Public Health England

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Dear Colleague,

GAP ANALYSIS ON SECURING DIPHTHERIA DIAGNOSTIC CAPACITY IN THE EU/EEA (Service Contract Number ECD.5731)

As part of ECDC's efforts to build and develop microbiology laboratory networks, the European Diphtheria Surveillance Network (EDSN) was established in 2009 where Public Health England (PHE) coordinated the laboratory activities. The EDSN performed External Quality Assessments (EQAs) to enhance and strengthen laboratory-based surveillance capacity. EQAs are an essential tool to enable laboratories to monitor, evaluate and improve their own performance. The last EQA report for the laboratory diagnosis of diphtheria under the auspices of EDSN was published in 2013. This EQA demonstrated that most participants correctly identified the specimens that were distributed; however, many had difficulties with toxigenicity testing, thus resulting in a relatively large number of unacceptable toxigenicity reports. These errors would have most likely prevented appropriate management and treatment of affected patients in a clinical setting. Whilst this EQA assessed the standard of performance of many national reference laboratories, many countries still do not have national reference laboratories recognised by their governments but may offer diphtheria diagnostic services.

Several European countries are also currently undergoing restructuring of their public health and healthcare systems which has now unfortunately led to a decrease in laboratory personnel and financial reductions for diagnostics (http://www.euro.who.int/en/about-s/partners/observatory/health-systems-in-transition-hit-series). The EDSN continued successfully until 2012 when funding for the activities was ceased by ECDC. Since 2012, there has been no formal activity and the diphtheria diagnostic capabilities for this specialised area of microbiology have therefore, diminished in many countries. This was recently highlighted by a fatal case in an unvaccinated child in Spain where not only diagnosis but procurement of the therapeutic drug (diphtheria antitoxin) was problematic.

As a consequence of this, ECDC have commissioned a gap analysis of diphtheria diagnostics among member states where the main objectives are to:

- Assess the current microbiological capability for the laboratory diagnosis of diphtheria in the EU/EEA.
- Assess the PH impact in individual Member States where diphtheria diagnostic activities have ceased.
- Assess the availability of specialised reagents for diphtheria diagnostics in the EU.

- Assess the training needs for scientists/medical and public health staff in this specialised area and identify best practices and gaps in diphtheria diagnostics to establish laboratory training workshops for scientists and medical staff.

- Assess availability of policy and guidelines related to the management and control of diphtheria.

This gap analysis is essential to the public health management and implications of diseases caused by toxigenic corynebacteria in Europe and will impact upon the mechanisms for diagnostics and management. Therefore, it is very important that this questionnaire is completed by each member state. Can you kindly forward this onto your relevant counterpart if you are no longer the person nominated as the operational contact for diphtheria? If you have any questions or queries please do not hesitate to contact me. I look forward to receiving your responses.

Yours sincerely

Professor Androulla Efstratiou
Head, WHO Global Collaborating Centre for Reference and Research on Diphtheria and Streptococcal Infections, Public Health England

Cc: Dr Ida Czumbel, ECDC, Stockholm, Sweden

Public Health England

GAP ANALYSIS ON SECURING DIPHTHERIA DIAGNOSTIC CAPACITY IN THE EU/EEA, including diphtheria antitoxin (DAT) availability

Service Contract Number: ECD.5731

ECDC Operational contact point for diphtheria (epidemiologist)

Name:
Country:
Address:

Email:
Telephone:

ECDC Operational contact point for diphtheria (microbiologist)

Name:
Country:
Address

Email:
Telephone:

Please state the country questionnaire is being completed for:
……

Completion Date of questionnaire …../…../…..

A. DIPHTHERIA SURVEILLANCE

1. Is there a surveillance system for diphtheria in your country, please tick all that apply?

	Yes	No
For C. diphtheria		
For C. ulcerans		
For C. pseudotuberculosis		

   a. If yes to question 1, is there an official link between epidemiology and microbiology in terms of diphtheria surveillance in your country?

      YES/NO

   b. Please describe the link between epidemiology and microbiology in terms of diphtheria surveillance in your country:
      .....
      .....
      .....

2. Do you provide a diphtheria reference service for your entire country?

      YES/NO

   a. If yes, since when? Please state the year the service started:
      .....
      .....

3. If your diphtheria reference service is not for the entire country, what geographical area do you cover?
   .....
   .....
   .....
   .....

4. Are you aware of other diphtheria reference activities/laboratories i.e. other labs that are able or planning to undertake toxin testing within your country?

      YES/NO

   a. If yes, please provide details:
      .....
      .....
      .....
      .....

5. Is there a legal obligation for laboratories to submit isolates/specimens to your laboratory for reference testing?

   YES/NO

6. How many laboratories sent isolates/clinical specimens to the National Reference Laboratory per year?

   .....
   .....
   .....

7. Do you provide a reference service for cultures sent from outside your own country?

   YES/NO

   a. If yes, please list countries below:

   .....
   .....
   .....

8. Is there an official agreement in place in between your country and another to allow the transfer of samples?

   YES/NO

   a. If yes, please describe the agreement:

   .....
   .....
   .....

9. a. In the table below please indicate the number of isolates/specimen received by your laboratory, which were originally isolated from patients within your country.

	C. diphtheriae								*C. ulcerans*		*C. pseudotuberculosis*	
Biotype	gravis		mitis		belfanti		intermedius					
Year	Tox+	Tox-	Tox+	Tox-	Tox+	Tox-	Tox+	Tox-	Tox+	Tox-	Tox+	Tox-
2013												
2014												
2015												

   b. If you have any further comments with regard to the table above please provide them below:

   .....
   .....
   .....

10. If you have reported any toxigenic strains in the above table, please indicate how toxigenicity was determined in the table below:

Test	Please tick if you used the test listed	If yes, please describe by year and pathogen?
PCR		
Elek test		
Both Elek and PCR		
Other tests *(please specify)*		

B. LABORATORY CAPACITY
DIAGNOSTIC SERVICES

11. Do diagnostic laboratories within your country screen, using Tellurite medium/Tinsdale medium throat swabs for the presence of potentially toxigenic corynebacteria?

    YES/NO

    a. Please state the approximate number of laboratories screening using this method: .....
    b. Please state the approximate number of laboratories not screening using this method: .....

    c. If the diagnostic laboratories within your country do not screen,

        i. When was the service terminated?
        .....
        ii. Why was the service terminated?
        .....
        .....
        .....
        .....
        iii. What was the impact of ceasing this service? Please describe:
        .....
        .....
        .....

12. How many of your regional laboratories/other laboratories in your country are expected to perform diphtheria reference services (i.e. undertake toxin testing) either?
    a. Currently:.....
    b. And also in the future:.....

13. Are there staff members in these laboratories that require specialist training on diphtheria reference diagnostics?

    YES/NO

14. When was the last occasion that the head of the diphtheria reference service attended a training workshop on diphtheria diagnostics?

    a. Please state the date of the training:
    .....
    b. Please state the training provider:
    .....

15. When was the last occasion that any of the laboratory staff working on diphtheria diagnostics attended a training workshop on diphtheria? YES/NO

    a. Please state the date of the training:

    .....

    b. Please state the training provider:

    .....

    c. Is this person(s) still employed to undertake these activities?

    YES/NO

16. What are the most urgent needs in your country related to laboratory capacity at this time? Please indicate the respective needs regarding their importance on a scale of 1 (not urgently needed) to 5 (very urgently needed)

Needs	1 Not urgently needed	2	3	4	5 Very urgently needed
EQA distribution					
Supply of Elek basal medium					
Supply of antitoxin					
Development of a dip stick /rapid test for toxin detection					
Laboratory training workshop					

17. What laboratory support would be required to maintain diphtheria diagnostics in your country?

.....

.....

.....

18. Do you have any formal or informal collaboration with other diphtheria reference laboratories within your country and or within the EU Diphtheria Surveillance Network? (eg: 'twinning initiatives).

    YES/NO

    a. If yes, please provide details below:

    .....

    .....

    .....

    .....

19. What steps have you taken to remedy, the gaps (if any) identified in the last diphtheria diagnostics EQA in 2013? Please provide details below:
Further information on the 2013 EQA can be found here:
http://ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispForm.aspx?ID=1157

.....
.....
.....

20. What was the impact in your country of cancelling the European Diphtheria External Quality Assurance and training following the EQA exercise IN 2013? Please describe below:

.....
.....
.....

IDENTIFICATION PROCEDURES:

21. In the table below briefly indicate your current methodologies for bio-typing and identification practices for *Corynebacterium* species in the table below.
Please include details of the primary selective media, screening tests used and method(s) for biochemical identification.

Primary selective media, screening tests used and method(s) for biochemical identification	**Please tick if you use the method listed**	If yes, which controls do you use?
Primary culture:		
Gram stain		
Other stains (*please specify*)		
Blood agar		
Tellurite agar		
Screening, biotyping and identification tests:		
Cystinase		
Pyrazinamidase test		
Nitrate reduction		
Urease hydrolysis		
Hiss serum water sugars (Glucose, Maltose, Sucrose)		
API Coryne		
Identification via sequencing genes (*please specify which genes*)		
MALDI-TOF (*please specify which system*)		
Other tests *(please specify)*		

CULTURE MEDIUM AVAILABILITY IN YOUR COUNTRY

22. Do you experience problems with obtaining culture media for diphtheria diagnostics?

    YES/ NO

    a. If yes, please describe:
    .....
    .....
    .....

TOXIGENICITY TESTING

23. Please indicate the method(s) currently used in your laboratory for toxin detection for *Corynebacterium* species.

Method	Please tick if you use the method listed	If yes, which controls do you use? Please specify species and culture collection reference number
Elek test		
PCR		
RT-PCR		
In vivo test		
Tissue culture (e.g. Vero cell bioassay)		
Other tests (*please specify*)		

    a. If Elek test performed, what is the source of your Elek medium?

Elek medium source	Tick all that apply
In house	
WHO/PHE	
Commercial	

        a. If the source is Commercial, please state which manufacturer you use?
        .....
        .....
        .....

b. What type of serum do you use for the Elek medium?

Serum for Elek medium used	Tick all that apply
Equine	
New-borne	
Bovine	
Other, please describe:	

c. Do you have problems in obtaining supplies of antitoxin for laboratory diagnostics?

YES/NO

i. If yes, please give details:

.....
.....
.....

d. What is the concentration of antitoxin used for the Elek test? Please tick all that apply?

Concentration of antitoxin used for the Elek test	Tick all that apply
500 IU	
1000IU	
Other, please specify:	

e. If PCR test performed, which primer sets do you use? Please state the sequence below.

i. Fragment A of toxin gene:

.....
.....
.....

ii. Fragment A and B (entire gene)

.....
.....
.....

iii. Which manufacturer and reference

.....
.....
.....

f. Do you use an internal template or inhibition control: eg: artificial template which Is amplified by the same primer pair as the template DNA, and is used to confirm the PCR reaction has not failed:

YES/NO

g. Are you using a RT-PCR assay?

YES/NO

i. If yes, please describe briefly and state the reference i.e. list Taqman®, Light Cycler or other:

.....
.....
.....

h. If you do not perform PCR:

i. Are there relevant facilities thermocycler, gel tanks, photographic equipment etc) available in your laboratory/institute:

YES/NO

ii. Would you like to introduce PCR toxin detection to your laboratory?

YES/NO

iii. Please describe why you would like to or do not want to introduce PCR toxin detection to your laboratory:

.....
.....
.....

i. Do you carry out any toxigenicity tests other than PCR and Elek test?

YES/NO

i. If yes, please describe your procedure or attach methods and/or references if available:

.....
.....
.....

ANTIBIOTIC SENSITIVITY

24. Do you routinely determine antibiotic sensitivities on ANY *C. diphtheriae*, *C. ulcerans* and *C. pseudotuberculosis* isolates received by your laboratory?

    YES/NO

25. Do you only determine antibiotic sensitivities if specifically requested to do so?

    YES/NO

    a. If yes, please indicate in the table below which methods you perform in your laboratory?

Antibiotic sensitivity method	Please tick if you use the method listed	If yes, which manufacturer are the reagents from?
E test		
Disc diffusion (please state method)		
Broth dilution		
Agar incorporation		
Commercially prepared MIC microtitre trays		
Other (*please specify*):		

26. If breakpoint values are measured, what interpretation guidelines are used? Please tick all that apply.

Interpretation guidelines used	Tick all that apply
CLSI (formerly NCCLS)	
BSAC	
EUCAST	
Other, please specify:	

MOLECULAR TYPING OF CORYNEBACTERIA

27. Do you perform molecular typing on the following:

	Yes	No
For C. diphtheria		
For C. ulcerans		
For C. pseudotuberculosis		

28. If yes, which of these typing methods do you perform in your laboratory?

Methods	Please tick if you use the method listed
Ribotyping	
Multilocus enzyme electrophoresis (MLEE)	
Pulsed-field gel electrophoresis (PFGE)	
Random amplified polymorphic DNA (RAPD) typing/PCR typing	
Amplified Fragment Length Polymorphism (AFLP)	
Multilocus sequence typing (MLST)	
Variable Number Tandem Repeats (VNTR)	
Other (*please specify*)	

29. If no, are you planning to perform molecular typing in the future?

YES/NO

30. How long might it take before capacity is in place? Please describe:
.....
.....
.....

31. Which molecular typing method would you find most useful? Please describe:
.....
.....
.....

32. Which analytical and/or database software do you use for molecular typing/microbiological data? Please tick all that apply.

Analytical and/or database software	Tick all that apply
Taxotron	
BioNumerics	
Other, please specify:	

33. Do you use the MLST database and submit your own MLST strains/ST types to the database?

YES/NO

C. *DIPHTHERIAE* POPULATION IMMUNITY SCREENING

34. When was the last diphtheria sero-prevalence study undertaken in your country?

    a. Please state the year the study was carried out:.....

    b. Please describe the study:

.....
.....
.....

35. Which of these serological methods do you perform in your laboratory?

Methods	Please tick if you use the method listed	If YES, In-house or commercial? Please state manufacturer
In vivo toxin neutralisation		
Tissue culture toxin neutralisation		
ELISA In house method		
ELISA commercial kit (please state which kit)		
Passive haemagglutination		
Other (*please specify*)		

36. What interpretation criteria do you use for your serological assay?

.....
.....
.....

37. How do you define the following:

    a. Immune/protective status:

.....
.....
.....

    b. Susceptible status:

.....
.....
.....

D. PAN EUROPEAN DIAGNOSTICS AND EQA

38. Is your laboratory accredited?

    YES/NO

    a. If yes, please state accreditation body?

.....
.....
.....

39. What would you consider the best approach(es) to maintain diphtheria laboratory expertise in Europe?

.....
.....
.....

40. What is the added value of having an EQA for diphtheria diagnostics?

.....
.....
.....

41. Are you aware of the diagnostics and EQA report results for diphtheria published on the ECDC website?

    YES/NO

42. Have these reports been disseminated to your relevant colleagues or to the decision makers? Please tick all that apply.

Who the reports have been disseminated to	No	Yes
Colleagues		
Decision Makers		

43. Would a Diphtheria EQA exercise for laboratory diagnostics and toxigenicity testing be essential for your laboratory accreditation?

    YES/NO

    a. If no, please state the reason.

.....
.....
.....

44. Is there a need for a laboratory workshop on diphtheria diagnosis and typing for your country?

    YES/NO

    a. If Yes, what areas do you specifically wish to see covered in this workshop? Please provide detasil below:

    .....
    .....
    .....

45. Do you perform sero-prevalence studies in your country?

    YES/NO

    a. When was the last time that a sero-prevalence study had been undertaken in your country?

    .....
    .....
    .....

    b. Please describe whether the study was at national or regional level?

    .....
    .....
    .....

46. Have you ever performed or participated in any diphtheria screening studies in your country?

    YES/NO

    a. IF YES, please state the year this took place and describe the study in more detail.:

    .....
    .....
    .....

E. PUBLIC HEALTH ASPECTS

47. Do you have national case management guidelines in place for diphtheria?

YES/NO

a. If no, do you use the the following:

Case management guidelines	Tick all that apply
WHO diphtheria guidelines	
Any other guideline, please provide details:	

48. Do you have national laboratory manual for diphtheria in your country?

YES/NO

a. If no, do you use the the following:

Laboratory manual	Tick all that apply
WHO diphtheria guidelines	
Any other guideline, please provide details:	

49. Do you have diphtheria antitoxin (DAT) procurement in place in your country?

YES/NO.

a. If yes, which body is responsible for it?

Body responsible	Tick all that apply
National	
Regional	
Local e.g. hospital	
Any other guideline, please provide details:	

b. Please provide further details of DAT procurement in place in your country:

.....

.....

50. Do you have an existing DAT stockpile in your country?

YES/NO

a. If yes, please describe the name of the product

.....

.....

b. Please, describe the name of the manufacturer

.....

.....

51. Considering the prevalence of the illness in your country, what would be the quantity of the stockpile that would be required to cover your countries needs? Please describe.

.....

.....

OTHER COMMENTS OR SUGGESTIONS
(Please continue on a separate sheet if necessary).

.....

.....

.....

.....

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Many thanks for your invaluable co-operation.

DEADLINE DATE FOR RETURN OF QUESTIONNAIRE: 4 APRIL 2016
Please return the completed questionnaire by email (preferable):

Professor Androulla Efstratiou
WHO Global Collaborating Centre for Diphtheria and Streptococcal Infections
National Infection Service
Public Health England
61 Colindale Ave
London NW9 5EQ
United Kingdom
Email: Androulla.efstratiou@phe.gov.uk

If you have any queries please do not hesitate to contact either Professor Androulla Efstratiou or Dr Ida Czumbel, (ECDC) ida.czumbel@ecdc.europa.eu