratory methods (18, 26). In this study, EQA enabled the comparison of *C. trachomatis* detection by differing assay types (EIA and NAAT) and also the comparison of the three main NAAT used by participants in the study (Abbott LCx, BDProbeTecET, and the Roche AMPLICOR/COBAS CT/NG method group), with two distinct sources of *C. trachomatis*.

The ability of laboratories to detect *C. trachomatis* in the EQA panels included in this study has been variable. In general, those using EIAs were significantly less likely to report detection of *C. trachomatis* than those using the molecule-based NAAT. With a specimen containing approximately 833 EB/ml, only 2.3% of laboratories using EIAs detected *C. trachomatis*, in comparison to 66.4% of those using NAAT. These findings are in concordance with those of other studies, and it is now accepted that NAAT are superior to EIA-based tests for detection of *C. trachomatis* (22, 28). However, in this study only those specimens that were designed to be challenging to participants were included, and at higher concentrations such a difference may not exist. Furthermore, NAAT are not without problems, including the effects of inhibitors in the specimens, contamination, sensitivity, reproducibility, and variation in the efficiency of nucleic acid extraction (4, 6, 7, 10, 11, 15, 16, 19, 23, 24).

At higher concentrations of *C. trachomatis*, detection by NAAT was excellent, irrespective of the method used or the strain under test. Interestingly, we found that the BDProbeTecET method was less likely to detect *C. trachomatis* than the Roche AMPLICOR/COBAS CT/NG method group at lower concentrations of the L2 strain only. With the clinical isolate no difference was seen, a finding similar to the results of Chan et al. (8). Further analysis indicated that differences in strain detection did not occur with the BDProbeTecET method or with the Roche AMPLICOR/COBAS CT/NG method group. To date at least 18 serotypes of *C. trachomatis* have been recognized (12, 21, 27). Serotypes are distinguished by differences in the sequence of the *ompA* gene, which encodes the major outer membrane protein (MOMP). Although MOMP types differ by geographical region, no differences are seen between the serotypes present in males and females in the same population (21). Specific serotypes are associated with particular infections: types A, B, and Ba are commonly associated with trachoma, types C to K are associated with lower and upper genital tract infections, and the lymphogranuloma venereum serotypes L1, L2, L2a, and L3 are associated with more-severe infections such as proctitis and lymphadenitis (2, 5, 21). Furthermore, a strain has been associated with concurrent infection with *Neisseria gonorrhoeae* (3). Although the serotype of the clinical isolate used in this study, 6498, is not known, it is possible that differences in performance could be attributed to the differential efficacy of the Roche AMPLICOR/COBAS CT/NG method group with different strain types. However, the product insert for the Roche AMPLICOR/COBAS CT/NG method group states that the lower detection limit is the same for all 12 serotypes tested: 80 inclusion-forming units/ml. It is possible that strain 6498 is also an L2 strain, although this would be unusual for a clinical isolate from the United Kingdom. In the future, it would be interesting to distribute defined serotypes at known concentrations in EQA panels to determine whether differences in detection and/or sensitivity actually occur with different serotypes in clinical laboratories. It is also possible that these results have highlighted the fact that the BDProbeTecET method is less able to detect the L2 strain at lower concentrations, and analysis comparing the Abbott LCx results to those of the BDProbeTecET method and the Roche AMPLICOR/COBAS CT/NG method group showed that the BDProbeTecET method was less likely to detect the L2 strain in the lower-concentration specimens whereas the Roche AMPLICOR/COBAS CT/NG method group was more likely to detect the L2 strain. Unfortunately, due to discontinuation of the manufacture of the Abbott LCx test, comparison with strain 6498 was not possible. Interestingly, McCartney et al. (20) found that the sensitivity and specificity of the BDProbeTecET method were comparable to those of the Abbott LCx in testing of male urine and (female) endocervical samples; however, a lower detection rate was found with the BDProbeTecET method for female urine specimens, which contain lower concentrations of *C. trachomatis*. Furthermore, the product insert information for the BDProbeTecET method states that detection levels differ depending on the serotype in question, ranging from 5 to 200 EB/reaction, and that 15 EB/reaction is the limit of detection for type LGV2. Differences in the sensitivity of detection may affect epidemiological data, and most importantly, the identification of infected patients may be missed, resulting in inappropriate treatment or no treatment and the development of serious physical sequelae, psychological effects, and further transmission of the pathogen.

The rate of false positives in this study was low (0.3 to 1.5%) and was not associated with any method. False positives were common in a single participating laboratory only, which reported three consecutive negative specimens as positive; all other false positives were reported by separate participants. The results found in this study are similar to those reported by Land et al. (0 to 3% false-positive *C. trachomatis* results with urine specimens [18]).

A major drawback of this study is the use of simulated specimens in place of real clinical specimens. However, it would be very difficult to obtain the required volume of endocervical swab medium with sufficient uniformity of consistency

and of *C. trachomatis* concentrations. Land et al. (18) reported that clinical urine specimens are problematic when used in EQA due to issues of stability and storage, and Kellogg et al. (17) noted that differences in detection are highly dependent on the quality of endocervical specimens. The benefits of using simulated specimens include the ability to define stability, concentration, and uniformity. The major difference between the two strains used in this study lies in the preparation of specimens with the chemical constituents of the medium used and the physical state of the specimens (liquid or freeze-dried). It is possible that these physical and chemical differences could have affected extraction and amplification efficiency, resulting in the differences in performance with the L2 strain. Strain 6498 was freeze-dried and was made with a liquid matrix different from that for the L2 strain. The process of freeze-drying could have damaged the *C. trachomatis* cells by disrupting cellular integrity, thereby releasing nucleic acid more readily. Freeze-drying could also have had an effect on the aggregation of the bacterial cells. However, the latter possibility is unlikely, because upon IF analysis, no aggregates were seen in freeze-dried specimens (data not shown). It is possible that the composition of the matrix could affect the detection of *C. trachomatis*, and specific chemical inhibitors and inhibitors in urine are known (4, 9, 19, 23). However, this study did not include inhibitors or their indicators such as hemoglobin, nitrates, and β-human chorionic gonadotropin in the specimens, although Earle's balanced salt solution with Bronidox and sucrose were used. Land et al. (18) included some specimens with inhibitors and noted that a significant number of participants reported a positive specimen with inhibitors as negative; these differences were not linked to specific methods.

A variety of sample types are tested for *C. trachomatis* in clinical laboratories. A recent poll of participants by UK NEQAS showed that 100% routinely test endocervical swabs and urine samples by molecular methods, with endocervical swabs representing the most frequently tested sample type. Of these laboratories, 51% also test other sample types (throat, rectal, and conjunctival swabs) less frequently. Varying the specimen types for EQA to include both endocervical swabs and urine specimens would more accurately reflect procedures in the clinical laboratory.

The current expanded gold standard for *C. trachomatis* detection includes the confirmation of a positive result with a second, different test (28). In 2002, meta-analysis showed that the majority of laboratories use one test only and do not confirm with a second, distinct test (28). In a recent UK NEQAS for Microbiology scheme (distribution 1734), 78% of participants reported that they routinely confirm positive results for *C. trachomatis* whereas 7% reported that they do not routinely confirm positives. Of those confirming positive results, only 1% used a different NAAT while 72% confirmed positive results by repeat testing with the same assay. Gaydos et al. (13) expressed concern at the cost of repeat testing. The retention of two separate NAAT platforms may simply be beyond the financial and staff resources of smaller laboratories.

In summary, the performance of participating laboratories in the detection of *C. trachomatis* in simulated EQA specimens was diverse. The detection of *C. trachomatis* by participants using NAAT was superior to that by those using EIAs. False positives were rare, and detection was excellent, at higher concentrations of *C. trachomatis* irrespective of the method used or the strain under test. The Roche AMPLICOR/COBAS CT/NG method group was more likely to detect the L2 strain than the Abbott LCx and BDProbeTecET methods in specimens containing lower concentrations, whereas no difference was noted between the Roche AMPLICOR/COBAS CT/NG method group and the BDProbeTecET method with clinical isolate 6498. The use of simulated specimens enabled powerful use of EQA results by testing differing strains and concentrations, reflecting the concentrations found in varying specimen types. The chemical matrix, physical presentation, and strain used in EQA specimens may affect results and should be considered in the design of EQA specimens. Future EQA studies should include a range of specimen types and a variety of *C. trachomatis* subtypes so as to more accurately reflect routine procedures in clinical diagnostic laboratories.

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