

Chlamydia trachomatis false-negative test results by Aptima Combo 2 CT/NG assay (Hologic Inc.) in the EU/EEA, 2019

Summary

In April 2019, Finland reported false-negative/equivocal results in patients tested for *Chlamydia trachomatis* (CT) using Aptima Combo 2® Assay (Hologic Inc., USA) (AC2). Between February and May 2019, over 190 specimens that tested negative/equivocal with AC2 (targeting the 23S rRNA gene) of 2314 specimens tested were positive with Aptima CT Assay (ACT) (targeting 16S rRNA gene). A patient tested in June 2018 was identified so far as the earliest case with discordant results. In the laboratories using AC2 in Finland, the cases that might have been missed due to false-negative/equivocal results may amount to 6 to 10% of CT positive cases.

In mid-May 2019, Hologic confirmed that a mutation in the 23S rRNA gene is the likely root cause of these discordant results and issued an Urgent Field Safety Notice in June 2019 with updated instructions for test results interpretation and reflex testing of samples to laboratories using AC2. Implementation of these measures will allow the appropriate diagnosis and management of the variant CT.

Two samples in Sweden have been confirmed as having the 23S rRNA gene mutation. At the time of writing of this risk assessment, additional Member States are investigating discordant AC2/ACT results.

In the short term, there is a need to understand the spread of the new CT variant in order to inform the need for patient re-call and re-testing in settings where AC2 is used. Member States may therefore consider the following:

- Review CT notification rates and investigate any unexplained changes in epidemiology and/or positivity rates;
- Investigate the presence of the new variant by using the proposed case definitions for possible (AC2-negative/equivocal sample with RLU ≥ 15 and positive result in another CT assay using an alternative CT target) and confirmed cases (positive result for CT which has the C1515T mutation in the 23S rRNA gene);
- Re-calling for testing with ACT/other platforms those patients who may have possibly received false negative results by AC2 (i.e. negative result with RLU ≥ 15) and, as a matter of urgency if larger numbers of confirmed cases are detected. A six-month look-back period may be considered initially, however each Member State should decide on the length of the look-back period based on local investigations and taking into account the duration of CT infection, social consequences for patients and their contacts, risk of reinfection and resource needs.

In the longer term, additional research questions need to be addressed including whether the variant strain has different severity/virulence/risk of complications, clarifications on the emergence of the strain and on its molecular epidemiology.

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Event background

In April 2019, experts from Finland informed ECDC and the STI Network in EU/EEA Member States through the Epidemic Intelligence System for Sexually Transmitted Infections (EPIS STI) of their discovery that certain *Chlamydia trachomatis* (CT) samples had tested falsely negative using the Aptima Combo 2[®] Assay (Hologic Inc., USA) (AC2). AC2 is a second-generation nucleic acid amplification test that mainly qualitatively detects CT 23S rRNA and/or *Neisseria gonorrhoeae* (NG) 16S rRNA.

In mid-February 2019, the Clinical Microbiology Laboratory of Turku University Hospital in Finland, observed a discrepancy in the test results of a patient whose sample was positive for CT by a multiplex sexually transmitted infection (STI) assay (Allplex STI Essential, Seegene, Seoul, Korea) yet negative when tested using AC2 in the Panther Instrument (Hologic Inc.). In addition, the patient had a partner who tested CT-positive in a laboratory from central Finland where a different diagnostic assay is used for chlamydia and gonorrhoea screening (Abbott m2000, Abbott Park, Illinois, USA). In the following week, samples from two other patients with clinical suspicion of CT infection were reported as negative by AC2 but positive in Allplex [1].

The results of AC2 are given in *relative light units* (RLU). Assay results are determined by a cut-off based on the total RLU and the kinetic curve type and reported as negative, equivocal or positive for CT and/or NG. If only a CT signal is detected and if RLU is <25, the equipment gives a negative CT result. The result is interpreted as equivocal or negative (depending on the Panther interpretation of the dual kinetic for the sample) if RLU is ≥ 25 and < 100 and positive result if RLU is ≥ 100. If both CT and NG signals are present, the range for CT negative results is RLU <85, for equivocal results ≥ 85 and < 250, and for positive results ≥ 250 [2]. The manufacturer recommends that equivocal samples should be retested and if equivocal on retesting a new sample should be requested [2]. Hologic Inc also markets the Aptima *Chlamydia trachomatis*[®] Assay (ACT). The ACT assay targets CT 16S rRNA and has cut-off values of RLU 100 for low-positive and 5,000 for positive [3]. There are no concerns about the ACT assay.

The AC2 RLUs for the samples of the three patients that were CT positive with the Allplex assay were between 23 and 28 RLU and were all interpreted as negative by the instrument. When these specimens were re-tested using ACT, the samples of the three patients were positive and had RLUs >6000 [1].

In Finland, between February and 24 May 2019, 2 314 specimens which were negative or equivocal with AC2 were retested with ACT. Of these, 196 resulted positive using ACT. These discordant samples had RLU values between 3 and 101 on AC2. Almost all AC2-negative or equivocal samples with RLU values between 20 and 84 retested positive on ACT (for example, 13 out of 15 in Turku, 87%). Demographic information was available for 25 patients from Turku, 14 were females, the mean age was 28 years (range 17-48 years) and they were predominately heterosexual.

Most cases were detected in southern and western Finland where AC2 is predominately used by clinical laboratories in both the public and private sectors. In addition, 17 samples that had been kept following a positive result with the Anyplex STI-5 II Detection test were available. Of these, one sample of a male case taken in late June 2018, was found to be negative with AC2 but positive with ACT. This has been the earliest AC2/ACT discordant specimen detected to date (AC2 RLUs of 22 and 19, sample tested twice).

Overall, approximately 50% of combined CT-NG diagnostic tests in Finland are performed with AC2. Based on estimates from one laboratory in Finland, AC2-negative or equivocal/ACT-positive cases may amount to 0.4% of all tested samples and an additional 6% to 10% of chlamydia diagnoses during the latter half of 2018 [1].

Considering the risk of complications and sequelae from CT infection and to reduce further transmission, laboratories in affected regions in Finland have recalled patients who tested negative with AC2 and had RLU values above 20. The period of recall varied by hospital district [4].

Specimens from 10 of the AC2-negative or equivocal and ACT-positive cases were sequenced (sequencing of 23S rRNA, 16S rRNA and typing based on the *ompA* gene) and when compared with the reference strain sequence, CT E/Bour (HE601870.1), there was a single nucleotide change in the CT23S rRNA gene in position 1515 (C→T) in the discordant specimens. All 10 analysed specimens had the same change, whereas this change was not found in CT reference strain sequences deposited in GenBank or in previously sequenced CT isolates from Finland, which had been AC2-positive [1]. In mid-May 2019, following receipt of samples from Finland, Hologic confirmed that the C1515T mutation in the 23S rRNA gene is likely to be the root cause of these false-negative AC2 results. A synthetic RNA transcript corresponding to the CT 23S rRNA with a C1515T mutation yielded a significantly suppressed CT detection probe-signal in AC2 as observed with clinical samples containing the mutated CT strain [5].

On 24 May 2019, the ECDC organised a teleconference with participants from 12 Member States, The International Union against Sexually Transmitted Infections (IUSTI) and the European Commission, to obtain more details about the investigation in Finland, understand if other countries have similar observations, share experience from Sweden in response to the Swedish new variant of CT (nvCT) that emerged in 2006 (see below) and agree on any further steps.

As of 13 June 2019, two AC2 false-negative CT cases with the C1515T mutation in the 23S rRNA gene have been verified in Sweden [6], which are the first two cases outside Finland. In addition, other Member States have begun investigating discordant results and a small number of samples are being sequenced. Results are expected shortly. Sequencing of the 23S rRNA gene is essential to determine whether these discordant results are due to the mutated CT strain found in Finland and not only due to the different performance characteristics of AC2 and ACT.

On 7 June 2019, Hologic Inc. started distributing an Urgent Field Safety Notice to laboratories using AC2 [7]. The notice recommends changes in test result interpretation and procedures for reflex testing with ACT. In addition Hologic has informed ECDC that they are developing a revised version of the AC2 test and, for the short term, a “variant-specific” research-use-only assay to support scientific investigations [5,7].

ECDC risk assessment for the EU/EEA

This event is the second documented CT test ‘escape variant’ following the incident of the 2006 Swedish nvCT, which was detected following an investigation into an unexpected decline in CT notifications in the Halland region of Sweden [8]. It was estimated that around 8,000 chlamydia cases were missed in Sweden due to the nvCT and that the strain had been circulating for several years before detection, likely with a prevalence of 1% of CT infections in late 2002 [9,10]. The prevalence of nvCT in Sweden varied between 20% and 65% in counties using NAATs which did not detect nvCT [11]. In the current event in Finland it is estimated that, in areas where AC2 is used, 6-10% of all CT diagnoses may have been missed because of falsely negative AC2 results [1]. This may have a major impact on the validity of CT testing in countries implementing widespread screening programmes using AC2. In 2017, 409 646 cases of CT were reported in the EU/EEA [12,13].

This finding that 6-10% of CT diagnoses may have been missed (based on discordant AC2/ACT results) in areas using AC2 in Finland is a cause of concern. Left untreated, CT infection can progress to damage the upper reproductive tract and cause serious reproductive tract complications including pelvic inflammatory disease, ectopic pregnancy and tubal factor infertility [14]. CT can also be transmitted from mother to baby during labour leading to infection in the neonate. Evidence from randomised controlled trials indicates that offering women CT tests and treatment can reduce the risk of pelvic inflammatory disease at 12 months after testing by 35% [14]. Repeated testing in 3–6 months to detect reinfections should be offered to young women and men (<25 years of age) who test positive for CT [15].

The Swedish nvCT did not spread widely in Europe [9,16]. The reasons for the lack of spread of the nvCT outside Sweden are not known, but might possibly be because the nvCT was rare in populations with more international sexual networks, e.g. men who have sex with men (MSM) [16]. Investigations so far have led to identification of confirmed cases in Sweden [6]. In addition, discordant AC2/ACT results are under investigation in additional EU/EEA Member States.

To date there is scarce information on the epidemiology of the variant CT cases detected in Finland. Results from the investigation so far and personal communication with the investigators, indicate that there is an even gender distribution and where the sexual orientation is known, these cases are reported to be heterosexual. The mean age was 28 years, which is slightly higher than the overall mean age of cases diagnosed in Finland and in the EU/EEA in 2017 - reported in TESSy to be 25 years in both cases [12]. There have been no confirmed variant CT cases reported among MSM so far.

Based on the information available so far, it appears possible that one or more CT strains carrying the C1515T mutation in the 23S rRNA gene are present in additional EU/EEA Member States, but investigations to provide evidence for this are still pending.

There is, so far, no evidence available on whether the detected variant CT strain has an increased risk of transmission, different severity or risk of complications.

Options for response

The actions already taken and which will be carried out by Hologic Inc. are described in the Urgent Field Service Notice [7] and a recent Eurosurveillance letter [5]. It may be expected that as laboratories implement the recommendations in the Notice, more false-negative AC2 results will be identified through re-testing with alternative platforms and managed appropriately. Despite this, there are a number of other important options for public health actions that need to be considered.

In the short term, in settings where AC2 is used, understanding the spread of the new CT variant(s) in order to inform the need for patient re-call and re-testing is essential:

- Member States need to review their chlamydia notification rates and investigate any unexplained changes in epidemiology and/or chlamydia test positivity rates. These analyses can provide some indication on whether CT cases are being missed in the country. Finnish laboratories, however did not observe any

major changes, which might indicate less spread of the CT variant than initially anticipated. 23S rRNA sequencing data of all cases in Finland is urgently needed. In Finland, the initial cases were detected following close collaboration between astute laboratory staff and clinicians, highlighting the importance of collaborative review of unusual observations, comparison to previous test results of the same patient as well as to partner test results where available.

- Member States with laboratories that use AC2 need to investigate the presence of the new variant(s). The following case definition is being proposed [5]:
 - Possible case: a person with an AC2-negative or equivocal sample yielding RLU ≥ 15 and a positive result in a reflex CT assay using an alternate CT target.
 - Confirmed case: a person with a positive result for CT which has the C1515T mutation in the 23S rRNA gene.
- Possible cases should be identified prospectively and if stored samples are available, retrospectively. Samples from possible cases should be kept and stored frozen. If any possible cases are identified, these need to be confirmed by sequencing or, when available, using alternative validated tests. This is critical in order to determine the need for recalling patients (if there are no cases with the C1515T mutation in the 23S rRNA gene detected in a country then no recalls are likely to be needed) which can require significant resources and have an important impact on patients. ECDC is discussing with the STI network optimal ways of facilitating a rapid, validated, sequencing service and ways of supporting Member States wishing to implement sequencing of the 23S rRNA gene nationally.
- Investigations at country level should include, where possible, the collection of epidemiological data, including at a minimum, age, gender, sexual orientation and travel history, both on possible and confirmed cases in order to describe the epidemiology of the new variant and inform patient recall. Following this, the collation of these data at EU/EEA level is important in order to provide a more complete picture of the distribution of the new CT variant in the EU/EEA which should help to support Member States in their public health response (protocols are under development and will be agreed with the STI network).
- Member States which detect at least one confirmed case, should consider re-calling for testing with ACT/other platforms those patients tested with AC2 who may have possibly received false negative results (i.e. negative result with RLU ≥ 15 , although the cut-off may be modified as additional data are analysed). Such a recall should be considered urgently if larger numbers of confirmed cases are detected. Considering the limited data available on the prevalence of the variant, the difficulties in identifying samples for retrospective testing and the challenges in sequencing samples, as well as the precautionary principle, the threshold for re-calling patients should be low. The look-back period for the re-call will need to be assessed by each Member State but should initially be around six months [14], although this also needs to be informed by the local investigations (taking into account the duration of CT infection, social consequences for patients and their contacts, risk of reinfection and resource needs). Assessment of positivity rates of re-tested patients will help inform look-back periods [5]. In Finland, the look-back period was determined by hospital districts. In Turku, patients were recalled from the beginning of 2018, six months before the first detected discordant result, whereas in other hospital districts patients were recalled for the six months before corrective measures were implemented. In Sweden, for the nvCT response, the lookback period varied by county and mainly ranged from 3 to 12 months.

In the longer term, additional scientific questions will need to be addressed, including whether the detected variant strain has different severity/virulence/risk of complications, more details on emergence of the strain and on the molecular epidemiology of the strain [5]. Considering that this event is the second CT test escape variant documented in the EU/EEA within 15 years, EU/EEA Member States and test manufacturers should consider the need to implement surveillance for such variants, particularly for diagnostic nucleic acid amplification tests targeting a single genetic region for CT and possibly other pathogens including NG [5]. Such an initiative could be coordinated at the EU/EEA level considering that the same commercial assays are used in many EU/EEA Member States.

Source and date of request

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All experts have submitted declarations of interest and a review of these declarations did not reveal any conflict of interest.

Experts from WHO reviewed the risk assessment, but the views expressed in this document do not necessarily represent the views of WHO.

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This report was written with the coordination and assistance of an Internal Response Team at the European Centre for Disease Prevention and Control. All data published in this threat assessment brief are correct to the best of our knowledge at the time of publication. Maps and figures published do not represent a statement on the part of ECDC or its partners on the legal or border status of the countries and territories shown.

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