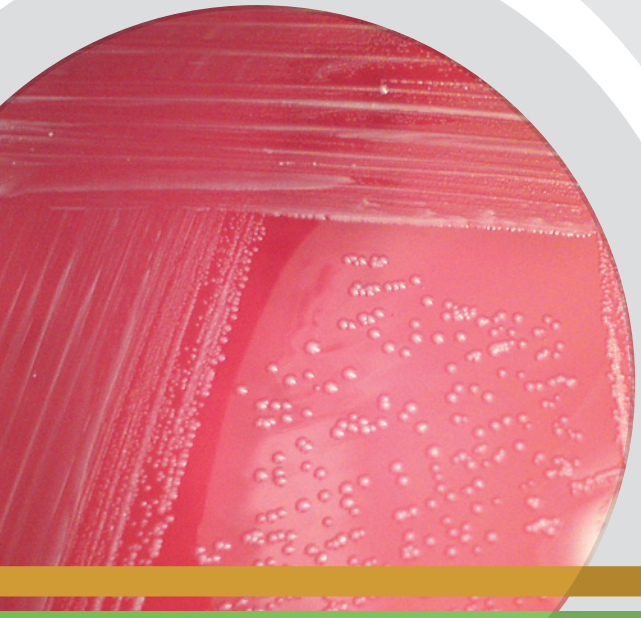

TECHNICAL REPORT

External quality assurance scheme for diphtheria diagnostics 2012

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ECDC TECHNICAL REPORT

External quality assurance scheme for diphtheria diagnostics 2012

As part of the European Diphtheria Surveillance Network (EDSN)

This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Dr Ida Czumbel and produced by Dr Shona Neal and Prof Androulla Efstratiou (Health Protection Agency, Streptococcus and Diphtheria Reference Section/WHO Global Collaborating Centre for Reference and Research on Diphtheria and Streptococcal Infections, London, United Kingdom), on behalf of the EU DIP-LabNet consortium (referring to Specific Contract No. 3228).

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Contents

Abbreviations

DIPNET	Diphtheria Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EDSN	European Diphtheria Surveillance Network
ELWGD	European Laboratory Working Group for Diphtheria
EQA	External Quality Assurance
eQAD	External Quality Assurance Department
EU	European Union
EU DIP-LabNet	EU Diphtheria Laboratory Network
HPA	Health Protection Agency (United Kingdom)
MALDI-TOF	Matrix-Assisted laser desorption ionization – time of flight
NEQAS	National External Quality Assessment Service
NIS	Newly Independent States
NTTB	Non-toxigenic, tox-gene bearing
PCR	Polymerase chain reaction
SDRS	Streptococcus and Diphtheria Reference Section (of the HPA, London)
WHO	World Health Organization

Executive summary

Effective control of an uncommon vaccine-preventable disease is dependent upon prompt and early recognition and diagnosis. It is often difficult to diagnose diphtheria clinically, particularly in those countries where the disease is rarely seen. Diphtheria is often confused with other conditions, such as severe streptococcal sore throat, Vincent's angina, or glandular fever. Therefore, accurate microbiological diagnosis is crucial and complementary to clinical diagnosis. It is fundamental that EU Member States have the capacity and ability to undertake the procedures relating to the microbiological diagnosis of not only diphtheria but also the related infections caused by all potentially toxigenic strains of corynebacteria.

ECDC has a role in building and developing microbiology laboratory networks; in 2010, the responsibility for the activities of the European Diphtheria Surveillance Network (EDSN) was transferred to ECDC.

This report comprises the findings of the second EQA dispatch for the laboratory diagnosis of diphtheria under the auspices of EDSN. A total of thirty countries participated in this EQA and were asked to isolate, identify and perform toxigenicity testing on any *Corynebacterium* spp. present in the six simulated throat specimens sent. Key findings are listed below; a description of the work involved and the outcomes of these exercises are detailed further in this report.

Key findings

- Seven out of thirty participants have introduced methods to enhance their diphtheria diagnostic capacities.
- However, the use of the phenotypic toxigenicity test, Elek, has declined, due to difficulties in obtaining reagents; this is a recurrent problem, and a number of countries requested and received these specialised components (Elek media, antitoxin strips, and antitoxin discs).
- Overall, 90% of the identification reports and 89% of the toxigenicity reports were correct – a slight improvement on the previous EDSN EQA from 2010.
- However, eleven countries reported worse results for this recent EQA exercise, mostly due to incorrect identification; specimen 6 revealed the most errors, as this was a problematic isolate to detect.
- The Elek test also generated false positives and negatives; participants reported problems interpreting the test and reading the plates after 48 hours, thus emphasising the need to regularly train personnel and perform EQAs.
- Specific countries have been identified that would benefit from attending the next diphtheria diagnostics workshop in January 2013, based on their average-to-poor performance in this EQA.
- These activities should be continued to maintain the level of capability and quality of results in all Member States.

Background

The European Centre for Disease Prevention and Control (ECDC) was formed as a European Union (EU) agency to identify, assess, and communicate current and emerging threats to human health from communicable diseases. Part of ECDC's mandate includes to 'foster the development of sufficient capacity within the Community for the diagnosis, detection, identification and characterisation of infectious agents which may threaten public health. The Centre shall maintain and extend such cooperation and support the implementation of quality assurance schemes.' (Article 5.3, EC 851/2004[1]).

An external quality assurance (EQA) exercise evaluates the performance of laboratories by an outside agency, which provides material especially for that purpose; this can be used as part of a quality management system. Thus, EQAs may identify areas for improvement in laboratory diagnostic capacities. ECDC support EQA schemes as they impact on the surveillance of the diseases listed in Decision No 2119/98/EC[2] and ensure comparability of results across laboratories from all EU/EEA countries. The main purposes of EQA schemes include:

- assessment of performance of national reference laboratories and of laboratories offering diphtheria laboratory service;
- assessment of the effects of analytical procedures (method principle, instruments, reagents, calibration);
- evaluation of individual laboratory performance;
- identification and justification of problem areas;
- provision of continuing education; and
- identification of needs for training activities.

Thus, the EQA process can increase the probability of correct diagnosis, case management and an improved quality of surveillance data by motivating the users to improve laboratory performance.

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 [2]. At the request of the WHO European Region, the European Laboratory Working Group for Diphtheria (ELWGD) was promptly established in 1993 to help strengthen the diphtheria diagnostic capabilities of the many countries affected [3]. At the time, screening for diphtheria from routine throat swabs was adopted in many European countries; currently, no EU countries are screening for diphtheria, due to the low incidence of *C. diphtheriae* and *C. ulcerans* now observed.

The first EQA exercise for diphtheria diagnostics in Europe was performed in 1994 under the auspices of the ELWGD, and since then, ten distributions for laboratory diagnostics have been performed (including two for serological immunity testing). Results from the last four distributions revealed that irrespective of the composition of the EQA panel or the countries participating, correct toxigenicity and identification reports have rarely exceeded 90% [4,5]. Therefore, continued EQA programmes for diphtheria diagnostics need to be maintained.

The European Diphtheria Surveillance Network (EDSN) was established in March 2010 and comprises nominated epidemiologists and laboratory experts for diphtheria from the 27 EU Member States and two of the three other EEA countries. The purpose of the EDSN is to establish a system of expertise for the prevention and control of diphtheria and to strengthen and harmonise the laboratory capacity at national level. The network has two components: epidemiological (conducted by ECDC and focused on data collection and analysis) and laboratory (outsourced to the Health Protection Agency (HPA), London and focused on EQA and training). The key objective of this work, as described in this report is:

- to assess and improve laboratory performance for standardised and appropriate methods to be used for the laboratory diagnosis of culture-confirmed diphtheria infections for ensuring accurate and comparative diphtheria surveillance across Europe.

[1] Regulation (EC) No 851/2004 of the European Parliament and of the Council of 21 April 2004 establishing a European Centre for Disease Prevention and Control.

[2] Decision No 2119/98/EC of the European Parliament and of the Council of 24 September 1998 setting up a network for the epidemiological surveillance and control of communicable diseases in the Community.

Materials and methods

EQA design

The design of the EQA scheme allowed the material to be tested by the individual reference laboratories, using their routinely available techniques within the allocated time period. All participating laboratories were able to compare their own submitted results with the intended results to determine any differences and implement any improvements, if required.

The EQA distribution was aided by the availability of the large collection of corynebacteria isolates and expert knowledge at the HPA's Streptococcal and Diphtheria Reference Section (SDRS, London, UK). It was supported by the United Kingdom National External Quality Assessment Service (UK NEQAS) for Microbiology, and facilities in the external Quality Assurance Department Microbiology Services Division, London, UK. UK NEQAS for Microbiology undertake several international EQA schemes for other organisms that also require freeze-drying, distribution, results analysis and web-based reporting.

Participants

The list of the participating reference laboratories can be found in Annex 1. In addition, Turkey requested the EQA panel, as they had previously participated in the 2007 DIPNET distribution. Turkey also accepted to pay for the shipping costs, and to justify their inclusion, Turkey is an EU candidate country.

All participants were contacted before the EQA distribution to confirm the address and contact details for dispatch of the potentially hazardous material. It was envisaged that some reference laboratories would wish to store the viable cultures and use them for their own quality processes. The distribution of the well-characterised material may become a resource within and between the reference laboratories.

Timeline

Table 1. Timeline for the second EDSN EQA exercise

Event	Date
Selection of EQA strains	February 2012
Building participants list	March – May 2012
Assessment of strains before freeze-drying	March 2012
Transfer of strains to eQAD UK NEQAS	March 2012
Freeze-dry panel produced (eQAD UK NEQAS)	May 2012
Pre-dispatch checks of freeze-dried panel (SDRS)	May 2012
Requests for specialised Elek media and antitoxin from participants	March – May 2012
EQA panel dispatch (eQAD UK NEQAS)	30 May 2012
Additional Elek media and antitoxin dispatch to various countries	30 May 2012
Reference laboratories testing EQA panel	June – July 2012
Final return of results	13 July 2012
Preliminary feedback to participants	August 2012
Intended results sent to participants	August 2012
Analysis and collation of results	July – September 2012
Preliminary results presentation at ESCAIDE meeting, Edinburgh	24 – 26 October 2012
Producing report	July – December 2012

The EQA simulated specimen panel

Six *Corynebacterium* spp. strains were selected based on their variability and toxigenicity. The strains had been referred to the WHO Collaborating Centre, London, and are all from clinical cases. Two strains had been submitted from English laboratories (2006–09) and one other was a recent wound swab from a patient from Dublin, Ireland, in 2011. Two other strains had been submitted as part of international collaborations; one from St Petersburg, Russia, in 1999 and the other from Seattle, USA, in 1994. The panel contained two non-toxigenic toxin-gene-bearing (NTTB) strains, where one was a *C. diphtheriae* biovar *intermedius*. One of the specimens (coded as DIPEQA12-3) contained no target organism, to test whether the participants would name or report an isolate. Therefore, this was a little more challenging than the previous EDSN EQA.

The strains were coded and prepared as simulated throat specimens by the addition of two or more commensal organisms and freeze-dried by the Quality Assurance Laboratory, HPA, London. Quality control of the specimens

was undertaken by the WHO Collaborating Centre both before and after freeze-drying to test for viability and retention of the organism's characteristic properties.

Full instructions were included in the dispatch, asking participants to isolate, identify, and perform toxigenicity testing on any *Corynebacterium* sp. present and report their results, the time taken to achieve a final result, and any problems encountered. The EQA was distributed in May 2012 to 29/30 EU/EEA countries (no laboratory counterparts for Liechtenstein) plus Turkey. Full instructions and a result form were enclosed and also sent electronically, with results to be submitted by 13 July 2012 (Annex 2).

A questionnaire was circulated in 2010 and reported in the previous EDSN report; therefore, details of this are not included. Also, participants were asked whether they had changed or introduced any methods for this 2012 EQA.

Additional dispatch of specialised media

Due to monetary restrictions, participants were not provided with a budget for laboratory consumables, reagents and media for the diagnosis of diphtheria. Therefore, all centres had to procure these items for this round of EQA. However, many countries still requested a shipment of Elek media and/or antitoxin strips or discs from the WHO Collaborating Centre for Diphtheria and Streptococcal Infections in London. Elek is the gold standard test for detecting toxigenicity, yet requires media which can be laborious to make, and antitoxin, which is hard to obtain.

In contrast to the last report in 2011, there does not appear to be any commercial product on the market to test the phenotypic expression of diphtheria toxin; thus it relies on good quality, reliable in-house preparations.

Eight countries received shipments, which in total comprised 87 vials of Elek media, 178 antitoxin strips and 110 antitoxin discs.

Data analysis

The intended results were sent to all participants in August 2012 for information and for each laboratory to rapidly assess how they performed (Annex 3). Participants' results were analysed against the intended results on the basis of isolation, identification, and toxigenicity testing of any *Corynebacterium* spp. present in the specimens. Results from each centre were evaluated as acceptable (fully correct results), acceptable with minor errors (incorrect biotyping results), or not acceptable (failure to isolate target *Corynebacterium* spp. and/or incorrect phenotypic toxigenicity result). To compare the countries' performance over time, results of the 2010 and 2012 EQA were allocated either 'fully correct', 'acceptable' or 'incorrect' for each specimen.

If any participant experienced problems, or a method was identified as generating incorrect results, EU DIP-LabNet offered direct advice and recommended repeat testing the specimens, following corrective action, in order to improve diphtheria diagnostics in EU/EEA countries.

Results

Laboratory capabilities

Luxembourg and Norway recently introduced a MALDI-TOF assay for identification, Hungary implemented the Elek test; Ireland, Latvia and Lithuania conduct a PCR targeting the *tox* gene; and Italy designed a real-time PCR for both identification and presence of toxin gene.

In addition, Iceland, who had not participated in the 2010 EQA, were able to accept this EQA, but only to perform identification, as they do not have the media for toxigenic testing. Spain, who also could not receive the last EQA, but had been participants in previous diphtheria diagnostic EQAs, also participated in this EQA, and while they were not able to perform a phenotypic toxigenic test, they were able to use molecular methods to detect the toxin gene and for identification (16S rDNA and *rpoB*). In addition, Greece could only report the identification.

There were also many countries that could not test for the toxin expression using Elek or another phenotypic assay, due to difficulties in obtaining the specialised reagents and antitoxin. Consequently, the number of Elek testing centres has dropped since the last EQA from 20 to 18. Some countries also reported problems with expired reagents or difficulties in interpreting the results.

Laboratory diagnostic EQA results

All 30 centres submitted results, including Turkey. The intended results, with a summary of the participants' findings, are shown in Table 2.

All bar one centre reported the correct species, biovar and toxigenicity for **specimen 1**. This centre reported an incorrect biovar; therefore, there were no unacceptable results for this first specimen.

Specimen 2 was a non-toxigenic toxin-gene-bearing *C. diphtheriae* biovar *mitis*; consequently, around a third of the centres experienced problems with the toxigenicity results. Five centres reported false toxigenic results with the phenotypic Elek test and three centres reported false negative results for the genotypic PCR test. For those four centres that performed both PCR and Elek, a positive diagnosis would have been made and treatment, screening contacts and public heath action would have ensued, had this been a clinical case. However, 20 centres reported a fully correct result, with two centres reporting wrong biovars.

There was no target organism in **specimen 3**; subsequently, most centres correctly reported 'none' for the target organism. However, three centres reported *Arcanobacterium haemolyticum* and one centre *C. striatum*. These are not potentially toxigenic corynebacteria and very rarely cause severe disease. In addition, three centres did report either *C. diphtheriae* or *C. ulcerans*; this may be due to transcriptional error as six specimens would have been processed at the same time and could be switched at any stage. This could have led to inappropriate clinical and public health management.

Centres scored well with **specimen 4**, where 25/30 centres gave a fully correct result for the *C. ulcerans* strain (non-toxigenic). Five centres misidentified it as a *C. pseudotuberculosis, C. amycolatum* or a non-pathogenic *Corynebacterium*, and one centre reported the *C. ulcerans* as toxigenic using the Vero cell assay.

Approximately two-thirds of the centres reported **specimen 5** correctly as a weak toxigenic *C. ulcerans* (21/30). A further three centres gave unacceptable identification results, reporting either *C. pseudotuberculosis* (2) or none (1). Worse still, six centres reported non-toxigenic results using various methods (see Table 2); if this had been a true case, treatment and public health action would have been missed.

Table 2. Summary of results for each of the EQA specimens for the 30 participating laboratories

Specimen number	Intended result	Toxigenicity (Elek and PCR)	Number of laboratories with		
			Fully correct result	Acceptable result	Unacceptable result
DIPEQA12-1	*C. diphtheriae* biovar *gravis*	Toxigenic	29	1	0
DIPEQA12-2	*C. diphtheriae* biovar *mitis*	Non-toxigenic (NTTB)	20	2	8 (5 reported toxigenic results with Elek, and 3 reported negative PCR results)
DIPEQA12-3	NO organism	N/A	23	4*	3 (2 reported *C. diphtheriae,* 1 *C. ulcerans*)
DIPEQA12-4	*C. ulcerans*	Non-toxigenic	25	0	5 (1 reported toxigenic result with Vero cell) (2 reported *C. pseudotuberculosis,* 1 *C. amycolatum* and 1 '*Coryne* spp., non-pathogenic')
DIPEQA12-5	*C. ulcerans*	Weak toxigenic	21	0	9 (2 reported *C. pseudotuberculosis* and 1 'none') (6 reported non-toxigenic results; 2 PCR, 1 Elek, 2 both PCR and Elek and 1 Vero cell)
DIPEQA12-6	*C. diphtheriae* biovar *intermedius*	Non-toxigenic (NTTB)	9	11	11 (3 reported toxigenic Elek results) (4 reported no isolate, 1 *Corynebacterium* spp., 1 *C. amycolatum*, 1 *C. striatum* and 1 *C. xerosis*)

NTTB = non-toxigenic toxin-gene-bearing strain

** Three centres reported* Arcanobacterium haemolyticum *and one centre* C. striatum*.*

Specimen 6 proved to be the most difficult, as only a third of centres (9/30) achieved a fully correct result and reported the *C. diphtheriae* biovar *intermedius* NTTB strain. In addition, one centre reported it as '*Corynebacterium* spp., possibility of *C. diphtheriae'*, and would normally have referred the isolate if it were a clinical case; this was scored as an acceptable result. The biovar was incorrectly identified as gravis (1), *mitis/belfanti* (2) or *mitis* (6); the latter differs only in colony size (0.5 – 1 mm in diameter for *intermedius*, compared with 1.5 – 2 mm for *mitis* biovars), and in *intermedius* strains fermenting dextrin. However, more alarmingly, centres reported false toxigenic (3) or incorrect identification results (8). The lack of detecting the target organism would have delayed the treatment of the case, contact tracing and any other public health interventions.

Overall, from the six specimens and 30 centres, there were 180 available reports, resulting in 18 (10%) incorrect identification reports and 20 (11%) incorrect toxigenicity reports. This was a slight improvement to the previous EDSN EQA in 2010 of 14% incorrect identification and 10% incorrect toxigenicity reports from 24 countries [5]. The time taken to generate a final result ranged from one to thirty-five days; the mean was seven days and the median was five. Nine countries managed to report acceptable or fully correct results for all six specimens: Austria, Cyprus, France, Iceland (ID only), Malta (ID only), the Netherlands, Norway, Slovakia and the United Kingdom. A further twelve centres achieved acceptable or fully correct results for 5/6 specimens. However, approximately a third of the participants (9/30, 30%) reported two or more of the specimens incorrectly, for identification and/or toxigenicity. Unfortunately, three countries only obtained correct results for 3/6 specimens, and one country reported only two specimens correctly; one of the centres reported a severe lack of resources due to the economic crisis.

Of the 26 countries that participated in both the 2010 and this EQA, only six had improved. Nine countries performed comparably between the two EQAs; most of these performed well (seven achieving 4/6 fully or acceptable results), including France, Malta and the United Kingdom, who reported 6/6 fully or acceptable results. Unfortunately, the remaining eleven countries reported worse results for the 2012 EQA; four of the countries have not had personnel attending a diphtheria diagnostic training workshop over the past three years.

Conclusions

Overall, the participating countries' capacities have continued to improve since the last EQA in 2010. Furthermore, countries which have expanded their repertoire of methods for diphtheria diagnostics have noticed an improvement in their performance in this EQA. This monitoring of performance through regular EQA dispatches has ensured that key personnel in EU countries have remained aware of diphtheria and maintained good standards in the use of specialised methods in diphtheria diagnostics.

One country that had performed extremely well on previous diphtheria diagnostic EQAs, only achieved two correct results on this occasion. Although they have not attended any EU-funded workshop since 2007, they were given some feedback on this poor performance, and after a few days they submitted 5/6 correct results. This example shows the importance of both workshop training and individual feedback as tools to improve diagnostic performance.

Although there was a decrease in incorrect reports, previous problems persist, including both phenotypic and genotypic toxigenicity testing. Two countries reported 3/6 toxigenic results incorrectly; both used the Vero cell toxin neutralisation assay and submitted different results for the various specimens. No other country seemed to have a specific problem with toxigenicity testing, and moreover, no specimen seemed to cause any problems.

Sporadic false positives and negatives with the Elek test were observed from nine countries, where some reported reading the Elek plates at 72 hours and with problems interpreting the test. It is recommended that the Elek test is not read after 48 hours, as this can give false positives, due to non-specific agglutination lines between the antitoxin and other organism derivatives [6]. False negative results were also generated using a PCR method from eight countries; no particular method/primer pair was used, although few countries use an internal positive control to detect any PCR inhibition.

Although PCR assays are rapid and relatively straightforward to implement, a PCR to detect the diphtheria toxin gene should be used with caution and only as an adjunct to the Elek test, which detects the expression of the toxin. However, performing the Elek test may not be possible or feasible in some countries. Furthermore, in the EQA were two NTTB strains; these were first described in the late 1990s during the decline of the NIS epidemic. While rare, the public health significance of these NTTB strains is still unknown and warrants continued surveillance and further investigation, in view of their emergence in other European countries.

Two of the specimens were to test the countries' ability to detect the target organism. Although specimen 3 did not contain any target organism, several countries reported other *Corynebacterium* species. In particular, four centres reported an *Arcanobacterium haemolyticum;* it is likely that a commensal was picked and identified with the API Coryne (two centres gave 0530161 as the profile). In addition, only potentially toxigenic corynebacteria were required to be reported; thus, these centres did not follow instructions adequately. Specimen 6 contained a *C. diphtheriae* var *intermedius* NTTB strain which was used in the DIPNET EQA in 2007 (original designation 94/129). Although still poor, performance has improved for this EQA (9/30 cft 6/34 in 2007), which is encouraging, as this biovar can go undetected due to its small colony size. Many countries either did not detect it or reported the wrong biovar. Participants should therefore incubate throat swab plates for at least 48 hours (and check at 16 to 24 hours) to ensure that *C. diphtheriae* var *intermedius* strains can be detected. Regular EQAs and attendance of diphtheria diagnostic workshops should enhance experience and raise awareness of all the potential toxigenic corynebacteria and biovars.

Many countries continued to request specialised media and reagents for the Elek test. Although not budgeted, there were sufficient funds to fulfil this exercise, and money was saved by sending the reagents with the EQA specimens. However this is not an ideal situation, as these media and reagents could be needed for real clinical specimens; therefore, regular consignments would be needed to ensure that most countries can perform this specialised test with in-date reagents.

Recommendations

- To continue the EQA dispatches regularly, as this has improved some countries' capabilities and gives all Member States essential experience in handling toxigenic strains of corynebacteria. This should also maintain quality and improve further the Member States' capabilities in diphtheria diagnostics.
- To train participants that did not perform well, by way of a training workshop.
- If PCR is only used for toxigenicity testing in some Member States, they must ensure that the isolate is sent to a participating centre that can test the expression of the toxin, using for example the Elek test. This should not, however, hamper the delay in clinical and public health management.
- To explore avenues to support countries that requires specialised media and reagents for the Elek test through the EDSN.
- To bring together the laboratory counterparts at the annual network meetings to discuss these issues face to face and to discuss recent developments within this specialised field and review objectives in relation to microbiological surveillance.

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Annex 1. Participating reference laboratories

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Turkey	Selin Nar Otgun	Refik Saydam National Hygiene Center Communicable Diseases Research Department E blok, 2.kat, 06100 Sihhiye, Ankara, Turkey

Note: Liechtenstein could not participate in the EQA.

Annex 2. Instructions and result form for the EQA dispatch

External Quality Assurance Study for the Laboratory Diagnosis of Diphtheria

EU DIP-LabNet (contract number ECDC/10/002)

Second Distribution: May 2012

INTRODUCTION

I am pleased to enclose a total of six freeze dried specimens for the microbiological diagnosis of diphtheria. Please read the attached information and instructions carefully before proceeding with the laboratory work. *Ensure all results are appropriately recorded on the attached questionnaire and the questionnaire is fully completed before returning to the co-ordinating centre*.

Results from individual laboratories will be treated in strict confidence and will remain the property of EU DIP-LabNet and ECDC. *The recipient shall use the Materials only for this diagnostic EQA and not for any commercial or research and development purposes without the prior written consent of the HPA.*

Please submit your results by 13th July 2012. The "intended results" will be sent to all participants two weeks after the submission deadline.

Good luck!

Dr Shona Neal
EU DIP-LabNet Scientist
WHO COLLABORATING CENTRE FOR DIPHTHERIA & STREPTOCOCCAL INFECTIONS

INFORMATION AND INSTRUCTIONS FOR THE SECOND EXTERNAL QUALITY ASSURANCE STUDY

1. This distribution contains six simulated throat specimens prepared as freeze-dried specimens in glass vials:

labelled DIPEQA12-1 , DIPEQA12-2 through to DIPEQA12-6.

2. The vials containing freeze-dried infectious material should be opened in an exhaust protective cabinet. Gloves should be worn during reconstitution and subsequent handling of the vials. For safe removal of the plastic tear-off seals, please proceed as follows:

With the arrow on the plastic flip top pointing away from you, carefully but deliberately pull the flip top up and away from you. When it reaches the far edge, pull downwards and to the right or to the left (depending on whether you are right or left-handed) until the seal separates; then still holding onto the plastic top, gently remove altogether and dispose into a sharps container.

Slowly remove the plug. Add 1mL of broth to the vial and allow 1 minute to reconstitute. Treat the resulting suspension as the simulated specimen *using a drop from a Pasteur pipette or dipped swab as the inoculum* before spreading.

3. Laboratories will achieve the maximum benefit from these specimens if they are treated as normal patient specimens without non-routine procedures or media being used. Pathogens, if isolated, should be identified only to the level normally attempted in your laboratory.

4. Examine the plate cultures for any potentially toxigenic corynebacteria that may be present. Perform and record the test results for characterising the organisms (i.e. biochemical tests, toxin tests, morphology, microscopy and other tests you may use).

5. Please record ALL your results on the form provided and check the form before sending.

NOTE: These specimens may contain toxigenic corynebacteria. It is suggested that you handle the specimens under your own local safety rules for toxigenic *Corynebacterium diphtheriae and Corynebacterium ulcerans*.

External Quality Assurance Study for the Laboratory Diagnosis of Diphtheria: Second Distribution, May 2012

REPORT FORM

Name of participant: .....

Address: .....

.....

.....

.....

Tel: .....

Fax: .....

E-mail: .....

RESULTS TABLE
(return by post, fax, e-mail; see details on next page)

SPECIMEN	**CORYNEBACTERIUM* SPECIES ISOLATED	BIOVAR	PHENOTYPIC TOXIGENICITY RESULT (e.g. Elek)	GENOTYPIC TOXIGENICITY RESULT (e.g. PCR)	OTHER TEST/CHARACTERISATION RESULTS (if performed)	TIME TAKEN TO OBTAIN FINAL RESULT
DIPEQA12-1						
DIPEQA12-2						
DIPEQA12-3						
DIPEQA12-4						
DIPEQA12-5						
DIPEQA12-6						

* If *Corynebacterium* spp. is not isolated, record as 'none'.

Date results sent to the organiser: ……

Signature of participant: ……

GENERAL COMMENTS

	Yes	No
Did you find this exercise useful?	☐	☐
Are you willing to participate in another exercise?	☐	☐

Please state any change of method(s) from those reported in the 2010 Laboratory Diagnostics Questionnaire, if any:

Any other comments/suggestions:

Please forward results by 13th July 2012.

Annex 3. Intended results for the EU DIP-LabNet 2012 EQA for the laboratory diagnostics of diphtheria

EQA code	Original ref	Organism	Biovar/API profile	Elek toxigenicity	PCR tox detection	Sequencing results (based on 16S, rpoB & pld)	Commensals
DIPEQA12-1	H06 489 0001	*C. diphtheriae*	*gravis* (1010326)	Tox	pos	*C. diphtheriae*	*S. oralis* *N. lactamica* *S. salivarius*
DIPEQA12-2	99/196	*C. diphtheriae*	*mitis* (1010324)	Non-tox	pos (NTTB)	*C. diphtheriae*	*S. salivarius* *S. mutans* *N. lactamica*
DIPEQA12-3	N/A	NO organism	-	-	-		*S. oralis,* *N. lactamica* *S. salivarius*
DIPEQA12-4	H11 398 0552	*C. ulcerans*	(0111326)	Non-tox	neg	*C. ulcerans*	*S. epidermidis* *S. sanguis*
DIPEQA12-5	H09 492 0187	*C. ulcerans*	(0111326)	v wk tox	pos	*C. ulcerans*	*S. salivarius* *S. mutans* *S. oralis*
DIPEQA12-6	94/129	*C. diphtheriae*	*intermedius* (1010324)	Non-tox	pos (NTTB)	*C. diphtheriae*	*S. epidermidis* *S. sanguis*