Can the Swedish new variant of Chlamydia trachomatis (nvCT) be detected by UK NEQAS participants from seventeen European countries and five additional countries/regions in 2009?

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In 2006, a new variant of *Chlamydia trachomatis* (nvCT) was reported in Sweden. The nvCT contains a deletion that includes the targets for the *C. trachomatis* genetic diagnostic single-target systems from Roche Diagnostics and Abbott Laboratories. Roche and Abbott have now developed certified dual-target assays that can detect the nvCT. This study examined the nucleic acid amplification tests (NAATs) currently used (in 2009) for *C. trachomatis* detection in laboratories from 17 European countries and five countries/regions outside Europe that are participating in the United Kingdom (UK) National External Quality Assessment Service (NEQAS). It further examined changes in these laboratories' testing strategy during the period from 2006 to 2009, and their performance regarding nvCT detection. A UK NEQAS blinded nvCT specimen was distributed to all 283 participating laboratories, which were asked to analyse the specimen according to their routine *C. trachomatis* diagnostic protocols for endocervical swabs. BD ProbeTec was the most commonly used NAAT, followed by Cobas Amplicor, Cobas TaqMan, and Aptima. From 2006 to 2009, the use of Cobas Amplicor, which does not detect the nvCT, decreased, but it was still used by 22% (n=57) of responding participants in 59% of the countries, 54 of these 57 used it as first assay. Virtually all of the other participants detected the nvCT correctly. Laboratories using commercial or *in house* NAATs that do not detect the nvCT are encouraged to carefully monitor their *C. trachomatis* incidence, participate in effective internal and external quality assurance and controls schemes, and to consider changing their testing system.

Introduction

In most middle- and high-resource settings nucleic acid amplification tests (NAATs) are the most commonly used tests for rapid, highly sensitive and specific detection of *Chlamydia trachomatis*.

In 2006, a new variant of *C. trachomatis* (nvCT), which contains a 377 bp deletion in the cryptic plasmid, was reported in Sweden [1,2]. This deletion includes the genetic targets for commercially available single-target systems that were at the time used worldwide, namely the Amplicor *C. trachomatis/Neisseria gonorrhoeae* (CT/NG) test, the Cobas Amplicor CT/NG test, and the Cobas TaqMan CT/NG test (Roche Diagnostics), as well as the RealTime CT/NG test (Abbott Laboratories). Subsequently, nvCT was identified in high proportions (10-65%) in most counties across Sweden. The affected NAATs were used in two thirds of the Swedish counties, and many thousands of false negative samples were reported [3-5]. Previous studies, using *ompA* gene sequencing and a new multilocus sequence typing (MLST), showed that the nvCT seems to be of clonal nature, belonging to genotype E and displaying a unique MLST sequence type [3,5]. Other commercial genetic diagnostic systems that are internationally available, such as a) the BD ProbeTec ET (Becton Dickinson), b) the Aptima CT and Aptima Combo 2 (Gen-Probe), c) the artus *C. trachomatis* PCR Kit (Qiagen), d) the artus *C. trachomatis* Plus PCR Kit (Qiagen), and e) the CHLAMYDIA tr. Q - PCR Alert Kit (Nanogen), were able to identify the nvCT; these NAATs target(s) are a) the cryptic plasmid (outside the deletion), b) specific 23S and 16S *rRNA* sequences, c) the *ompA* gene, d) both the *ompA* gene and the cryptic plasmid (outside the deletion), and e) the cryptic plasmid (outside the deletion), respectively.

Both Abbott Laboratories and Roche Diagnostics have now designed new sensitive and specific dual-target assays, namely the Abbott RealTime CT/NG (Abbott; new version, CE mark-certified in January 2008) that targets another sequence of the cryptic plasmid in addition to the sequence affected by the nvCT deletion, and the Cobas TaqMan CT v2.0 (Roche; CE mark-certified in June 2008) that detects the chromosomal *ompA* gene in addition to the sequence affected by the nvCT deletion [4]. Despite active surveillance and a number of studies performed in many countries [6], only sporadic cases of nvCT have so far been reported outside Scandinavia, e.g. in France [7], Ireland [8], and Scotland [9].

The aims of this report were to describe the NAATs currently used (in 2009) for *C. trachomatis* detection in laboratories from European countries (n=17) and countries/regions outside Europe (n=5) that are participating in the United Kingdom (UK) National External Quality Assessment Service (NEQAS). It further aimed to identify changes in these laboratories' testing strategy during the

period from 2006 to 2009, and to highlight their performance regarding detection of the nvCT.

Materials and Methods

The UK NEQAS distributes clinically relevant and educational specimens for external quality assessment (EQA). In the UK NEQAS scheme for *C. trachomatis* detection ('Molecular'), at present there are 283 participating laboratories (274 laboratories from 17 European countries and nine laboratories from five countries/regions outside Europe). However, most of the participating laboratories are in the UK (see Table 1). For surveillance and educational purposes, a blinded EQA specimen (Specimen 9119 in UK NEQAS Distribution 2402, issued in January 2009, as well as blinded specimens of three wildtype *C. trachomatis* strains) containing the nvCT, 1.67-3x10^4 elementary bodies per ml of reconstituted lyophilised specimen, was prepared as previously described [10]. Vacuum integrity and moisture content (<2%) of the freeze-dried specimen were validated and approved before distribution to all 283 participants. The laboratories were requested to reconstitute the specimen in molecular grade water and analyse the specimen according to their routine protocols for detecting *C. trachomatis* from an endocervical swab.

Results

Nucleic acid amplification tests (NAATs) used in 2009 for C. trachomatis diagnostics and changes in testing strategy during 2006-2009

Of the 283 laboratories participating in the scheme, 261 (92.2%) returned results on the nvCT specimen. In 2009, BD ProbeTec was the most commonly used main NAAT (39.5% of laboratories), followed by Cobas Amplicor (20.7%), Cobas TaqMan (16.1%), and Aptima (5.7%) (Table 1).

During the period from 2006 to 2009, the use of Cobas Amplicor decreased. However, it was still used as main NAAT in 2009 by 54 participants in 13 (59.1%) of the countries. In contrast, the numbers of laboratories using Cobas TaqMan, Abbott, and Nanogen Q-PCR have increased (Figure 1).

Detection of the Swedish new variant of C. trachomatis (nvCT)

The reporting laboratories used more than seven different commercial assays, *in house* single-target (n=7) or multi-target (n=3) real-time PCR assays, or did not specify their method (n=8). Twelve of the laboratories used two different assays (Table 2). However, specific testing algorithms used for routine diagnostics in these laboratories were not accessible.

Table 1

Countries and laboratories, including the main diagnostic assay used, participating in the UK NEQAS scheme for molecular detection of *Chlamydia trachomatis* in 2009

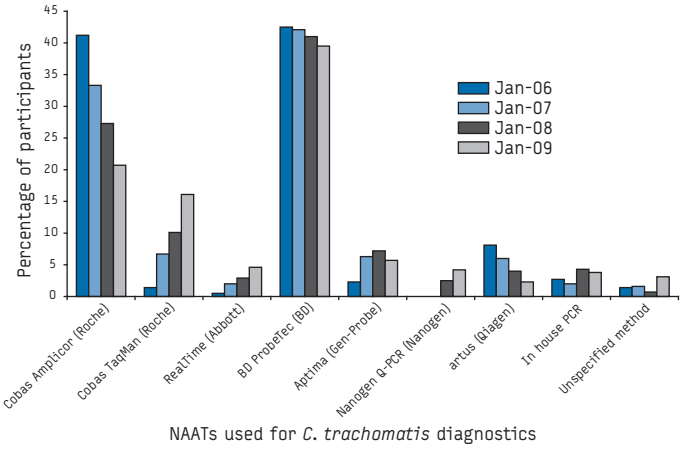
Country	No. of participating laboratories	Cobas Amplicor (Roche)[a]	Cobas TaqMan v2.0 (Roche)	Abbott RealTime (Abbott)	BD ProbeTec (Becton Dickinson)	Aptima Combo 2 (Gen-Probe)	Nanogen C. tr. Q-PCR Alert (Nanogen)	artus (Qiagen)[b]	In house single-target real-time PCR[c]	In house multi-target real-time PCR[c]	Unspecified method	Not returning results
Austria	5	2	-	-	1	-	-	1	-	-	1	-
Belgium	5	1	-	-	2	-	-	2	-	-	-	-
Croatia	1	1	-	-	-	-	-	-	-	-	-	-
Denmark	4	-	1	-	1	-	-	-	1	-	-	1
Finland	5	-	2	-	1	-	-	-	-	-	-	2
Germany	1	-	-	-	-	-	-	-	-	-	-	1
Greece	1	-	-	-	-	-	-	-	-	-	-	1
Hong Kong	2	1	-	-	-	-	-	-	-	-	-	1
Ireland	10	3	2	1	1	1	-	-	-	1	-	1
Israel	3	3	-	-	-	-	-	-	-	-	-	-
Italy	42	6	3	3	7	-	7	2	2	-	4	8
Kuwait	1	-	-	-	-	-	-	-	-	-	-	1
Macao	1	1	-	-	-	-	-	-	-	-	-	-
Malta	1	1	-	-	-	-	-	-	-	-	-	-
Netherlands	7	2	-	-	3	1	-	-	1	-	-	-
Norway	5	-	2	-	2	-	-	-	-	-	1	-
Portugal	5	-	-	1	2	-	1	-	-	-	1	-
Slovenia	2	-	1	-	-	-	-	-	1	-	-	-
South Africa	2	1	-	-	-	-	-	-	-	-	-	1
Sweden	8	-	1	1	4	-	-	-	-	-	-	2
Switzerland	14	10	-	2	1	-	-	-	1	-	-	-
United Kingdom	158	22	30	4	78	13	3	1	1	2	1	3
Total	283	54	42	12	103	15	11	6	7	3	8	22

[a] A few laboratories used Amplicor CT/NG (Roche). However, it was not possible to determine the exact number.
[b] Both artus *C. trachomatis* PCR Kit (*ompA* gene; Qiagen) and artus *C. trachomatis* Plus PCR Kit (*ompA* gene and cryptic plasmid; Qiagen) were used. However, it was not possible to determine how many laboratories used which kit.
[c] Details about *in house* assays were often reported and could not be accessed retrospectively.

Eighty percent (n=209) of the laboratories correctly reported the presence of *C. trachomatis* in the nvCT specimen (Figure 2). The majority (94%, 51/54) of the laboratories using Cobas Amplicor as their first assay reported a false negative result, as expected. However, one laboratory using Cobas Amplicor, an assay that can not detect the nvCT, reported a false positive result. Furthermore, two additional laboratories reported an equivocal result: They used Cobas Amplicor, which was negative, but to confirm their results used Aptima and Cobas TaqMan, which detected the nvCT correctly. The reasons for using this double testing strategy were not available. Presumably it does not reflect their routine diagnostics of all *C. trachomatis* samples. Furthermore, one laboratory using the Abbott system reported a negative result. All remaining laboratories reported a positive result (Figure 2).

Figure 1

Diagnostic assays (main NAAT) used by participating laboratories in the UK NEQAS scheme for molecular detection of *Chlamydia trachomatis* from 2006 to 2009*



*The total number of participating laboratories and laboratories returning results (in parenthesis) was 221 (100%), 263 (95.8%), 278 (100%), and 283 (92.2%), in 2006, 2007, 2008, and 2009, respectively.
NAAT: nucleic acid amplification test; NEQAS: National External Quality Assessment Service.

Discussion and conclusions

This report highlights the NAATs currently used (in 2009) for *C. trachomatis* detection in laboratories from 22 countries participating in the UK NEQAS scheme, alterations in their testing strategy during the period from 2006 to 2009, and their performance regarding detection of the nvCT.

Most of the laboratories (94%) using Cobas Amplicor, the second most common assay, as their first assay, reported an expected false negative result for the nvCT. However, two laboratories reported an equivocal result, i.e. negative with the Cobas Amplicor, but positive with an additional assay that detected the nvCT. One laboratory using the Cobas Amplicor assay reported a false positive result. This result suggests incorrect reporting either of the type of assay that was used or of the result, misinterpretation of the results, mix-up of specimens or contamination with other *C. trachomatis* strain or PCR amplicon.

One laboratory that was using the Abbott system and should have detected the nvCT, reported a negative result. A possible explanation could be that the older RealTime CT/NG test, the single-target assay that does not detect the nvCT, was used instead of the new Abbott RealTime CT/NG dual-target test. It is unlikely to reflect a sensitivity issue because the nvCT specimen contained a high number of elementary bodies per ml.

All other assays including the new Abbott RealTime CT and Roche Cobas TaqMan v2.0 performed well.

Laboratories that are still using Amplicor CT/NG, Cobas Amplicor CT/NG, and *in house* NAATs targeting the nvCT deletion in the cryptic plasmid are encouraged to monitor their C. trachomatis incidence in order to quickly identify unexplained significant declines in the normal or estimated local incidence and to alert reference centres about it. In addition, they are strongly encouraged to consider the feasibility of changing to a diagnostic method that can detect the nvCT, because using an additional NAAT on all negative samples is not feasible in the longer term.

Ideally, clinicians submitting samples to these laboratories should be objectively informed about the problem to diagnose the nvCT. An unexplained significant decline in incidence may be due to the emergence of nvCT. However, as other undetected mutants may emerge, monitoring of the incidence rate and participation of all laboratories in effective internal and external quality assurance and controls schemes are crucial.

Table 2

Combination of assays used in laboratories reporting using more than one assay for molecular detection of *Chlamydia trachomatis* in the UK NEQAS scheme in 2009

First assay	Second assay	No. of laboratories
Cobas Amplicor (Roche)	Cobas TaqMan v2.0 (Roche)	3
Cobas Amplicor (Roche)	Aptima Combo 2 (Gen-Probe)	1
Cobas TaqMan v2.0 (Roche)	Cobas Amplicor (Roche)	1
Cobas TaqMan v2.0 (Roche)	Aptima Combo 2 (Gen-Probe)	1
Cobas TaqMan v2.0 (Roche)	Nanogen C. tr. Q-PCR Alert (Nanogen)	1
BD ProbeTec (Becton Dickinson)	Cobas Amplicor (Roche)	1
BD ProbeTec (Becton Dickinson)	Aptima Combo 2 (Gen-Probe)	1
BD ProbeTec (Becton Dickinson)	In house single-target real-time PCR	1
In house single-target real-time PCR	artus (Qiagen)	1
Unspecified assay	Cobas Amplicor (Roche)	1

Based on the present study, it is obvious that a substantial number of laboratories in many European countries can still not detect the nvCT. However, the study only included laboratories participating in the UK-NEQAS scheme and thus gives a far from complete picture regarding the situation in the whole of Europe. The coverage in many participating countries was limited and it cannot be excluded that by selecting laboratories that are members of EQAS such as UK NEQAS a bias for high performance centres is introduced. Furthermore, no countries in eastern Europe were represented. In several of these countries, there are many shortcomings in the diagnosis of *C. trachomatis* and use of internationally available commercial NAATs is rare [11,12]. Some of the nationally produced and *in house* NAATs that are in use for diagnosis of *C. trachomatis* [11] may have their target in the nvCT plasmid deletion.

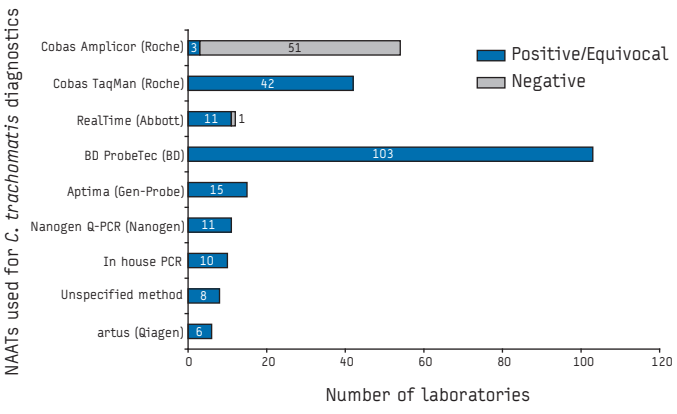
Even if the nvCT so far has been mainly detected in the Scandinavian countries, regular national and international surveillance, evaluation of the *C. trachomatis* diagnostic assays that are used, participation in external quality assessments including different diagnostic methods, and general evaluation of diagnostic guidelines are crucial. It cannot be excluded that the nvCT or other undetected mutants, e.g. *C. trachomatis* variants that do not contain the cryptic plasmid [13], are in a stage of early transmission in several countries. These mutants have a diagnostic selective advantage, can spread rapidly due to an accumulation of undetected and untreated cases that escape contact tracing, and may even possess biological advantages.

In comparison with wildtype *C. trachomatis* strains, no significant differences in symptoms and signs, sequelae, antimicrobial susceptibility, bacterial growth characteristics, cells/DNA load in NAAT samples have been associated with nvCT [3,4,14]. However, the incidence of nvCT in many Swedish counties has remained high and is even increasing in several counties using BD ProbeTec, an assay targeting a sequence outside the nvCT deletion. It has still not been ruled out whether the nvCT possesses particularly strong survival capabilities or other biological advantages over wildtype *C. trachomatis* strains. Further studies will soon be reported, which undertake a comprehensive phenotypic and genetic characterisation of the nvCT strain, estimate statistically the time point of emergence of the nvCT in certain Swedish counties, and follow the transmission of the nvCT in several Swedish counties, using Roche/Abbott and BD ProbeTec.

In general, more frequent and comprehensive internal and external quality assessment and quality assurance of different diagnostic methods may be required for many infectious agents worldwide, not just for *C. trachomatis*. The distributed control samples included in these exercises should reflect not only currently transmitted strains, but also temporally, geographically and genetically diverse strains. Ideally, most NAATs would use several species-specific targets in multicopy essential genes, giving diagnostic assays high sensitivity, specificity, and preventing false negative results due to different types of mutations.

FIGURE 2

Results and diagnostic method for detection of the new variant of *Chlamydia trachomatis* (nvCT) from 261 NEQAS laboratories in 22 countries in 2009



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