

Rapid communications

THE SWEDISH NEW VARIANT OF *CHLAMYDIA TRACHOMATIS* (nvCT) REMAINS UNDETECTED BY MANY EUROPEAN LABORATORIES AS REVEALED IN THE RECENT PCR/NAT RING TRIAL ORGANISED BY INSTAND e.V., GERMANY

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The May 2009 round of INSTAND's ring trial "*Chlamydia trachomatis* detection PCR/NAT" included a sample with high amount of the Swedish new variant of *C. trachomatis* (nvCT). A spectrum of at least 12 different commercial diagnostic nucleic acid amplification tests (NAATs) and many different *in house* NAATs were applied by the 128 participating laboratories which reported 152 results. Approximately 80% of the results correctly reported the presence of *C. trachomatis* in the nvCT specimen. The nvCT sample was mainly missed, as expected, by participants using the Roche COBAS Amplicor CT/NG (15.5% of reported results) but also by several participants using *in house* NAATs. The trend towards using nvCT-detecting NAATs is obvious and in addition to the new dual-target NAATs from Roche and Abbott, and BD ProbeTec ET, also a number of new CE mark-certified commercial tests from smaller diagnostic companies as well as many different *in house* NAATs were used. Laboratories using commercial or *in house* NAATs that do not detect the nvCT are encouraged to carefully monitor their *C. trachomatis* incidence, participate in appropriate external quality assurance and controls schemes, and consider altering their testing system. The reliable detection of low amounts of the wildtype *C. trachomatis* strain in other samples of the ring trial set indicates a good diagnostic performance of all applied commercial NAATs while also detecting the nvCT strain.

Introduction

With the increasing acceptance of nucleic acid amplification tests (NAATs) in the field of diagnostic microbiology and the broad availability of open platforms to perform an exponentially growing spectrum of *in house* and/or commercially prefabricated NAATs, there is a growing demand for appropriate internal and external quality control (QC) activities. One comprehensive external quality assessment scheme (EQAS) for diagnostic NAATs was established in 2002 by the German Society for Promotion of Quality Assurance in Medical Laboratories, INSTAND e.V. (www.instand-ev.de). This subscheme of INSTAND's well-established quality control initiatives, named "bacterial genome detection PCR/NAT", offers certified proficiency testing panels for prominent bacterial pathogens on a biannual basis. A detailed discussion of the current and the previous EQAS schemes can be found at: http://www-nw.uni-regensburg.de/~re24900.mmh.klinik.uni-regensburg.de/INSTAND_e.htm.

In 2006, a new variant of *Chlamydia trachomatis* (nvCT) was identified in the Swedish county of Halland by Ripa and co-workers [1]. This mutant strain is characterised by a 377-bp deletion in ORF-1 of the multicopy cryptic plasmid, which includes the target region of both the Roche and Abbott *C. trachomatis* NAATs available at that time [2]. The currently available new redesigned dual-target assays, namely the Abbott RealTime CT/NG (CE mark-certified in January 2008) that targets another cryptic plasmid sequence in addition to the sequence affected by the nvCT deletion, and the Roche COBAS TaqMan CT v2.0 (CE mark-certified in June 2008) that detects the chromosomal *ompA* gene in addition to the sequence affected by the nvCT deletion, have replaced the former assays [3].

Immediately after the first report on the nvCT, international studies were conducted to determine whether the nvCT was present in different settings across Europe, the United States, Australia [3-5]. Only sporadic cases have so far been reported outside the Nordic countries [3-5], however, current knowledge regarding the presence and prevalence of nvCT in other countries is highly limited due to few recent studies and the fact that many European laboratories can still not detect the nvCT [4], and those that can are not aware of it because no nvCT-specific or other distinguishing NAATs are used. Ideally all laboratories should use NAATs that detect nvCT, because a wider geographic spread of this variant can not be excluded.

To supplement a recent Eurosurveillance publication on a United Kingdom National EQAS (UK NEQAS) distribution [4], the present report provides a concise reflection on diagnostic performance and NAATs used by European laboratories participating in the May 2009 INSTAND e.V. ring trial regarding detection of the nvCT. Assuming that most diagnostic laboratories are participating in one external QC scheme only, the intersection between UK NEQAS and INSTAND ring trials should be very limited and the present study represents an additional exploratory piece in the jigsaw puzzle of European *C. trachomatis* NAAT testing regimens.

Materials and methods

The May 2009 round of INSTAND's EQAS "bacterial genome detection PCR/NAT" included two panels for *C. trachomatis* detection. One set of four lyophilised blinded samples was offered

for participants using combined detection of *C. trachomatis* and *Neisseria gonorrhoeae* (RV 530), and a separate set for those detecting *C. trachomatis* only (RV 531).

The latter set (*C. trachomatis*; RV 531) contained a sample with ~105 inclusion forming units (IFUs) of the nvCT strain per ml of reconstituted lyophilised specimen. This set was completed by two samples containing ~103 IFUs/ml of a wildtype *C. trachomatis* strain and one sample without *C. trachomatis* in a natural background of human and bacterial cells. The laboratories were requested to reconstitute the specimen in 300 µl of molecular grade water and analyse a 100 µl portion of the specimen, according to their routine protocols for detecting *C. trachomatis* from an endocervical swab.

Results

Response rate

NAAT results for distribution RV 531, which included the nvCT sample, were returned by 128 laboratories (100% of participants), including 115 laboratories from Germany, 12 from nine other European countries and one from United Arab Emirates (Table). For unknown reasons, some laboratories applied more than one *C. trachomatis* NAAT, which probably does not reflect their routine diagnostic workup for *C. trachomatis*. Due to this reporting of results from multiple assays and/or lack of assay specifications in the reports, the effective number of results (n=152) is higher than the number of participants (n=128).

Nucleic acid amplification tests (NAATs) used for *C. trachomatis* diagnostics

The change in the spectrum of NAATs applied by the participants in the German INSTAND schemes from 2006 to 2009 is depicted in Figure 1. Especially in the current round (2009), the spectrum of NAATs used in the German INSTAND ring trial substantially differed from the recent UK NEQAS ring trial [4]. In 2009, Roche COBAS Amplicor CT/NG (15.5% of participants) and BD ProbeTec ET (Becton Dickinson; 15.5%) were the most commonly used main NAATs, followed by Roche Cobas TaqMan (14.8%) and Abbott RealTime CT (11.0%). Nevertheless, from 2006 to 2009, the use of Roche COBAS Amplicor CT/NG and Roche Amplicor

CT/NG rapidly decreased from 37.4% to 15.5%, and 6.8% to 0%, respectively. In contrast, the numbers of laboratories who have shifted to the new dual-target assays Abbott RealTime CT/NG and Roche COBAS TaqMan CT v2.0 significantly increased. Furthermore, especially in the recent years several new or at least less popular commercial *C. trachomatis* NAATs as well as many *in house* NAATs were in use (Figure 1).

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

Twelve different commercial assays were used for reporting results (n=106) on the nvCT sample. Furthermore, use of "other commercial assays" was indicated in 19 results, *in house* real-time PCR assays in 23, and in four results the NAAT was not specified (Figure 2). In 80% (n=122) of the results the presence of *C. trachomatis* was reported correctly. As expected, the nvCT sample was missed by those using the Roche COBAS Amplicor CT/NG (n=15). One laboratory that used the Abbott system reported a negative result, which suggests that the older single-target RealTime CT/NG test (not detecting the nvCT) was used. Furthermore, participants using "other commercial kits" (n=5), *in house* PCRs (n=7), and completely unspecified assays (n=2) reported negative results (Figure 2).

Aside from the nvCT sample, a very good performance was observed for the detection of small amounts of the wildtype *C. trachomatis* strain in the other two positive specimens (~103 IFUs/ml) and the negative specimen of the QC panel RV 531. Ninety-two percent of all laboratories reported correct results for these three samples. A mean accuracy rate of 97% was observed among participants using commercial assays, whereas the mean accuracy rate was 86% when *in house* or "other" assay formats were used.

Discussion and conclusions

Pathogen- and method-specific ring trials (EQAS) organised by independent institutions have repeatedly proven to be valuable external quality control measures. In addition to assessing the diagnostic performance (analytical sensitivity and specificity) of different assays at individual laboratories, the statistical analysis of the results provides an actual snapshot on the technology and use of commercial or *in house* NAATs for detection of a given pathogen among the participants.

The results of the latest UK NEQAS [4] and German INSTAND (present study) quality assessment distributions for molecular detection of *C. trachomatis* clearly show that a substantial number of laboratories can still not detect the nvCT. A broader spectrum of NAATs, including many different internationally less popular and recognised commercial NAATs and *in house* NAATs, was applied in the INSTAND ring trial. This may reflect a trend, at least in Germany (90% of participants), towards the use of individual PCR assay formats and amplicon detection platforms mainly observed in smaller laboratories. These laboratories are typically facing a smaller number of samples per day but still try to keep the test frequency high enough to end up with short turn-around-times for their PCR results. Under these circumstances, diagnostic tests (or assay platforms) designed for really large sample numbers can usually not be operated economically and the use of customised kits and/or assay formats indeed makes sense, i.e. as long as they are thoroughly validated and reliable. As an aid to orientation, the inclusion of as many assays as possible from "smaller companies" in challenging EQAS schemes is appreciated in this respect.

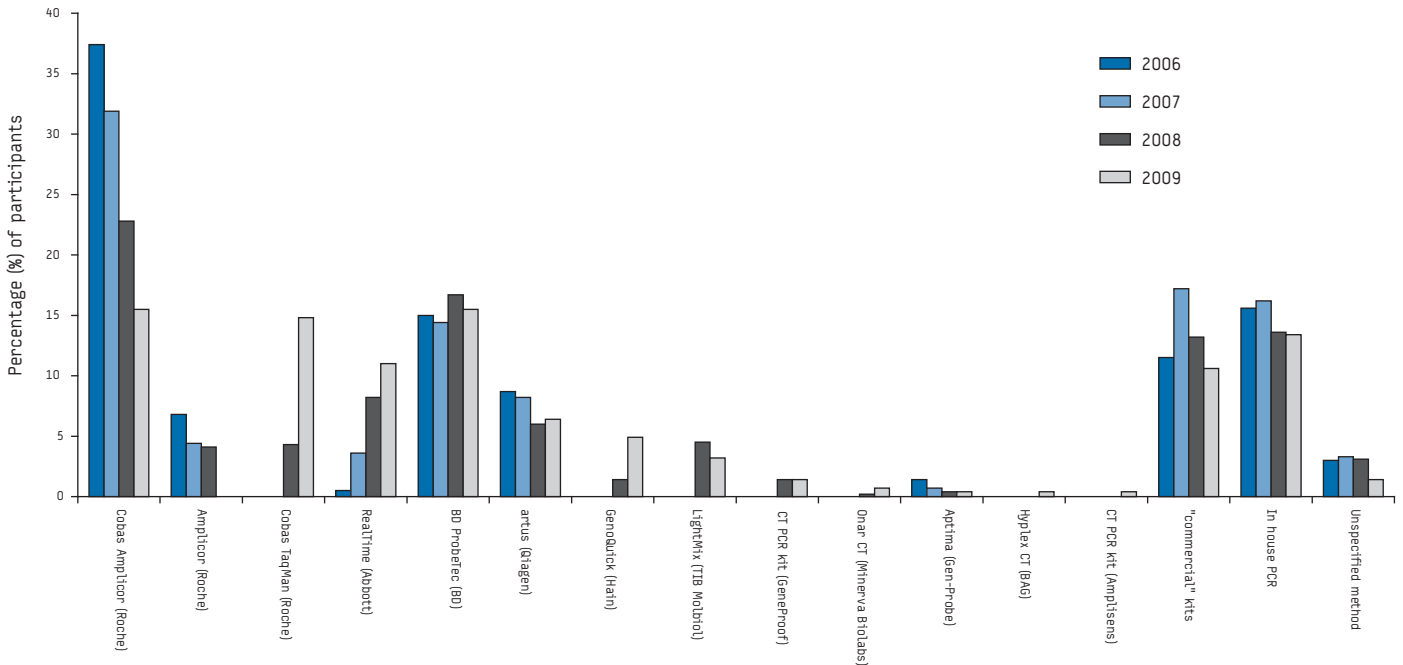
TABLE

Number and geographic location of the laboratories participating in the INSTAND scheme "bacterial genome detection PCR/NAT", distribution RV 531, May 2009

Country	Number of participants
Germany	115
Czech Republic	3
Slovakia	2
Austria	1
Bulgaria	1
Cyprus	1
France	1
Hungary	1
Russian Federation	1
Switzerland	1
United Arab Emirates	1
Total	128

FIGURE 1

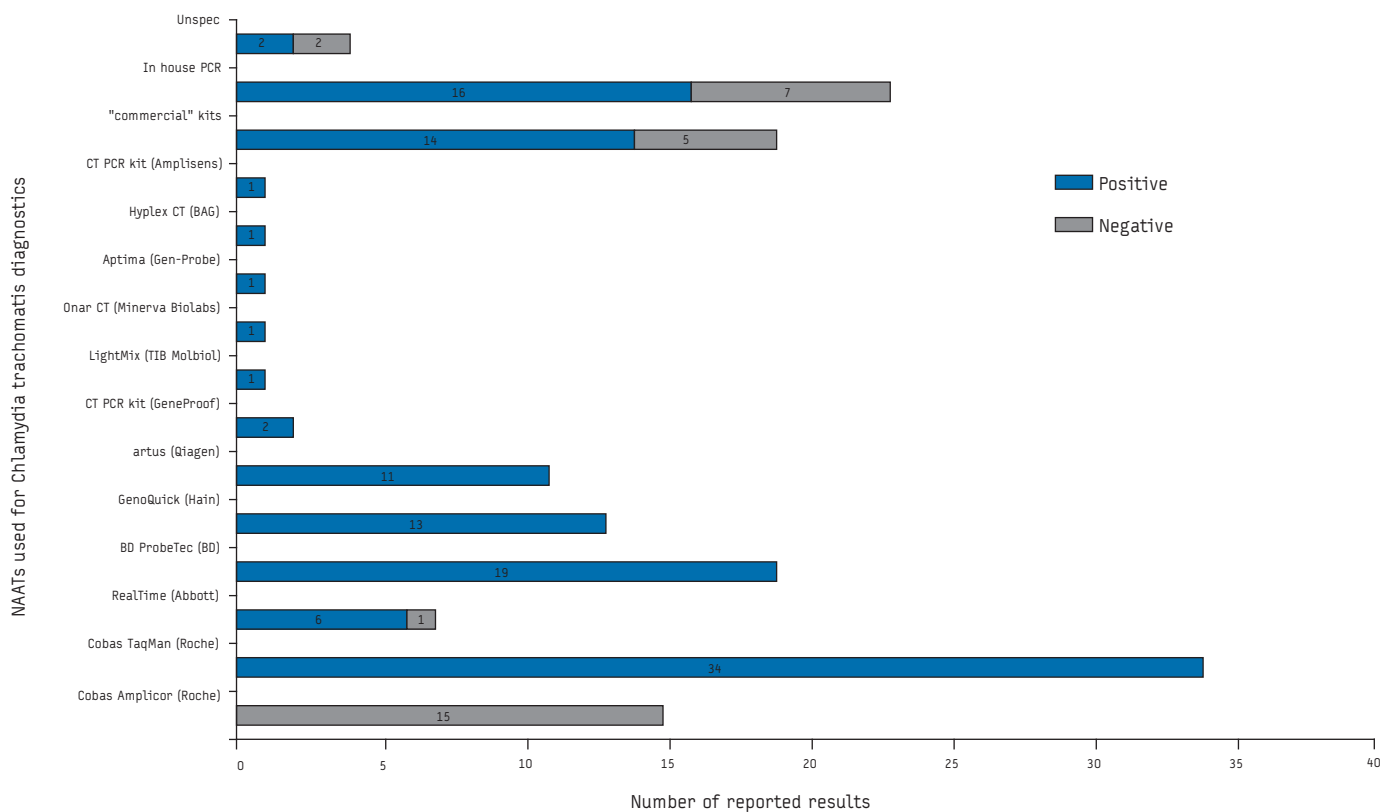
Main nucleic acid amplification tests (NAATs) used by participating laboratories in the German INSTAND schemes RV 530 and 531 for molecular detection of *Chlamydia trachomatis* from 2006 to 2009



NAATs used for *Chlamydia trachomatis* diagnostics

FIGURE 2

Methods and corresponding results regarding detection of the new variant of *Chlamydia trachomatis* (nvCT). Data from the INSTAND's RV 531 distribution were analysed (152 results from 128 laboratories in 11 countries)



As also reported from the previous UK NEQAS study [4], the use of the former versions of Roche Cobas Amplicor CT/NG and Amplicor CT/NG, which do not identify the nvCT, has rapidly declined. However, a substantial number of laboratories are still using Roche Cobas Amplicor CT/NG [4, present study] and these laboratories should consider changing their testing system. Another worrying aspect revealed by the present study is the continued use of some *in house* NAATs, which were not specified in detail by the participants, that also miss the nvCT. In order to detect the nvCT, laboratories using these *in house* PCR assays are recommended to consider changing their testing system, altering the probe and/or primer set in their *in house* NAAT, introducing an additional target in their *in house* NAAT, or introducing an additional assay not affected by the mutation, i.e. for dual testing. Dual testing is however often restricted by a more complicated workup procedure, including specimen splitting, different methodological protocols, and additional costs. Considering the currently still presumed low prevalence of the nvCT strain outside northern Europe, routine diagnostic application of nvCT-specific NAATs is not necessary. Nevertheless, as already mentioned above, at present the true prevalence of the nvCT outside the Nordic countries is mainly unknown.

In conclusion, laboratories using commercial or *in house* NAATs that do not detect the nvCT are encouraged to (a) carefully monitor their *C. trachomatis* incidence for unexplained declines, (b) frequently participate in effective internal and external quality assurance and control schemes, and (c) ideally to consider changing their testing system. This is crucial for an early detection as well as reliable surveillance of the nvCT, but also of other possibly undetected mutants, and, accordingly, the first two points are advisable for all diagnostic laboratories.

The nvCT strain will certainly be included again in one of the future rounds of INSTAND's PCR/NAT *C. trachomatis*-specific ring trials. It will be interesting to see whether the "affected" laboratories have learnt their lessons and switched to NAATs that also detect the nvCT.

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