Gap analysis on securing diphtheria diagnostic capacity and diphtheria antitoxin availability in the EU/EEA
ECDC TECHNICAL REPORT

Gap analysis on securing diphtheria diagnostic capacity and diphtheria antitoxin availability in the EU/EEA
This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Dr Ida Czumbel and produced by Professor Androulla Efstratiou, Vicky Head, Amy Trindall, Daniel West and Dr Mark Reacher (Public Health England, Executive Agency of Department of Health, 61 Colindale Avenue, London, NW9 SEQ, United Kingdom) in relation to Specific Contract No. ECD.5731.

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Abbreviations

DIPNET  Diphtheria Surveillance Network (from 2010 EDSN)
ECDC   European Centre for Disease Prevention and Control
EDSN   European Diphtheria Surveillance Network
ELWGD  European Laboratory Working Group for Diphtheria
EQA    External Quality Assessment
EU/EEA European Union/European Economic Area
EU DIP-LabNet EU Diphtheria Laboratory Network
MALDI-TOF Matrix Assisted Laser Desorption Ionization – Time of Flight
NTTB   Non-toxigenic tox-gene bearing
PCR    Polymerase Chain Reaction
PHE    Public Health England
RT-PCR Reverse Transcription Polymerase Chain Reaction
WHO    World Health Organization
Executive summary

This gap analysis demonstrated that there are significant gaps in diphtheria diagnostic capacity within the EU/EEA, with only six Member States fulfilling the minimum criteria in terms of surveillance, specialised laboratory diagnostics and expertise (see Methods section). The areas with the greatest gaps are related to training and surveillance of all three potentially toxigenic corynebacteria - *Corynebacterium diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis*. The following areas were highlighted as requiring further action.

- Surveillance systems should be in place for the three pathogens, with appropriate methods to determine toxigenicity;
- EU diagnostic capability should be enhanced to isolate, detect toxigenicity, and undertake molecular characterisation of the above pathogens. Consequently there is also an urgent need for a laboratory training workshop, especially for ‘newer’ EU Member States;
- An EQA needs to be re-established, with adequate availability of media/reagents;
- The availability of updated guidelines (national and WHO) [20] on laboratory diagnosis of diphtheria and other related infections caused by potentially toxigenic corynebacteria should be considered a priority;
- Risks relating to the lack of EU availability and procurement of diphtheria antitoxin (DAT) should be addressed.

Background

Diphtheria is an acute infectious disease affecting the upper respiratory tract, and occasionally the skin, caused by the action of diphtheria toxin produced by *Corynebacterium diphtheriae*, *C. ulcerans* and in rare instances *Corynebacterium pseudotuberculosis*. Early and accurate microbiological diagnosis of each suspected case is essential to inform management and treatment of the case and close contacts. Microbiological diagnosis will also help to influence regional vaccination policies and ensure that essential medicines such as diphtheria antitoxin are distributed appropriately. The first indication of the likelihood of the disease is often given by the microbiology laboratory reporting the presence of the causative organism in routine throat swabs and other swabs taken from the respiratory tract and other sites. Clinical diagnosis, particularly in countries where the disease is uncommon, is not easy and may be confused with other causes, such as tonsillitis or streptococcal sore throat. This highlights the importance of both diagnostic and reference laboratories in providing simple, rapid and reliable methods to assist clinicians in achieving the correct diagnosis. However, microbiological diagnosis should be regarded as complementary to, and not a substitute for clinical diagnosis. The diagnostic laboratory should also refer any presumed *C. diphtheriae*, *C. ulcerans* or *C. pseudotuberculosis* isolates to their national reference laboratory for confirmation and toxigenicity testing. If a Member State does not have such a laboratory, arrangements need to be in place for urgent referrals to another reference laboratory or to the WHO Global Collaborating Centre in the UK.

Due to their epidemic patterns, the emergence of new strains, novel reservoirs and their dissemination to susceptible human and animal populations, *Corynebacterium diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* infections are usually difficult to detect [1]. Although *C. diphtheriae* is largely controlled through mass immunisation programmes, diphtheria escalated to epidemic proportions within Russia and the Newly Independent States (NIS) in the 1990s, highlighting the continuing potential of this disease to cause morbidity and mortality when immunisation programmes are disrupted [2].

The European Laboratory Working Group on Diphtheria (ELWGD) was established at the request of WHO's Regional Office for Europe in 1993 in response to the devastating epidemics of diphtheria in the former Soviet Union. The ELWGD comprised selected representatives from different diphtheria reference centres in France, Finland, Germany, Russia, Romania, Ukraine, UK and USA [3]. The aims of the ELWGD were to assess laboratory capabilities, develop laboratory guidelines and outline future study needs. The group now encompasses more than 40 countries globally. In 2003, funding was secured from the European Commission (DG SANCO) to establish a network of microbiologists and key epidemiologists responsible for diphtheria within their country; this network was called the Diphtheria Surveillance Network (DIPNET). As part of ECDC's efforts to build and develop disease surveillance networks the diphtheria network was transferred to ECDC as the European Diphtheria Surveillance Network (EDSN) in 2010 and Public Health England (PHE) was awarded a contract to coordinate the outsourced laboratory activities. EDSN performed External Quality Assessments (EQAs) and training in laboratory diagnostics to enhance and strengthen laboratory-based surveillance capacity. EQAs are an essential tool to enable laboratories to monitor, evaluate and improve their performance. The last EQA report for the laboratory diagnosis of diphtheria under the auspices of EDSN was published in 2013 [10]. It demonstrated that most participants correctly identified the corynebacteria to species level in the specimens that were distributed; however, many had difficulties with toxigenicity testing, resulting in a relatively large number of unacceptable toxigenicity reports. These errors would most likely have prevented appropriate management and treatment of affected patients in a clinical setting. The EQA assessed the performance standard of many national reference laboratories as satisfactory in being able to...
enhance laboratory-based surveillance capacity. However, as the results of the questionnaire show, six countries reported that they still do not have officially recognised national reference laboratories for diphtheria, although they do offer diphtheria diagnostic services.

Several European countries are also currently restructuring their public health and healthcare systems which has led to a decrease in laboratory personnel and financial reductions for diagnostics [4]. The EDSN continued successfully until 2012 when, due to competing priorities, ECDC restructured and temporarily discontinued funding for the EQA and training activities. Reportedly, since 2012 the diphtheria diagnostic capabilities for this specialised area of microbiology have decreased in many countries (information shared via email from the diphtheria operational contact points in countries requesting support from PHE ). Diphtheria remains a public health threat in the EU for pockets of unvaccinated individuals. This was recently highlighted by a fatal case in an unvaccinated child attending a summer camp in Spain where there were difficulties with access to diphtheria diagnostics. Other cases reported recently within EU have also highlighted the problems of access to diphtheria antitoxin therapy [7][19].

To collect more systematic information about laboratory capacity related to diphtheria control, ECDC commissioned a gap analysis of diphtheria diagnostics among Member States. The main objectives were to assess:

- the current microbiological capacity for laboratory diagnosis of diphtheria in the EU/EEA;
- microbiological surveillance and the public health impact on individual Member States where diphtheria diagnostic activities have reportedly been suspended;
- the availability of specialised reagents for diphtheria diagnostics in the EU;
- training needs for scientists/laboratory/medical and public health staff in this specialised area and identify best practices and gaps in diphtheria diagnostics in order to establish laboratory training workshops;
- availability of policies and guidelines related to the management and control of diphtheria;
- supply and access to diphtheria antitoxin (DAT) within Member States for both therapeutic and diagnostic use.

The findings from this gap analysis are essential to public health management and the implications for diseases caused by toxigenic corynebacteria in Europe and will have an impact on mechanisms for diagnostics and management.
Materials and methods

This section describes the methods used to develop and administer the questionnaire and to analyse the data. It also sets out the criteria used to analyse any potential gap in diphtheria diagnostic capacity.

Questionnaire design and pilot

A pilot survey was developed by Androulla Efstratiou (PHE) in conjunction with Ida Czumbel (ECDC). This was initially shared with six EU Member States via email for comment and completion. The questionnaire was finalised based on the feedback received.

Participants

The final questionnaire was distributed to 30 EU/EEA Member States, with the option of completing it online via a survey tool (SelectSurvey) or submitting a hardcopy version. SelectSurvey is a web-based tool for creating online surveys. It allows the user to distribute surveys by email or via a web page, and to create a report of all responses. SelectSurvey allows the collection of sensitive and non-sensitive information via a secure public web interface. The data collection was closed in June 2016 after which the reference link was deactivated.

Responses were received from all 30 EU/EEA Member States that were invited to participate, with some Member States submitting multiple responses. A list of participating countries can be found in Annex 1.

Data validation

Where available, responses submitted online formed the initial dataset. Responses received in hardcopy were entered into the SelectSurvey online survey by the analysis team. Some countries submitted multiple responses, for example by completing both the pilot survey and the final survey, by supplying both an online and a hard copy response, or by submitting responses from more than one person in the Member State. In these cases the different versions were compared for consistency. Responses to the final survey were assumed to be correct, although information from the pilot questionnaire was used to complete missing details, if available. Any significant contradictions were checked with the respondents. Similarly, where more than one final response was received, ‘missing’ information was derived from other responses and significant contradictions were checked with the respondents. In addition, where close reading and analysis of the data highlighted contradictory information, this was queried with the respondents. Where questions required a yes/no response, a missing response was assumed to mean ‘no’.

Following preparation of the draft report, ECDC requested additional validation of responses from Member States. To facilitate this, the epidemiological and microbiological operational contacts points (OCP) in each Member State were sent a copy of their responses to the questionnaire and given eight days to notify the analysis team of any corrections they wished to make or further information they wished to provide. In addition, Member States that did not provide information on the impact of the EQA cessation or DAT procurement were specifically asked to do so. Member States were informed that if they did not respond to this request, it would be assumed that the initial response was accurate and no changes were required. Overall, responses were received from 21 Member States. Five indicated that no changes were required and one provided updated contact details. Fifteen Member States provided amendments or further information. The majority of changes did not materially affect the results of the gap analysis; only two countries changed their status against the minimum criteria (minimum criteria should be fulfilled by Member States in order to have functioning surveillance in place - see specification below).

Data analysis

The online data were exported into MS Excel which was used to construct the final dataset. Analysis was undertaken using MS Excel, including compilation of the figures included in this report and DAT availability.

Describing the ‘gap’

The final section of this report considers the extent to which a ‘gap’ exists in diphtheria diagnostic capacity across the EU/EEA. This has been evaluated by comparing the responses received against the criteria set out below. These criteria have been developed based on the expert opinion (experts from PHE, ECDC and WHO) discussed at a meeting organised by the main contractor on 13 May 2016 at PHE in London. Note that this analysis highlights potential gaps only, based on the information provided through the questionnaire and the validation process. Additional checks may be required before using this information to inform action.
Minimum criteria

Area 1: Microbiological/epidemiological surveillance
- Every Member State should have a surveillance system in place for all three potentially toxigenic species.
- Every Member State should have close cooperation between microbiology and epidemiology for diphtheria surveillance.

Area 2: Laboratory diagnostic capability
The reporting laboratory in each Member State was evaluated at one of four levels indicated below, based on the information received. Each Member State should ideally have at least one laboratory at the ‘reference laboratory’ level, with additional expertise available through the WHO reference centre.
- Minimum or no diagnostic capability (Diagnostic laboratory level): Member State does not meet the standard for laboratory diagnostics.
- Partial diagnostic capability (Diagnostic laboratory level): Member State has reported at least one method for primary culture or at least one method for biochemical identification.
- Reference diagnostic capability (Reference laboratory level): Member State has reported at least one method for primary culture and at least one method for biochemical identification and toxigenicity is determined by either PCR or Elek.
- WHO reference centre level: Member State is of ‘reference laboratory’ standard but also reports using molecular and phenotypic tests for toxigenicity and at least one method for molecular typing and at least one method for serological assay, as well as provision of expert advice for microbiological diagnosis, molecular epidemiology and serological immunity studies. This is in accordance with the WHO terms of reference for a WHO Collaborating Centre for Reference and Research on Diphtheria and Streptococcal Infections (http://apps.who.int/whocc/Detail.aspx?cc_ref=UNK-1946cc_code=unk).

Area 3: Expertise in laboratory diagnostics
- At least one current laboratory staff member should have received recent training on laboratory diagnosis of diphtheria (during 2013 or thereafter).
Results

The questionnaire was sent to 30 EU/EEA Member States and responses were received from all 30 countries. A total of 18 Member States replied using the online questionnaire available through SelectSurvey and 19 Member States submitted hardcopy responses (six countries replied using both methods). Details of how each Member State submitted their response(s) are set out in Annex 1.

This section summarises the responses to the questionnaire. The first section examines the extent to which surveillance systems are in place across the EU/EEA and sets out the number of diphtheria isolates identified by species/biovar and year. The second section describes laboratory capacity in the EU/EEA in terms of the diagnostic and reference services in place. The third section sets out Member States’ training, EQA and support needs. The fourth section describes the serological methods in place and population immunity studies. The final section focuses on public health aspects such as screening studies, use of guidelines and diphtheria antitoxin procurement and stockpiles.

Diphtheria surveillance

Surveillance systems

The World Health Organization (WHO) outlines the rationale for the surveillance of diphtheria as follows [5]:

‘Diphtheria is a widespread severe infectious disease that has the potential for causing epidemics. Surveillance data can be used to monitor levels of coverage and disease as a measure of the impact of control programmes. Recent epidemics have highlighted the need for adequate surveillance and epidemic preparedness.’

Two different case definitions are currently applied in public health settings across the European region: the 2012 EU case definition, which considers disease caused by \( C. diphtheriae \), \( C. ulcerans \) and \( C. pseudotuberculosis \) [6] and the 1994 WHO case definition, which only considers classical respiratory diphtheria cases caused by \( C. diphtheriae \) (‘epidemic diphtheria’) [5]. However, all EU Member States are obliged to use the 2012 EU case definition when reporting cases to ECDC.

All of the Member States (n=30) reported having a surveillance system in place for \( C. diphtheriae \). Surveillance was less common for \( C. ulcerans \) (n=22), and only 20 Member States reported having surveillance for all three species. See Table 1.

Table 1. EU/EEA Member States reporting surveillance for diphtheria, by species, 2013-2015

<table>
<thead>
<tr>
<th>Surveillance system for</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C. diphtheriae )</td>
<td>30 (100%)</td>
</tr>
<tr>
<td>( C. diphtheriae ) and ( C. ulcerans )</td>
<td>22 (73%)</td>
</tr>
<tr>
<td>( C. diphtheriae, C. ulcerans and C. pseudotuberculosis )</td>
<td>20 (67%)</td>
</tr>
</tbody>
</table>

All 30 of the EU/EEA Member States responding to the survey (100%) reported the existence of surveillance system involving both epidemiology and microbiology although one country reported that there was no cooperation between epidemiology and microbiology. A total of 14 Member States explained that the link involved mandatory reporting; eight commented on the existence of a surveillance system or database and eight described arrangements for informing relevant colleagues. In all, 28 countries (93%) stated that they provided a nationwide diphtheria reference service; of these, 13 (43%) stated that there was a legal obligation for laboratories to submit isolates/specimens to their laboratory for reference testing.

Only three Member States reported the provision of a reference service for cultures submitted from outside their own country (France, Germany and the United Kingdom). The Global WHO Collaborating Centre for Diphtheria (based at Public Health England) is obliged to accept cultures from any country in the world. Only one country (the United Kingdom) stated they would have material transfer agreements in place to allow the transfer of samples, if requested by a specific country.

Isolates/ specimens

From 2013 to 2015, a total of 200 toxin-positive (tox-positive) isolates were identified from 20 countries, along with 623 toxin-negative (tox-negative) isolates from clinical specimens, mainly throat and skin cultures (pers. comm. A. Efstratiou). The numbers of both tox-positive and tox-negative isolates increased over the three years, with a marked increase in 2015. \( C. diphtheriae \) was the most common species identified, making up around half of the tox-positive isolates and the majority of the tox-negative isolates (see Tables 2 and 3). Eleven countries reported having no positive isolates/specimens for corynebacteria during the period surveyed 2013–2015 (see Annex 2).
Table 2. Number of isolates reported, by species and year

<table>
<thead>
<tr>
<th>Year</th>
<th>C. diphtheriae</th>
<th>C. ulcerans</th>
<th>C. pseudotuberculosis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tox+</td>
<td>Tox-</td>
<td>Tox+</td>
<td>Tox-</td>
</tr>
<tr>
<td>2013</td>
<td>35</td>
<td>153</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>2014</td>
<td>30</td>
<td>210</td>
<td>26</td>
<td>8</td>
</tr>
<tr>
<td>2015</td>
<td>51</td>
<td>232</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>116</td>
<td>595</td>
<td>84</td>
<td>27</td>
</tr>
</tbody>
</table>

Table 3. C. diphtheriae isolates by biovar

<table>
<thead>
<tr>
<th>Year</th>
<th>gravis</th>
<th>mitis</th>
<th>belfanti</th>
<th>intermedius</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tox+</td>
<td>Tox-</td>
<td>Tox+</td>
<td>Tox-</td>
</tr>
<tr>
<td>2013</td>
<td>30</td>
<td>66</td>
<td>4</td>
<td>55</td>
</tr>
<tr>
<td>2014</td>
<td>15</td>
<td>99</td>
<td>14</td>
<td>72</td>
</tr>
<tr>
<td>2015</td>
<td>13</td>
<td>98</td>
<td>32</td>
<td>85</td>
</tr>
<tr>
<td>Total</td>
<td>58</td>
<td>263</td>
<td>50</td>
<td>212</td>
</tr>
</tbody>
</table>

Note: Two countries were unable to characterise C. diphtheriae by biovar, which is why the totals in this table differ slightly from those in Table 2.

Of the 13 Member States reporting toxigenic isolates, 12 stated that the toxigenicity status of these isolates had been microbiologically confirmed by a combination of PCR and the Elek test (noting that in some cases the Elek test was performed elsewhere). In just one Member State, toxigenicity was determined by PCR only.

Comparison with other data sources

Diphtheria caused by C. diphtheriae, C. ulcerans and C. pseudotuberculosis is a notifiable disease in the EU and cases are reported to ECDC in accordance with the EU case definition for communicable diseases. The number of isolates reported here is notably higher than the number of cases reported to ECDC and published in a Rapid Risk Assessment [7]. For comparison purposes, numbers of cases reported to ECDC are summarised in Table 4.

The differences between the sources may reflect the different definitions: the questionnaire requested the number of isolates, however ECDC requests notification of confirmed cases of diphtheria and there may be multiple isolates per case (although this is rare). Alternatively, the difference could reflect under-reporting or under-ascertainment or could refer to the number of carriers around cases.

Table 4. Cases of C. diphtheriae and C. ulcerans in the EU/EEA, reported to ECDC, 2013 and 2014 [7]

<table>
<thead>
<tr>
<th>Year</th>
<th>C. diphtheriae</th>
<th>C. ulcerans</th>
<th>Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013</td>
<td>19</td>
<td>12</td>
<td>0</td>
<td>31</td>
</tr>
<tr>
<td>2014</td>
<td>24</td>
<td>13</td>
<td>1</td>
<td>38</td>
</tr>
<tr>
<td>Total</td>
<td>43</td>
<td>25</td>
<td>1</td>
<td>69</td>
</tr>
</tbody>
</table>
Laboratory diagnostic and reference services in place across the EU/ EEA

This section describes the laboratory diagnostic and reference services in place across the EU/EEA. This includes the methods used for the identification of Corynebacterium species (diphtheriae, ulcerans and pseudotuberculosis), toxin detection, antibiotic susceptibility testing and molecular typing. The importance of speed coupled with accuracy is essential when performing these procedures. However, the range of investigations is very much dependent upon the expertise of laboratory staff and the availability of reagents and financial resources. Due to the low number of diphtheria cases within Member States, screening of throat swabs among the population groups potentially at risk for diphtheria (e.g. orphanages, military, etc.) is not undertaken routinely.

Diagnostic procedures

Figure 1 shows the number of identification methods listed by countries. Twenty-nine Member States listed the use of at least one primary culture method for morphological identification and at least one test for biochemical properties of corynebacteria species. Over half of Member States (n=17) listed the use of four to seven methods/tests, with five Member States listing three or fewer and eight listing eight or more. One Member State did not report data on the identification methods.

Figure 1. Number of primary morphological and biochemical methods used for identification of corynebacteria species in the EU/EEA, 2013-2015 (n=30)
**Figure 2a.** Methods used for morphological and microscopic identification of potentially toxigenic *Corynebacterium* spp. species in the EU/EEA, 2013–2015 (n=30)

Figure 2a shows the number of Member States reporting the use of each test. The most common primary method used by 26 Member States was Gram stain, followed by blood agar culture (n=25). The use of tellurite was reported by 23 Member States and the use of another stain was reported by one Member State.

The screening and biochemical characterisation of potentially toxigenic species of diphtheria bioware is shown in Figure 2b. The most frequently used tests were API Coryne (n=23), followed by urease hydrolysis (n=15) and nitrate reduction (n=11), Hiss serum water sugars (Glucose, Maltose, Sucrose) (n=11), Cystinase (n=10) and the Pyrazinamidase test (n=8), as reported by 29 Member States.

Fifteen countries reported using the MALDI-TOF system and a single biochemical test such as urease hydrolysis.

The least commonly used methods were identification via molecular sequencing of specific genes (as reported by five Member States).
Five (17%) Member States experienced problems in obtaining culture media for diphtheria diagnostics, specifically the supply of basal medium for the Elek test (four countries) and tellurite blood agar (one country). The supply of antitoxin for laboratory diagnostics is discussed later in this report.

**Toxigenicity testing**

Twenty-eight Member States reported using at least one method for toxin detection. The most common method was the Elek test, used by 23 Member States, followed by PCR, used by 16 Member States and RT-PCT, used by 10 Member States (33%). A total of 19 Member States (63%) reported using both the Elek test and PCR or RT-PCR (see Figure 3).

**Figure 3. Methods currently used for toxin detection**

Among the 23 Member States that reported using the Elek test, the source of Elek medium was provided as follows:

- eleven (48%) used medium from WHO/PHE;
- nine (39%) produced their own medium in-house (as per WHO recipe [8]); and
- four (17%) used commercially-sourced medium (one Member State specified the manufacturer as Microgen, Russia; the others did not state the manufacturer).

The source of serum used for the Elek medium was reported as:

- equine - nine (39%)
- new-born bovine - four (17%)
- bovine – eight (35%)
- other (stated as rabbit) – one (4%)
- not stated – four (17%).

The concentration of antitoxin used (note that Member States may have reported using more than one) was:

- 15 (65%) 500 IU
- 2 (9%) 1000 IU
- 4 (17%) other (stated as 1780 IU/ml, 400 IU, ‘dry’ and ‘10 IU for modified; 500 IU for full plate’)
- 4 (17%) did not state the concentration used.

Overall, 18 Member States (60% of the total) reported problems in obtaining supplies of antitoxin for laboratory diagnostics. A total of 12 commented on the lack of supplier in their country or in Europe. Three noted they had only been able to obtain antitoxin from PHE/WHO. One Member Stated described a long delivery period associated
with procuring antitoxin from Microgen in Russia (www.microgen.ru), and another stated that, in addition to the problem with lack of supply, their country did not allow toxin to be imported without the CE marking.

In all, 16 Member States reported using PCR to determine toxigenicity. Of these, ten reported using primer sets for fragment A of the toxin gene and six reported using primer sets for the entire gene. Eight Member States indicated that they used an internal template or inhibition control to confirm that the PCR reaction had not failed.

Ten Member States reported that they used RT-PCR to detect toxigenicity.

Of the seven Member States that did not perform PCR or RT-PCR, only three had the relevant facilities to do so, and only three stated that they wanted to introduce PCR toxin detection in their laboratory.

Three Member States reported that they carried out toxigenicity tests other than PCR or Elek: metabolic-inhibition colorimetric test on BSC-1 cell tissue cultures; diphtheria Schick/test toxin and diphtheria antitoxin (NI BSC); in vitro (for publications only, not routinely); and tissue culture (e.g. Vero cell bioassay).

**Antibiotic susceptibility testing**

Ten Member States routinely determined antibiotic susceptibility on any *C. diphtheriae, C. ulcerans* or *C. pseudotuberculosis* isolates received. There were 15 Member States that determined antibiotic susceptibility if specifically requested to do so. As shown in Figure 4, a total of 21 Member States (70%) used the E test and 17 (57%) used the disc diffusion method. The following interpretation guidelines were used to determine breakpoints:

- 21 Member States: EUCAST
- 6 Member States: CLSI (formerly NCCLS)
- 2 Member States: Other.

**Figure 4. Methods currently used for antibiotic susceptibility testing**

<table>
<thead>
<tr>
<th>Antibiotic susceptibility methods</th>
<th>Number of Member States</th>
</tr>
</thead>
<tbody>
<tr>
<td>E test</td>
<td>21</td>
</tr>
<tr>
<td>Disc diffusion</td>
<td>17</td>
</tr>
<tr>
<td>Commercially prepared MIC microtitre trays</td>
<td>10</td>
</tr>
<tr>
<td>Broth dilution</td>
<td>5</td>
</tr>
<tr>
<td>Agar incorporation</td>
<td>3</td>
</tr>
</tbody>
</table>

**Molecular typing**

Only nine Member States reported performing molecular typing (see Table 5). Of these, most were able to type both *C. diphtheriae* and *C. ulcerans*; only three performed molecular typing on all three species. The most common method was multilocus sequence typing (MLST), performed by eight countries (Figure 5). Two countries carried out pulsed-field gel electrophoresis and ribotyping independently.

**Table 5. Member States reporting performing molecular typing, by species performing molecular typing, by species**

<table>
<thead>
<tr>
<th>Molecular typing performed on the following species:</th>
<th>Yes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. diphtheriae</em></td>
<td>9 (30%)</td>
</tr>
<tr>
<td><em>C. diphtheriae</em> and <em>C. ulcerans</em></td>
<td>8 (27%)</td>
</tr>
<tr>
<td><em>C. diphtheriae, C. ulcerans and C. pseudotuberculosis</em></td>
<td>3 (13%)</td>
</tr>
</tbody>
</table>
The nine Member States carrying out molecular typing reported results based on the following analytical software:

- 78% (n=7) Bionumerics
- 11% (n=1) Taxotron®
- 11% (n=1) Other (stated as ‘MLST database’).

Of these Member States, eight used the MLST database [9] and submitted their own MLST strains/sequence types to the MLST public database.

A further four countries provided information on the software used but also stated that they did not perform molecular typing on *C. diphtheriae*, *C. ulcerans* or *C. pseudotuberculosis*.

Eight Member States said they were planning to perform molecular typing in the future. Of these, one reported having whole genome sequencing facilities in place and felt that this would be the most useful molecular typing method.

Another noted that methods were in place for other pathogens that could be applied to corynebacteria with appropriate training; this country stated that MLST would be the most useful method. Two other countries also felt that MLST would be the most useful method, one noting that they had the necessary equipment but would require training. One Member State felt that AFLP would be the best method and finally another Member State reported that whole genome sequencing (WGS) would be the most useful, and that they already had a platform in place.
Member States’ training, External Quality Assessment (EQA) and support needs

This section describes the current situation across the EU/EEA in terms of specialist training in diphtheria reference diagnostics, international collaboration and EQA. The section also summarises Member States’ views on the value of EQA going forward, their most urgent needs with respect to diphtheria diagnostics and the support they require in order to maintain diphtheria diagnostics.

Training

In total, 11 Member States (37%) stated that there were members of laboratory staff in regional/other laboratories that required specialist training on diphtheria reference diagnostics. Many of these were countries that joined the EDSN after the network was transferred to ECDC or countries that had not attended a training course within the last five years (pers. comm. A. Efstratiou). For 13 Member States (43%), training was not required and six countries did not provide an answer.

A total of 23 countries (77%) provided information on the last occasion when the head of the diphtheria reference service attended a training workshop on diphtheria diagnostics. Of these, 12 Member States (52%) reported that such training was undertaken between 2011 and 2015. Seven countries (30%) reported that it was last undertaken between 2007 and 2010 and one country reported it was last undertaken during the 1990s. Three Member States reported that the head of the diphtheria reference service had never attended a training workshop on diphtheria diagnostics. There were 18 Member States that specified the training provider - all were associated with either the DIPNET or EDSN programmes. Seven Member States cited the European Workshop on Laboratory Diagnosis of Diphtheria and eight Member States specified Health Protection Agency/Public Health England (HPA/PHE) Dr Androulla Efstratiou as the provider. Three specified other providers, including WHO and the European Commission DG SANCO (now DG SANTE).

In all, 19 countries provided information on the last occasion when any laboratory staff working in the national reference laboratory had attended a training workshop on diphtheria diagnostics. Of these, 16 Member States (84%) reported that such training was undertaken during the period 2011 to 2013. Two countries reported that it was last undertaken in 2010. There were 17 Member States that specified the training provider – again all were associated with either DIPNET or EDSN activities. In nine cases Member States cited an ESDN training which took place in Athens in 2011, and eight Member States specified HPA/PHE/EDSN (Dr Androulla Efstratiou) as the provider. Overall, 18 out of 19 Member States reported that there were laboratory staff still in employment who had attended training workshops on diphtheria during or after 2013.

In all, twenty-one Member States stated there was a need for a laboratory workshop on diphtheria diagnosis and typing (Figure 7).

International collaboration

Overall, 11 (37%) Member States reported a formal or informal collaboration with other laboratories within their own country and/or diphtheria reference laboratories within the EU Diphtheria Surveillance Network. The most common collaboration, cited by seven Member States, was with PHE, specifically the WHO Global Collaborating Centre for Reference and Research on Diphtheria and Streptococcal Infections, National Infection Service, though personal communication with Androulla Efstratiou was also mentioned. As a WHO Collaborating Centre for diphtheria infections, PHE offers microbiology reference services and assists WHO and Member States in promoting laboratory-based surveillance at national and global levels. If necessary, it can also receive strains for confirmation of toxigenicity and molecular characterisation, both from Member States and countries outside the EU (http://apps.who.int/whocc/Detail.aspx?cc_ref=UNK-194&cc_code=unk). Three Member States mentioned collaboration with other countries and two mentioned former and current European networks (DIPNET, pre 2010), EU DIP-LabNet, EDSN).

External Quality Assessment (EQA) and accreditation

EQA studies are essential in enabling laboratories to monitor, evaluate and improve their own performance and therefore provide significant benefits.

The last European EQA exercise took place in 2013 under the auspices of the EDSN [10]. A total of 32 countries, including Croatia as a new member of the European Union, participated and were asked to isolate, identify and perform toxigenicity testing on any Corynebacterium spp. present in the six simulated throat specimens sent. In this survey, 25 (83%) of Member States reported that they were aware of the report on this exercise available on ECDC’s website [10]. Of these countries, 17 (57%) said that the EQA report had been disseminated to colleagues and 12 (40%) said that it had been disseminated to decision-makers.
Overall, 27 Member States commented on whether they had taken any steps to remedy gaps identified during the 2013 EQA exercise. Of these, eight countries indicated that the EQA had not identified gaps and therefore no steps were required. Fourteen Member States indicated that they had taken steps to address gaps. Five Member States (17%) reported having taken no steps to remedy gaps that had been identified.

Respondents were asked to describe how the discontinuation of the European Diphtheria EQA and training after 2013 had had an impact in their country. Twenty-five Member States responded and among these, two stated that there had been no effect and one had not previously participated in the EQA. Overall 22 Member States (73%) described negative impacts, which are summarised in Figure 6 below. The most commonly cited difficulties were associated with perceived loss of accuracy, expertise or confidence (nine Member States) and quality assessment and validation of methods (eight Member States). Five Member States stated that the lack of EQA was problematic for laboratory accreditation and/or maintenance of the reference function. Three countries noted that the discontinuation had led to a delay in obtaining final toxin confirmation, as samples had to be sent externally. Two countries had other responses: one reported that they ‘missed the EQA’ and another noted that they had not been aware of the cessation of the EQA but would be open to participating in the future.

**Figure 6. Local impact of the discontinuation of the European Diphtheria EQA and training**

Overall, 20 (67%) Member States stated that their lab was accredited for diphtheria diagnostics while 22 (73%) indicated that an EQA exercise would be essential for their laboratory accreditation. Of the eight Member States that felt an EQA exercise was not needed for accreditation, the main reasons given were:

- the laboratory is not a reference laboratory for diphtheria/they are not seeking accreditation (n=4);
- in the absence of EQA, another approach would be acceptable for accreditation (n=1);
- low incidence of diphtheria in the country meant that this was not a priority area (n=1).

A total of 26 countries commented on the benefit of an EQA for diphtheria diagnostics. Of these:

- 11 (42%) commented in favour of EQA values for quality assurance and/or accreditation (they use EQA results for quality assurance and accreditation for diphtheria diagnostic services);
- 5 (19%) commented on the importance for maintaining knowledge and expertise;
- 5 (19%) felt EQA was essential for adequate reference activities;
- 3 (12%) felt it assisted with preparedness in the event of a diphtheria case;
- 3 (12%) reported they had no other practical experience;
- 3 (12%) commented on the value for standardisation.

**Laboratory expertise: urgent needs**

In total, 23 (77%) Member States suggested approaches for maintaining laboratory expertise in Europe. Of these:

- 15 (65%) stated the importance of EQA;
- 8 (35%) suggested workshops/training;
- 6 (26%) mentioned the re-establishment of ESDN or European-wide network;
- 5 (22%) suggested provision of central laboratory/scientific support;
- 2 (9%) suggested standardised methods/media/antitoxin provision;
- 2 (9%) felt that the low number of cases/samples led to inexperience, which would be difficult to address;
- 1 (4%) required financial support.
The survey asked respondents to rate how urgently (range 1–5 from 'not urgently required' to 'very urgently needed') five aspects of laboratory capacity were required. Overall, the supply of diphtheria antitoxin was most commonly considered to be 'very urgently needed', with 15 of the Member States rating their need in this category. If needs rated in the two highest categories are taken together (Figure 7), overall every category of laboratory capacity was rated as urgently needed ('4' or '5') by over 43% or more of the Member States, indicating a high level of need across the EU/EEA. Supply of antitoxin and EQA distribution were rated as urgently needed by the highest number of countries (19, 63%). A laboratory training workshop was rated as urgently needed ('4' or '5') by the lowest number of Member States (13, 43%). A total of 16 Member States felt the development of a 'dip stick' / rapid test for toxigenicity was urgently needed and 14 identified a supply of Elek basal medium as urgently needed.

Figure 7. Most urgent needs related to laboratory expertise

In all, 27 Member States provided details of the laboratory support they felt would be required to maintain diphtheria diagnostics in their country (see Figure 8). The most commonly cited support required was the supply of diphtheria antitoxin for toxigenicity testing, which was mentioned by 11 Member States (37%), followed by the distribution of EQA and the supply of Elek basal medium (nine Member States each, 30%) and staff training (seven Member States, 23%).

Figure 8. Support required to maintain diphtheria diagnostics in Member States
Serology and population immunity screening

This section covers the methods in use across the EU/EEA for performing serological assays to establish diphtheria immunity, along with implementation of sero-prevalence studies for determination of population immunity. Accurate determination of anti-diphtheria toxin antibodies is essential in order to obtain reliable information on the immune status of a person in a given population. It is also vital for evaluating the immunogenicity of diphtheria vaccines in clinical trials, monitoring long-term immunity and providing recommendations for vaccination policy. Therefore, serological methods must be accurate, reproducible, specific and sensitive. The last EQA for serology was undertaken in 2009, among 11 European countries [11].

Methods

In all, 19 (63%) Member States reported performing at least one serological method. Of these, the most common method was the ELISA using a commercial kit (11, 37%), as shown in Figure. Tissue culture toxin neutralisation and ‘other’ were the next most common (4 for each, 13%). ‘Other’ methods stated (9 in total) were Luminex/multiplex [12] and DELFIA. One Member State reported performing ELISA using an in-house method and one reported performing the passive haemagglutination assay.

Figure 9. Serological methods performed by Member States, 2016

Interpretation criteria

Of the 19 countries undertaking serological assays, most used interpretation criteria as defined by WHO. Three countries reported using the criteria of the commercial kit used but did not state what these were.

As shown in Table 6, most Member States carrying out serological assays reported using interpretation criteria consistent with those defined by WHO. Divergence from the WHO criteria mostly reflected a different interpretation of the immune/protected status (i.e. fully protective cut-off versus threshold for reliable protection) or the framing of the answer in terms of a requirement for booster vaccinations rather than IU/ml.

Table 6. Interpretation criteria given by Member States performing serological assays

<table>
<thead>
<tr>
<th>Definition of</th>
<th>Definition given</th>
<th>Number of Member States (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune/protective status</td>
<td>&gt;0.1 IU/ml (WHO standard)</td>
<td>13 (68%)</td>
</tr>
<tr>
<td></td>
<td>Did not specify</td>
<td>2 (11%)</td>
</tr>
<tr>
<td></td>
<td>Stated different criteria</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>Susceptible status</td>
<td>&lt;0.01 IU/ml (WHO standard)</td>
<td>13 (68%)</td>
</tr>
<tr>
<td></td>
<td>Did not specify</td>
<td>2 (11%)</td>
</tr>
<tr>
<td></td>
<td>Stated different criteria</td>
<td>2 (11%)</td>
</tr>
</tbody>
</table>
Seroprevalence and population immunity screening studies

A total of 22 Member States (73%) reported having performed seroprevalence studies in their country. Of these, 10 Member States had performed a seroprevalence study in the last ten years and 12 (40%) had performed one between 1995 and 2003. The most recently completed study was undertaken in Lithuania in 2014. A study is currently underway in Portugal, three countries have studies planned for the period 2017–21 (Norway, Spain and the Netherlands). A seroprevalence study was conducted in Belgium between July 2013 and January 2015; analyses of the results are ongoing. Most studies have been at a national (or international) level.

Furthermore, EUPert-LabNet and ECDC are organising a seroprevalence study of pertussis among the age groups 40 – 49 years and 50 – 59 years in the Member States. The sera will be tested for diphtheria and also for tetanus antibodies.
Public health aspects

This section covers public health aspects of diphtheria diagnostics and case management. This includes methods to determine the carriage of corynebacteria in the population (through routine screening of throat swabs or screening studies), the guidelines in place within individual Member States for case management and/or laboratory procedures, procurement of diphtheria antitoxin (DAT) and existing DAT stockpiles.

Carriage of corynebacteria: routine screening of throat swabs and diphtheria screening studies

Eight Member States (27%) stated that diagnostic laboratories in their country undertake screening of throat swabs for the presence of potentially toxigenic corynebacteria, using a combination of tellurite and Tinsdale media. Of these, five provided information on the number of laboratories that performed primary culture. In two countries primary culture was reported to take place in all relevant laboratories; in one country it occurred in 25% of laboratories; in another it occurred in two laboratories; and in one country it occurred in a single laboratory.

A total of 21 Member States (70%) reported that their diagnostic laboratories did not screen throat swabs for potentially toxigenic corynebacteria. Four of those responding reported that this had never been done. Four reported that screening had ceased during the 1980s, two reported that it ceased during the 1990s and one that it ceased between 2000 and 2010 (three countries stated that they did not know when this activity had ceased and seven provided no answer to the question). A total of 11 Member States provided a reason for the cessation of this activity. The most common reason, cited by eight Member States, was the lack of cases or public health requirement, with three Member States explicitly stating that a lack of money/resources had contributed. One Member State reported that a loss of expertise due to retirement had led to an unofficial termination of this service, and one Member State reported that commercial media were not available and the laboratories did not prepare the media in-house because reagents were expensive and the stability and shelf life was short.

Two Member States specifically commented that the cessation of throat swab screening had led to a loss of expertise. Three Member States felt that there had been an impact in terms of knowledge regarding carriage in their population including, in two cases, carriage within their migrant/refugee population.

In total, 12 (40%) Member States reported performing or participating in diphtheria screening studies. Of these, six countries (20% of total) referenced the DIPNET multicentre European study carried out in 2007–2008 (Wagner et al.). Five local studies were described, including one on carriage in pigs and pig farmers (Germany).

Guidelines for case management and laboratory procedures

In all, 27 Member States reported using either national guidelines or the WHO diphtheria guidelines for the management of individual diphtheria cases and contacts. Specifically:

- 12 Member States (40%) use national guidelines.
- 15 Member States (50%) use the WHO diphtheria guidelines [1].
- Three Member States (10%) use other guidelines (two of which also use the WHO guidelines), including those from the US CDC [13], PHE [14] and a regional guideline.
- Three (10%) did not state the guidelines they used.

There were 23 Member States that reported using either a national laboratory manual or the WHO laboratory diphtheria guidelines to guide their laboratory protocols. Specifically:

- Eight Member States (27%) use a national laboratory manual.
- 15 Member States (50%) use the WHO laboratory diphtheria guidelines [15].
- Two Member States (7%) use their local lab SOP (one of which also applies the WHO guidelines).
- Six Member States (20%) did not state the guidelines they used.

Diphtheria antitoxin (DAT) procurement

Fourteen Member States reported that they had procurement for DAT in place but this did not clarify whether they were currently able to procure new stock. Procurement was always at national level, though in two cases a hospital was responsible rather than a national organisation/department.

Countries where national procurement for DAT is currently in place include Bulgaria, Denmark, Estonia, Finland, Germany, Ireland, Latvia, Lithuania, Luxembourg, the Netherlands, Norway, Spain, Sweden and the United Kingdom.

One country (Belgium) commented that they relied upon other countries having DAT and were recently able to source DAT twice from the Netherlands within a few hours. Work was currently underway in Belgium at the national (federal) level to make doses of DAT available in the country.
Another country (Ireland) reported that they had been unable to source DAT since 2012.

**Diphtheria antitoxin stockpile**

In total, 14 Member States (47%) reported that they had an existing DAT stockpile in their country. Of these, DAT was sourced from:

- The Institute of Immunology in Croatia (six Member States); respondents generally noted this had passed its initial use-by date, though efficacy tests showed that it was still potent and suitable for use.
- Microgen in Russia (three Member States).
- Other manufacturers (BulBio-NCIPD in Bulgaria, Vins Bioproducts Ltd in India and Intervax) (three Member States).
- Two unknown/not stated.

The questionnaire asked Member States to indicate the quantity of stockpile that would be required to meet their country's needs. Five Member States did not respond or did not know the answer. Of the remaining 25 Member States:

- Six felt that, as they had no cases, that they did not require a stockpile or could not estimate the size of the stockpile they might require.
- Among the 14 Member States with a stockpile, three responded that they had sufficient quantities of DAT for a low number of cases (i.e. <5); one for approximately ten cases (Norway) and two for 20 cases. One Member State maintains a stockpile of 40 doses. Four Member States specifically noted they had a sufficient amount of DAT for their needs. Three countries did not specify details of their stockpile.
- Among the 16 Member States without a stockpile, six indicated that they required a stockpile sufficient for up to five cases; two for five to ten cases and one for 10–20 cases.
- Two countries noted that the migrant crisis could affect their requirements; Greece has no stockpile and has requested procurement of sufficient DAT for five to ten cases.

**Diphtheria vaccination**

Currently all children in the EU/EEA are offered primary vaccination against diphtheria within their first 12 months of life via a three-dose schedule. This is followed by booster doses in later childhood and adolescence. The number of doses of diphtheria-toxoid-containing vaccine that a person receives before they are 18 years old varies among EU/EEA countries. The number of doses recommended by the age of 18 ranges from at least four in Denmark to seven in Bulgaria [16].

Data from WHO for 2014 estimated that 27 EU/EEA countries had diphtheria vaccination coverage at or above 95% for the first dose and 20 countries had coverage above 95% for the third dose. No countries had coverage rates under 90% for the first dose but two countries did for the third dose: Austria and Bulgaria. Liechtenstein was the only country where coverage data were not available for comparison [17].

The immunity induced by childhood immunisations can wane in adulthood without exposure to natural diphtheria-causing organisms or booster doses of diphtheria. This can result in people becoming susceptible to the disease as they get older. In response, WHO recommends people should receive booster doses with diphtheria toxoid every 10 years throughout life, and a combination of diphtheria and tetanus toxoid (DT or dT) should be used when tetanus prophylaxis is required following injury, to further promote immunity against diphtheria [18].

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1 Please note that the information on the use of commercial products mentioned in this report has been provided by Member States. The fact that ECDC has included the information in the report does not constitute any endorsement by ECDC of such products.
Analysing the gap in EU/ EEA diphtheria diagnostics

This section considers the extent of the current gap in diphtheria diagnostics across the EU/EEA. This has been evaluated by comparing the responses received against the criteria set out in the Methods section. Note that the gaps described below should only be considered as potential gaps, based on the information provided through the questionnaire and validation process. Further information may be required to inform action.

**Area 1: Microbiological/ epidemiological surveillance**

- Every Member State should have a surveillance system in place for all three diphtheria species.
- Every Member State should have close collaboration in place between microbiology and epidemiology for diphtheria surveillance.

These criteria were met for 19 of 30 Member States, thus indicating that there could be a significant gap in terms of diphtheria surveillance. Whilst most Member States had surveillance in place for *C. diphtheria* (n=30), fewer countries (n=20) had surveillance in place for all three pathogens: *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis*. One country reported that there was no collaboration between microbiology and epidemiology.

**Area 2: Laboratory diagnostic capability**

- Each Member State should have one laboratory reaching ‘reference laboratory’ level, with additional expertise available through WHO reference centres.

To achieve reference laboratory level, the Member State has reported at least one method for primary culture (Gram stain, other stain, blood agar or Tellurite agar) and at least one method for biochemical identification (API CORYNE and all other conventional tests, MALDI-TOF) and toxigenicity is determined by either PCR or Elek.

To achieve WHO reference centre level, the Member State meets reference laboratory standards and in addition reports using phenotypic tests for at least one method when doing molecular typing and at least one method when doing serological assay.

Overall, 24 of 30 Member States reported applying the methods required to achieve reference-laboratory or WHO-reference-centre level case confirmation (Table 7). On the other hand, six Member States did not report facilities meeting reference-laboratory-level criteria. This suggests that there is a potential gap in laboratory diagnostic services, as one in five Member States did not apply the methods required to identify diphtheria species and determine toxigenicity.

Table 7. Laboratory diagnostic levels across Member States as determined by the gap analysis

<table>
<thead>
<tr>
<th>Laboratory diagnostic level</th>
<th>Number of Member States (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum or no diagnostic capability</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Partial diagnostic capability</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>Reference diagnostic capability</td>
<td>19 (63%)</td>
</tr>
<tr>
<td>WHO reference centre level</td>
<td>5 (17%)</td>
</tr>
</tbody>
</table>

**Area 3: Expertise in laboratory training**

- At least one current laboratory staff member should have received training between 2013–2015.

This criterion was met by 19 of 30 Member States (37%). A total of 11 Member States (37%) stated that there were laboratory staff members in regional or other laboratories that required specialist training on diphtheria reference diagnostics. Many were those countries who joined the EDSN after the network was transferred to ECDC or countries that had not attended a training course within the last five years (pers. comm. A. Efstratiou).
Discussion

Although it is a rare disease, diphtheria remains a threat to public health in Europe. During periods of financial constraint many countries may not have sufficient resources to maintain the necessary level of preparedness to diagnose diphtheria – e.g. through the loss of laboratory expertise, and laboratories not being able to maintain laborious diagnostic infrastructure (specific media, specialised assays, training of staff. Given the purportedly high proportion of adults with diphtheria toxin antibodies below protective levels due to waning immunity [22, 23], the importance of good surveillance is crucial. Well-functioning surveillance systems including careful investigation and follow-up of cases are a reliable means of monitoring the disease and detecting new trends and changing epidemiology. The reliability of surveillance systems depends on good quality laboratory data. The gaps identified within this analysis can be used to inform options for better prevention and control of diphtheria in the EU.

Diphtheria surveillance

Overall, remarkable advances have been made since the epidemic of the 1990s, in terms of reducing diphtheria case numbers, case management and laboratory diagnostics. However, considerable challenges remain for the surveillance of the disease, as was evident from the data in this gap analysis. The majority of Member States have surveillance in place for the main pathogens, C. diphtheriae and C. ulcerans, but laboratory diagnosis appears to vary considerably between countries. Those countries that have reported cases in recent years have good laboratory capacity for isolation and confirmation or strong cooperation with other Member States for isolate referrals. These countries include Belgium, Denmark, France, Finland, Germany, Italy, Norway, Spain and the United Kingdom.

Case definitions do not appear to be consistent across countries. This represents a challenge when analysing data and limits the ability to pool data across countries, which in turn reduces the opportunities for understanding risk factors (e.g. for C. ulcerans).

Diphtheria caused by C. diphtheriae, C. ulcerans and C. pseudotuberculosis is a notifiable disease in the EU and cases should be reported to ECDC according to the EU case definition. The number of isolates reported in this study is notably higher than the number of cases routinely reported to ECDC and published in a recent rapid risk assessment or the Annual Epidemiological Report [7]. For comparison, the numbers of cases reported to ECDC are summarised in Table 4. The variations may reflect the use of different definitions. The questionnaire used for the current analysis requested the number of isolates and allowed for multiple isolates per case (although this is likely to be minimal), whereas ECDC requests routine notification of confirmed diphtheria cases (case-based data). The variations could also reflect the laboratory methodologies used in the individual countries, as discussed below in the section on laboratory capacity. Further differences from reports already published may be explained by the year of variation and/or different source of information [21].

Laboratory capacity and diagnostic services

It is vital that cases can be identified and treated in a timely manner. This entails ensuring that clinicians are aware of the various clinical presentations of diphtheria, as well as risk factors for infection including those specific to C. ulcerans, and that microbiologists have sufficient skills and resources for microbiological diagnosis. Maintaining this expertise in the face of the low incidence of disease is one of the key challenges for diphtheria surveillance. It is evident that laboratory methodologies are not consistent among Member States. The range of investigations is dependent upon the expertise of laboratory staff and the availability of reagents and financial resources. Many (approximately 50%) European laboratories are now using more modern technologies, such as MALDI-TOF and RT - PCR, which improve the identification at diagnostic laboratory level quite considerably. Unfortunately, due to the low incidence of diphtheria within Member States, throat swabs are not screened on a routine basis.

Based on the survey report, almost all Member States (97%, n=29) perform one primary culture for morphological orientation and one test for biochemical identification of the corynebacterium species. In addition, 23 Member States perform Elek tests for diphtheria toxigenicity analysis. Nearly all countries (93%, n=28) provide a diphtheria reference service for their entire country.

Although laboratories report the provision of reference and diagnostic laboratory services, there are deficiencies and problems related to supplying laboratories with the media necessary to deliver these services. The most commonly cited difficulties were associated with problems in obtaining the culture media for diagnostics or supply of basal medium Elek test. More than half of the (n=17) Member States had difficulty in procuring the medium and 11 countries reported that they use the medium provided by Public Health England/WHO’s diphtheria reference laboratory. Key issues that need to be addressed for diphtheria diagnosis include adequate procurement and supply of the specialised media for toxigenicity testing. Other difficulties mentioned in the survey were associated with loss of accuracy and expertise in toxigenicity testing. The recommended basal medium is not commercially available.
Member States’ training, External Quality Assessment (EQA) and support needs

Availability of definitive external quality assessment for this specialised area of laboratory diagnostics provides significant benefits to the majority of laboratories and is crucial for case identification and management as well as surveillance. Therefore, it is important to continue offering training and EQA programmes within this specialised area of microbiology.

Molecular typing

Ribotyping (used in two Member States in 2016) still remains the ‘gold standard’: the most accurate test possible, in conjunction with Multilocus Sequence Typing (MLST). It offers good discrimination and reproducibility, but is time-consuming and technically laborious; hence its ‘replacement’ by MLST (used in eight Member States in 2016). Databases exist for both methods and are available in the public domain (see http://www.pubmlst.org/cdiphtheriae and http://www.dipnet.org/ribo.public.php).

However, the wide variety of other methods that have been used - including pulsed-field gel electrophoresis (PFGE), Random Amplification of Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Multilocus Enzyme Electrophoresis (MEE) and spoligotyping - indicate a lack of consensus on which test is generally accepted as the most accurate. The need for a standard alternative is accepted and MLST is now being used increasingly by many laboratories globally.

The application of typing for surveillance of pathogen evolution relies on high-quality, public health laboratory-based surveillance to ensure representative specimens. It also requires strong partnerships and expertise in laboratory diagnosis, as exemplified by the partnerships between WHO and the various European diphtheria networks such as the ECDC European Diphtheria Surveillance Network (EDSN).

In contrast to more conventional typing techniques, such as pulsed-field gel electrophoresis, ribotyping, and MLST, whole genome sequencing allows the determination of relatedness with a maximum of resolution and, in addition, provides further genetic information on resistance genes and virulence factors. This technique may, therefore, provide crucial information on the relatedness of C. diphtheriae strains, and inform outbreak management in situations where clusters of cases are observed. Whole genome sequencing offers the potential to describe outbreaks in very high resolution and is a helpful tool in infection tracking and identification of transmission routes.

One option within the EU could be a tiered referral approach to typing, with eight reference laboratories having the potential to undertake molecular typing such as MLST.

Serology and population immunity screening

Accurate determination of diphtheria antibodies is essential to obtaining reliable information on the immune status of a population. This makes it possible to evaluate the success of immunisation programmes, identify the most susceptible groups within a population and offer recommendations for vaccination policy and scheduling. An international standard preparation or a calibrated secondary reference preparation must be used to determine diphtheria antibody levels and is important for the comparison of data obtained from different clinical trials and population immunity studies. Despite the EQAs undertaken in recent years and documented recommendations in terms of serological assays, considerable variation still exists between Member States. This was demonstrated by the gap analysis, where only four countries were using the in vivo toxin neutralisation test (TNT), which is regarded as the ‘gold standard’ method for determining protective levels of serum antitoxin. The fact that many countries appear to be using a diverse range of commercial ELISA assays and other technologies, such as the Double Antigen ELISA (DAE), Double antigen, time-resolved fluorescence immunoassay (dDA-DELFIA), Passive Haemagglutination Assay (PHA), Toxin Binding Inhibition assay (ToBI) and Bead-Based Multiplex assay, is a matter of concern.

Seroprevalence studies have been only undertaken in a few countries in recent years. There is a need to address the issue of assay standardisation across Europe and to conduct seroprevalence studies in targeted areas.

Public health aspects

In total, 27 out of 30 Member States use either national guidelines or follow the WHO guidelines for management of diphtheria; most countries follow the WHO manual for laboratory diagnosis [20] or use country-specific protocols that follow a similar algorithm.

An important issue addressed by the gap analysis is the availability of diphtheria antitoxin (DAT) within Member States as overall global supply and access to DAT, for both therapeutic and diagnostic applications, remains insufficient. This situation is unlikely to change in the near future as several international manufacturers have
ceased DAT production. Only 14 Member States have DAT procurement in place, and the source in six countries is primarily from entities that have reportedly ceased production. DAT is also the cornerstone of diphtheria diagnostics but several diagnostic reference laboratories across Europe and elsewhere routinely face problems in sourcing DAT for diphtheria toxigenicity testing.

The problems with DAT availability were highlighted when sporadic cases of diphtheria were reported recently in several European countries. In such cases the delay in DAT availability may result in fatalities. Problems with access to DAT are being reported by EU Member States on the ECDC Epidemic Intelligence Information System (EPIS) for Vaccine-Preventable Diseases. The gap analysis has shown that it would be useful to monitor DAT availability within countries and that it would be beneficial if a small stockpile of DAT was available for all the European countries concerned.
Conclusions

This analysis has demonstrated potentially significant gaps in diphtheria diagnostic capacity for all three pathogens in the EU/EEA, with only six Member States (one in five) fulfilling minimum standards established by the expert group in terms of surveillance, laboratory methods and training for all three diphtheria pathogens.

Although the majority of Member States reported surveillance in place for *C. diphtheriae*, the availability of primary media culture was reported to be a problem in some Member States. Eleven Member States reported use of medium from WHO/PHE. In addition, obtaining supplies of antitoxin for laboratory diagnosis was reported as a challenge. Overall, 19 countries reported problems with obtaining supplies of antitoxin for laboratory diagnosis, irrespective of the pathogen.

Fewer than half of the Member States had procurement for DAT in place and fewer than half reported having a DAT stockpile.

The criteria with the most significant gap related to training and surveillance for all three diphtheria pathogens. This is of particular concern given the increasing numbers of toxigenic *C. ulcerans* isolates reported between 2013 and 2015.

Nineteen Member States (63%) felt that a supply of DAT was urgently needed. Fewer than half of the Member States had DAT procurement procedures in place and fewer than half reported having a DAT stockpile. Eighteen Member States reported problems in obtaining supplies of DAT for laboratory diagnostics.

Given the importance of antitoxin in both the laboratory identification of toxigenic isolates and the clinical management of diphtheria cases, the sourcing difficulties reported and the low levels of stockpiling present a potentially significant risk to public health in the EU. This risk should be considered in the light of both rising numbers of toxigenic isolates and the movement into Europe of high numbers of migrants and refugees from countries with lower diphtheria vaccination coverage.

In addition, challenges still exist for the accurate monitoring of vaccine coverage in all age groups and in order to maintain consistently high vaccination coverage across Europe. Owing to the rarity of this disease, the understanding of the threat posed by diphtheria and the consequent demand for vaccination has diminished. Furthermore, anti-immunisation sentiment may actively discourage vaccination in some countries.

Overall, this study has highlighted a number of key areas requiring further investigation or action.

Key options for action

- Surveillance systems need to be in place to identify all three pathogens, with appropriate methods to determine toxigenicity.
- EU diagnostic expertise is needed to isolate the pathogens, detect toxigenicity, and in the event of an outbreak or epidemic, to undertake molecular characterisation. Therefore, there is an urgent need for a laboratory training workshop.
- The EQA scheme needs to be re-established.
- Availability of media/reagents needs to be ensured.
- Updated guidelines on laboratory diagnosis of diphtheria and other related infections caused by potentially toxigenic corynebacteria need to be available.
- DAT availability and procurement systems need to be defined in the EU/EEA Member States. This is particularly important given the increasing number of toxigenic isolates and population movements into Europe.
- Clear public health messages and strong efforts are essential in order to achieve the minimum 95% coverage recommended by WHO.

Since public health investigation of human cases may include the testing of pets as potential sources for *C. ulcerans* (and subsequent treatment if indicated), good collaboration with veterinary services would be beneficial.
References

### Annex 1. List of Member States participating

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Annex 2. Annual numbers of potentially toxigenic corynebacteria isolates and the number of confirmed toxigenic isolates reported by Member States, 2013–2015

Table B. Summary of isolates and toxigenic isolates reported by Member States, by year, 2013–2015

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Note: Countries highlighted in green reported at least one toxigenic isolate during the three-year period.
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