RAPID RISK ASSESSMENT

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

17 June 2019

Summary

In April 2019, Finland reported false-negative or equivocal results in patients tested for *Chlamydia trachomatis* (CT) using Aptima Combo 2 Assay (Hologic) (AC2). From February–May 2019, over 190 specimens that tested negative and/or equivocal with AC2 (targeting the 23S rRNA gene) of 2 314 specimens also tested positive with Aptima CT Assay (ACT) (targeting the 16S rRNA gene). A patient tested in June 2018 has been identified as the earliest case with discordant results. In laboratories using AC2 in Finland, the cases that may have been missed due to false-negative or equivocal results may amount to 6–10% of CT positive cases.

In mid-May 2019, Hologic confirmed that a mutation in the 23S rRNA gene is the likely root cause of the discordant results and issued an Urgent Field Safety Notice in June 2019 with updated instructions for test result interpretation and reflex testing of samples to laboratories using AC2. The implementation of these measures will allow for 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 issuing 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 recall and retesting 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 relative light units (RLUs) ≥ 15 and positive result in another CT assay using an alternative CT target) and confirmed cases (positive result for CT that has the C1515T mutation in the 23S rRNA gene); and
- recall for testing with ACT or other platforms 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, 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 and/or risk of complications and clarifications on the emergence of the strain and 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 CT samples had tested falsely negative using the Aptima Combo 2 Assay (Hologic) (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 tested positive for CT by a multiplex sexually transmitted infection (STI) assay (Allplex STI Essential, Seegene, Seoul, South Korea), but negative when tested using AC2 in the Panther instrument (Hologic). 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, US). 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 RLUs. Assay results are determined by a cut-off based on the total RLUs 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 the RLUs are <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 the RLUs are ≥ 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 RLUs <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 also markets the Aptima *Chlamydia trachomatis* Assay (ACT). The ACT assay targets CT 16S rRNA and has cut-off values of 100 RLUs for low-positive and 5 000 RLUs 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 23–28 RLUs and all interpreted as negative by the instrument. When these specimens were retested using ACT, the samples of the three patients were positive and had RLUs >6 000 [1].

In Finland, from February–24 May 2019, 2 314 specimens that were negative or equivocal with AC2 were retested with ACT. Of these, 196 tested positive using ACT. The discordant samples had RLU values of 3–101 on AC2. Almost all AC2-negative or equivocal samples with RLU values of 20–84 retested positive on ACT (for example, 13 of 15 in Turku; 87%). Demographic information was available for 25 patients in 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 was 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 have amounted to 0.4% of all tested samples and an additional 6–10% 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 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, but this change was not found in CT reference strain sequences deposited in GenBank or in previously sequenced CT isolates from Finland that 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 probably the root cause of the 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 experiences from Sweden in response to the Swedish new variant of CT (nvCT) that emerged in 2006 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], 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 the 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 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

The 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 Halland County, Sweden [8]. It was estimated that around 8 000 chlamydia cases were missed in Sweden due to the nvCT and that the strain had circulated for several years before detection, probably with a prevalence of 1% of CT infections in late 2002 [9,10]. The prevalence of nvCT in Sweden varied from 20– 65% in counties using NAATs that 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].

The 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 child 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 localised spread of the nvCT only Sweden are unknown, but may 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 the identification of confirmed cases in Sweden [6]. In addition, discordant AC2/ACT results are under investigation in additional EU/EEA Member States.

To date, information is scarce 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 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 no evidence available so far 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 those that will be carried out by Hologic 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 retesting 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 recall and retesting 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 certain indications on whether CT cases are being missed in the respective country. However, Finnish laboratories did not observe any major changes, which may indicate less spread of the CT variant than initially anticipated. The 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 and comparison with previous test results from the same patients, 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 definitions are proposed [5]:
  - possible case – person with AC2-negative or equivocal sample yielding RLU ≥ 15 and positive result in reflex CT assay using alternate CT target; and
  - confirmed case – person with positive result for CT that has C1515T mutation in 23S rRNA gene.

- Possible cases should be identified prospectively and retrospectively if stored samples are available. Samples from possible cases should be kept and stored frozen. If any possible cases are identified, they need to be confirmed by sequencing or using alternative validated tests when available. This is critical in order to determine the need for recalling patients, which can require significant resources and have an important impact on 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. ECDC is discussing optimal ways of facilitating a rapid validated, sequencing service and means to support Member States wishing to implement sequencing of the 23S rRNA gene nationally with the STI Network.
- 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 the 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 that detect at least one confirmed case should consider recalling for testing with ACT and/or 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 recalling patients should be low. The look-back period for the recall 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 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 retested 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, while in other hospital districts, patients were recalled for the six months before corrective measures were implemented. In Sweden, the look-back period varied by county for the nvCT response and mainly ranged from 3–12 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 and more details on the emergence of and molecular epidemiology of the strain [5]. Given 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 can 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

ECDC internal decision, 10 June 2019.

Consulted experts

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All experts have submitted declarations of interest and a review of the 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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