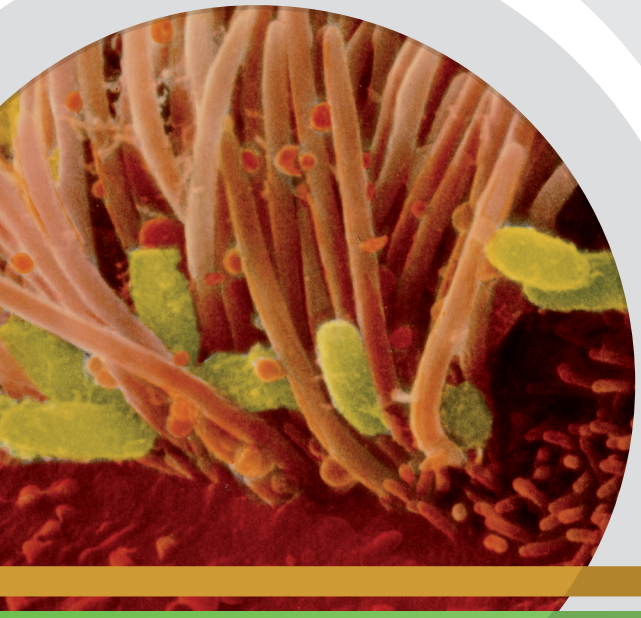


TECHNICAL REPORT

External quality assessment for the detection of *Bordetella pertussis* by PCR, 2018

On behalf of EUPert-LabNet network

www.ecdc.europa.eu

ECDC TECHNICAL REPORT

External quality assessment for the detection of *Bordetella pertussis* by PCR, 2018

On behalf of EUPert-LabNet network

This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), referring to Specific Contract No. 4 ECD.7289 implementing Framework Contract no. ECDC/2015/009 coordinated by Dr Sabrina Bacci, Dr Csaba Ködmön and Dr Lucia Pastore Celentano and produced by members of the EUPert-LabNet consortium as part of the coordination of activities for laboratory surveillance of whooping cough in Member States/EEA countries.

Authors
Dr Norman K. Fry, Dr David Litt, Public Health England – National Infection Service, London, UK; Dr Kevin Markey, National Institute for Biological Standards and Control, NIBSC, Potters Bar, UK; Alex-Mikael Barkhoff, Prof Qiushui He, University of Turku, Turku, Finland.

Acknowledgements
We acknowledge the expert technical assistance of John Duncan, Public Health England - National Infection Service, London, UK, in the preparation, testing and dispatch of this EQA panel. We also gratefully acknowledge the participation from all the laboratories in the EU/EEA Member States that took part in this study.

Suggested citation: European Centre for Disease Prevention and Control. External quality assessment for the detection of *Bordetella pertussis* by PCR, 2018 – On behalf of EUPert-LabNet network. Stockholm: ECDC; 2019.

Stockholm, January 2019

ISBN 978-92-9498-301-5
DOI 10.2900/172389
Catalogue number TQ-02-19-016-EN-N

Contents

Tables

Abbreviations

BP485	Reported as specific target sequence for *Bordetella pertussis*
Cq	Crossing threshold (for quantitative PCR)
DSM (DSMZ)	Deutsche Sammlung von Mikroorganismen und Zellkulturen, German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany
EUPert-LabNet	European Pertussis Laboratory Surveillance Network
EUVAC.NET	European surveillance network for selected vaccine-preventable diseases
fla	Flagellin gene
IS	Insertion sequence element
hIS*1001*	*Bordetella holmesii*-specific IS*1001* target
NTC	No template control
PCR	Polymerase chain reaction
pilT	*Bordetella bronchiseptica* twitching mobility gene
ptxA	Pertussis toxin subunit A
ptxP	Pertussis toxin promoter region
qPCR	Real-time (quantitative) PCR
recA	Housekeeping gene

Executive summary

Laboratory confirmation of pertussis is integral to surveillance, especially to monitor the effectiveness of vaccination strategies and inform any changes to national policies. Although there are now at least two commercially available molecular (DNA-based) external quality assessment (EQA) schemes for *Bordetella pertussis*, the European Centre for Disease Prevention and Control (ECDC) has organised previous and current studies in order to ensure maximum participation of microbiology reference laboratories across EU/EEA countries and highlight any issues of concern and make recommendations.

This report presents the results of the second European Pertussis Laboratory Surveillance Network (EUPert-LabNet) EQA scheme for *Bordetella pertussis* PCR funded by ECDC on behalf of EUPert-LabNet. The EQA study was conducted between February and March 2018.

The panel included dilutions of purified genomic DNA from *Bordetella pertussis* at three concentrations designated 'high', 'medium' and 'low'. The 'low' dilution was designed to be challenging. Duplicate samples of the *B. pertussis* 'high' dilution were included to test the reproducibility of polymerase chain reaction (PCR) assays. Genomic DNA from other *Bordetella* species — *B. parapertussis*, *B. holmesii*, and *B. bronchiseptica*, which can cause pertussis-like symptoms in humans — were also included in the panel, together with DNA from *Haemophilus influenzae*. Two 'blank' samples, i.e. with no added DNA, were included to check for potential contamination.

Twenty-eight laboratories from 27 EU/EEA countries participated in the current EQA round. One laboratory submitted two datasets – one using an in-house assay and one using a commercial assay – with both using real-time PCR (qPCR). As a result, 29 datasets were submitted.

Of the 29 datasets, all reported the successful detection of all three concentrations of *B. pertussis* and reported them as *B. pertussis* (n=28) or *Bordetella* spp. (n=1). Accordingly, all 29 datasets from all 28 laboratories achieved the intended results for the four samples in the panel containing *B. pertussis*.

The number of participating laboratories using qPCR assays for *Bordetella* PCR compared to conventional PCR increased from 13/21 (62%) in 2012 to 26/28 (93%) in 2017. However, no difference was seen in the sensitivity of qPCR compared to conventional PCR in this EQA, illustrated by the detection of *B. pertussis* DNA at all three dilutions by both platforms.

The most common targets for detection of *B. pertussis* and *B. parapertussis* were the insertion elements IS*481* (26 of 29) and IS*1001* (23 of 29) respectively. The pertussis toxin promoter region *(ptxP)* was the most common *B. pertussis*-specific target (15 of 29). Three commercial kits did not provide details of their *Bordetella* species targets.

However, IS*481* and IS*1001* are not completely specific for *B. pertussis* and *B. parapertussis* , which resulted in incorrect *Bordetella* species assignments by 6 to 14 laboratories, i.e. *Bordetella holmesii* was reported incorrectly as *B. pertussis* and *B. bronchiseptica* was reported incorrectly as *B. parapertussis* respectively.

Interpretative comments accompanying results using only these targets should be revised. Results that are PCR-positive for IS*481* only should more accurately be described as *Bordetella* spp. together with an explanatory statement.

The interpretation of results using commercial kits with unspecified targets for the detection of *B. pertussis* and/or *B. parapertussis* should also be carefully reviewed in light of the above statements.

1 Introduction

Pertussis (whooping cough) is a highly contagious acute bacterial respiratory infection caused by *Bordetella pertussis*, which is an exclusively human pathogen affecting people of all ages. A similar illness is caused by *Bordetella parapertussis*, but this is unaffected by current pertussis vaccines. Infants, particularly those who are unimmunised, are the most vulnerable group with the highest rates of morbidity and mortality, while older children and adults usually display milder symptoms. Despite being a vaccine-preventable disease, pertussis continues to be a major public health problem in many countries, typically showing cyclical outbreak periods every three to five years. The resurgence of pertussis in several countries with long-standing vaccination programmes has also been reported and pertussis remains one of the leading causes of vaccine-preventable deaths worldwide. The major contributory factors to pertussis outbreaks are the inability of both existing pertussis vaccines and natural infection to confer long-term immunity and asymptomatic transmission of pertussis to susceptible individuals [1–4].

Pertussis is a notifiable disease in EU/EEA countries, although there are differences in reporting systems between countries. The key prevention strategy for pertussis is high immunisation coverage starting early in life with an effective vaccine. The rationale for pertussis surveillance is the monitoring of the impact of national immunisation strategies, including the identification of high-risk groups and detection and investigation of clusters and outbreaks. Suspected pertussis cases may be notified on clinical suspicion usually according to national, ECDC or WHO case definitions. Many countries also report laboratory confirmation of pertussis to a national surveillance network [5–6].

ECDC has coordinated the pertussis surveillance at the European level since the transfer of EUVAC.NET (European surveillance network for selected vaccine preventable diseases, hosted by Statens Serum Institut, Denmark) to ECDC in 2011 and the formation of the EUPert-LabNet (European pertussis laboratory network) [7].

EUPert-LabNet is funded by ECDC and its main aims are to coordinate the activities for laboratory surveillance of whooping cough in European Member States and EEA countries and improve surveillance of the disease in Europe, and offer training in different laboratory methods to achieve this. EQA schemes and seroprevalence studies are also integral to network activities.

The role of the clinical microbiology laboratories is to provide front-line testing of suspected cases of pertussis, which can include investigation by culture, PCR or serology and the reporting of laboratory-confirmed cases to a surveillance network. As for other infectious diseases, the role of national reference microbiology laboratories can vary between countries, but usually includes the following core functions: provision of specialist reference tests, source of reference material, scientific advice, collaboration, research and monitoring and alert and response.

Laboratory confirmation of clinically suspected cases of pertussis can be made by culture and isolation of the causative organism, *B. pertussis*, detection of its DNA from nasopharyngeal/pernasal swabs or nasopharyngeal aspirates or throat swabs or antibody detection performed on serum or oral fluids. Each of these techniques has their strengths and limitations [8].

Laboratory confirmation of pertussis is integral to surveillance and it is imperative that the tests offered are fit for the purpose. The utility of PCR in the detection of *B. pertussis* DNA in respiratory specimens is well established and can offer increased sensitivity over culture. Improvements in both nucleic acid extraction and qPCR technologies have also led to decreased turnaround times.

While attempts to culture *B. pertussis* have declined in certain countries, the use of PCR for *B. pertussis* detection has increased. There is now an increasing number of commercial kits available for the detection of *B. pertussis* (and other *Bordetella* species) and clinical and reference laboratories may perform in-house testing.

Since the lack of EQA programmes for *B. pertussis* PCR in European diagnostic laboratories was reported by Muyldermans et al in 2005 [9] two commercial EQA programmes for *B. pertussis* PCR became available from INSTAND e.V., Düsseldorf, Germany and Quality Control for Molecular Diagnostics, Glasgow, Scotland.

Although there are now at least two commercially available molecular EQA schemes for *Bordetella pertussis*, ECDC has organised previous and current studies to ensure maximum participation from microbiology reference laboratories across the EU/EEA countries to highlight any issues of concern and make recommendations. Commercial schemes supply both simulated clinical specimens for extraction and testing by molecular (DNA-based) methodologies, while ECDC-funded schemes have comprised panels with extracted genomic DNA samples. There are strengths and limitations to both approaches. Provision of purified genomic DNA is less onerous and less costly than preparation of simulated clinical specimens. The DNA purification and extraction step is bypassed and such EQA schemes allow a more direct comparison of performance across the various platforms. However, this means that the whole analytical process is not assessed.

This is the third ECDC-funded *B. pertussis* PCR EQA. The first, 'Evaluation and standardisation of real-time PCR for detection of *B. pertussis*', was under the auspices of EUVAC.NET (ECDC Grant ECD.2042 Work Area 4) in January 2011 and 24 laboratories from 19 European countries participated. The second, the first on behalf of EUPert-LabNet, was conducted in 2012 and involved 21 laboratories in 21 EU/EEA countries [7].

This EQA programme builds upon previous collaborative studies on qPCR organised by the EUVAC.NET and EUPert-LabNet consortia. The current programme is designed to support ongoing initiatives to standardise laboratory techniques and reporting for confirmation of pertussis infection in individuals and outbreak situations. Through EQA schemes, the performance of national reference laboratories from each EU/EEA Member State can be independently assessed. Furthermore, through the scheme, recommendations for improvements to methodologies and areas for further training can be identified.

The specific aims of this *Bordetella pertussis* PCR EQA were to:

- evaluate sensitivity, specificity and reproducibility of current assays to detect *B. pertussis* DNA
- evaluate the ability of assays to differentiate between *B. pertussis* and other Bordetella species associated with respiratory disease, specifically *B. parapertussis*, *B. bronchiseptica* and *B. holmesii*
- assess good laboratory practice of the PCR technique by checking for evidence of contamination
- assess differences in interpretation and reporting of Bordetella PCR results
- identify training needs; and
- assist in the establishment of 'best practices' in current assays, interpretation and reporting.

2 Study design and methods

2.1 Organisation

The second EUPert-LabNet *B. pertussis* PCR EQA was organised by Public Health England's (PHE) National Infection Service, Colindale (London, UK) and intended for National Reference Laboratories in the European Union countries and Iceland and Norway.

Invitations to participate in the scheme together with a short questionnaire were initially sent via email to ECDC-designated pertussis laboratory experts in 30 EU/EEA countries in September 2017 (Annex 1).

Replies were received from all laboratories. Two laboratories from the UK participated, one from England and one from Scotland, together with 26 other laboratories (one per country), for a total of 28 laboratories from 27 EU/EEA countries, including Iceland and Norway (Annex 2). Reasons given for non-participation included not offering routine *Bordetella* PCR service (two laboratories) and participation in commercial EQA (one laboratory).

2.2 Panel characteristics

Clinical isolates of *B. pertussis*, *B. parapertussis*, *B. holmesii*, *B. bronchiseptica* and *Haemophilus influenzae* were selected from the culture collection at the Respiratory and vaccine preventable bacteria reference unit, PHE – National Infection Service, London, UK (Table 1) [7].

Purified genomic DNA was prepared from bacterial growth harvested from agar plates using the QIAsymphony DSP DNA Mini Kit and QIAsymphony SP automated instrument (Qiagen) using the manufacturer's recommended tissue extraction protocol for Gram-negative bacteria (including a one-hour pre-incubation with proteinase K in ATL buffer and RNAse A treatment). DNA concentrations were measured using the Qubit dsDNA Broad-Range Assay Kit (Life Technologies, Paisley, UK).

The panel was designed to include dilutions of purified genomic DNA from *B. pertussis* at three concentrations: 'high' (2.0 pg/µl), 'medium' (0.2 pg/µl) and 'low' (0.02 pg/µl). Duplicate samples of the *B. pertussis* 2.0 pg/µl dilution were included to test reproducibility. The DNA concentration of all other *Bordetella* species included in the panel (*B. parapertussis*, *B. holmesii*, *B. bronchiseptica)* and *Haemophilus influenzae* was 2 pg/µl. Two 'blank' samples containing the buffer only (10 mM Tris buffer, pH 8.0; i.e. no added DNA) were included to check for potential contamination. These latter samples are also referred to as 'no template control' (NTC) samples.

The panel was tested prior to dispatch by the sending laboratory. Each panel comprised 10 samples labelled 1 to 10 containing 200 µl final volume per sample. The panels were prepared, packed according to local regulations, collected by courier on 24 January 2018 from PHE Colindale, London and dispatched to the 28 laboratories at ambient temperature (Annex 2). The majority of packages were received in a timely manner (within 48 hours) allowing laboratories ca. 2.5 to 3 weeks from receipt to meet the target deadline for submission of results of 17:00 on 16 February 2018. For 25 of 28 laboratories where delivery or receipt dates were available, 17 of 25 were received within 24 hours; seven were received within 48 hours and one within nine days post-dispatch.

Table 1. Characteristics of strains used to prepare genomic DNA for inclusion in second EUPert-LabNet *Bordetella pertussis* PCR EQA (February 2018)

PHE reference number	Organism	Isolation date	Original specimen type	Clinical details
H114260371	*Bordetella pertussis* serotype 1.3	October 2011	Pernasal swab	Whooping cough
H114560403	*Bordetella parapertussis*	November 2011	Pernasal swab	Respiratory infection
H104780607	*Bordetella holmesii*	November 2010	Blood culture	Pyrexia
H111580382	*Bordetella bronchiseptica*	April 2011	Sputum	Not provided
H120420371	*Haemophilus influenzae* non-capsulated	January 2012	Blood culture	Bacteraemia

Testing

Participants were instructed to treat the panel as 'extracted DNA samples', test them using their usual '*B. pertussis*' PCR assay and report their qualitative and/or quantitative results. Instructions for testing, a reporting sheet for the results and a further questionnaire to ascertain details of each test were sent to each participant by e-mail as Word (Microsoft) documents prior to dispatch of the panel, with a deadline for return. The main questions in this questionnaire were concerning each participant's use of PCR for the diagnosis of *Bordetella* infections; the type of assay used (i.e., 'in-house', published or commercial kit); whether qPCR or traditional block-based thermocycler; which gene targets; the types of controls used including internal controls to check for inhibition and approximate number tested per year and any other comments on the EQA testing. The full questionnaire is listed in Annex 3.

2.3 Data analysis

The intended results of the organising laboratory (Table 2) were used as the basis for the scoring.

Participants were expected to:

- detect *B. pertussis* at both the 'high' and 'medium' dilutions
- differentiate between *B. pertussis* and other *Bordetella* species
- accurately interpret and report results depending on the targets used and results obtained
- obtain a 'negative' result (for *Bordetella*) with the two blank samples; and
- obtain a 'negative' result (for *Bordetella*) with the sample containing *H. influenzae*

The 'low' dilution of *B. pertussis* was designed to be challenging, so it was not included into certification criteria.

Qualitative EQA data scoring system

Results were scored according to a possible 10 out of 10 (100%). Results were also considered acceptable if '*Bordetella* spp.' was reported for samples containing *Bordetella* DNA (including *B. pertussis*), but incorrect if the wrong *Bordetella* species was reported.

3 Results

Twenty-six of 28 laboratories from the 27 EU/EEA countries submitted results and completed the second questionnaire by the deadline. One laboratory enquired about participating late (one week prior to the deadline). A panel was dispatched that arrived on the day of the deadline and the results were submitted three days later (first working day after arrival). One laboratory was unable to meet the deadline due to workload and reagent supply issues (results submitted ca. 4.5 weeks after the deadline). Data from these two laboratories are included for completeness. One laboratory submitted two separate datasets, one using a commercial assay and one using an in-house assay, so 29 datasets from 28 laboratories are presented.

The intended results are shown in Table 2 and were sent to all participants following the submission of the last set of results.

The overall results for the 29 datasets from 28 EU/EEA laboratories are shown in Table 3.

The majority of participants (24 of 29) used only qPCR platforms (using fluorescent detection of amplified products) and three used only conventional PCR (i.e., traditional block-based PCR). For two of these, three laboratories amplification products were detected by gel electrophoresis, staining with ethidium bromide and image capture under ultra-violet light or equivalent and the other used the Seeplex PneumoBacter ACE Detection Kit (Seegene), which offers detection of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Chlamydophila pneumoniae*, *Legionella pneumophila*, *Mycoplasma pneumoniae* and *Bordetella pertussis* and uses conventional PCR for amplification, but employs a microchip electrophoresis system to separate, quantify and size PCR products and fluorescence detection to perform analysis. Two laboratories used both qPCR and conventional PCR.

Bordetella pertussis dilution series

Of the 29 datasets from 28 countries, 28 (97%) reported the 'high', 'high duplicate', 'medium' and 'low' concentrations as positive for *B. pertussis* and one reported all four samples as positive for *Bordetella* spp., matching the manufacturer's recommendation. Accordingly, all 29 (100%) datasets from all 28 laboratories achieved the intended results for the four samples in the panel containing *B. pertussis*.

Bordetella holmesii

Twenty-two of 29 (76%) laboratories reported the *Bordetella holmesii* sample (no. 8) as *B. holmesii* or *Bordetella* spp. and 6 reported it as *B. pertussis* (or probable *B. pertussis*). Of the six, five used IS*481* as the target for *B. pertussis* and reported the IS*481* positive result as *B. pertussis*. The other used IS*481* together with *ptxP*, but reported the result as positive *B. pertussis* as (although the Cq result for *ptxP* was reported as negative).

Bordetella parapertussis and *Bordetella bronchiseptica*

Twenty-three of 29 (79%) datasets scored the *Bordetella parapertussis* sample as positive for *B. parapertussis* or *Bordetella* spp. Five reported this sample as negative for *B. pertussis* and one as negative for *B. pertussis* and *Bordetella* spp.

Twenty-two of 29 (76%) datasets scored the *Bordetella bronchiseptica* sample as positive for *Bordetella* spp. and seven specifically identified it as *B. bronchiseptica*. Fourteen of 29 incorrectly reported it as *B. parapertussis* (including two as 'probable B. parapertussis' or 'suspected *B. parapertussis*'.

Three of the remaining 29 reported this sample as negative for *B. pertussis*, one as negative for *Bordetella* spp. and one as negative for *B. pertussis* and negative for *Bordetella* spp.

Negative/no template controls

Of 29 datasets from 28 countries, all (100%) reported one of the two no template control (NTC) samples (number 10) in the panel as *B. pertussis* not detected or *Bordetella* spp. not detected and 27 (93%) also reported the other NTC sample (number 4) in the panel as *B. pertussis* not detected or *Bordetella* spp. not detected. However, two laboratories reported this sample positive for *B. pertussis* or *Bordetella* spp.; one as *Bordetella* spp. detected (very weak) and one as *B. pertussis* detected.

Haemophilus influenzae

All 29 (100%) datasets reported the sample containing *Haemophilus influenzae* as negative for *B. pertussis* or *Bordetella* spp. (including two who specifically scored this sample as negative for *B. pertussis*, *B. parapertussis* or

B. holmesii or negative for *B. pertussis* or *B. parapertussis)*. Four of 29 datasets reported the sample containing *Haemophilus influenzae* as positive for *Haemophilus influenzae*.

Reproducibility

Twenty-six of the 29 datasets reported the use of qPCR and 21 of these provided crossing thresholds (Cqs) [10,11] for *B. pertussis* and/or *Bordetella* spp. The reproducibility for the duplicate samples (numbers 1 and 5) within laboratories was very high. For 19 laboratories reporting Cqs for *B. pertussis*-specific targets, the difference was <3 Cq (range 0.00 to 3.00; average 0.5 Cq; median 0.2 Cq). For 12 laboratories reporting Cqs for the IS*481* target, the difference for the duplicate samples (numbers 1 and 5) was <1.5 (range 0.00 to 1.10; average 0.5 Cq; median 0.4 Cq).

Table 2. Intended results for second EUPert-LabNet *Bordetella pertussis* PCR EQA, February 2018

Sample number	Qualitative result		Sample details	
	B. pertussis	Bordetella spp.	Strain	Concentration of genomic DNA
1	Positive	Positive	*Bordetella pertussis* (H114260371)	2 pg/µL[1]
2	Positive	Positive	*Bordetella pertussis* (H114260371)	0.2 pg/µL
3	Positive	Positive	*Bordetella pertussis* (H114260371)	0.02 pg/µL
4	Negative	Negative	Tris buffer (10mM, pH 8.0)	Not applicable
5	Positive	Positive	*Bordetella pertussis* (H114260371)	2 pg/µL[1]
6	Negative	Positive	*Bordetella parapertussis* (H114560403)	2 pg/µL
7	Negative	Positive	*Bordetella bronchiseptica* (H111580382)	2 pg/µL
8	Negative	Positive	*Bordetella holmesii* (H104780607)	2 pg/µL
9	Negative	Negative	*Haemophilus influenzae* (H120420371)	2 pg/µL
10	Negative	Negative	Tris buffer (10mM, pH 8.0)	Not applicable

[1]*: Duplicate samples to test reproducibility.*

Table 3. Number of correct qualitative results achieved per panel member and analytical technology type

Panel number	Sample content	Sample DNA concn. (pg/µl)	Number of datasets achieving intended result n=29 (%)	PCR					
				qPCR			Conventional PCR		Other
				Commercial n=6 (%)	In-house n=15 (%)	Both n=3 (%)	Commercial n=1 (%)	In-house n=2 (%)	qPCR + conventional n=2 (%)
1	*B. pertussis*	2.00	29 (100)	6 (100)	15 (100)	3 (100)	1 (100)	2 (100)	2 (100)
2	*B. pertussis*	0.20	29 (100)	6 (100)	15 (100)	3 (100)	1 (100)	2 (100)	2 (100)
3	*B. pertussis*	0.02	29 (100)	6 (100)	15 (100)	3 (100)	1 (100)	2 (100)	2 (100)
4	NTC	NA	27 (93)	6 (100)	14 (93)	3 (100)	1 (100)	2 (100)	1 (50)
5	*B. pertussis*	2.00	29 (100)	6 (100)	15 (100)	3 (100)	1 (100)	2 (100)	2 (100)
6	*B. parapertussis*	2.00	29 (100)	6 (100)	15 (100)	3 (100)	1 (100)	2 (100)	2 (100)
7	*B. bronchiseptica*	2.00	15 (52)	1 (17)	9 (60)	3 (100)	1 (100)	0 (0)	1 (50)
8	*B. holmesii*	2.00	23 (79)	4 (67)	12 (80)	3 (100)	1 (100)	1 (50)	2 (100)
9	*Haemophilus influenzae*	2.00	29 (100)	6 (100)	15 (100)	3 (100)	1 (100)	2 (100)	2 (50)
10	NTC	NA	29 (100)	6 (100)	15 (100)	3 (100)	1 (100)	2 (100)	2 (100)

Shading indicates duplicate samples.
Concn: concentration
NTC:no template control
NA: not applicable.

Table 4. Summary of responses to questionnaire

	Number out of laboratories – n=28 (%)
Molecular (DNA-based) diagnostic assay(s) for pertussis performed routinely	25 (89)
Typical number of *Bordetella* PCR assays performed per year	Of 26 laboratories reporting numbers tested. Range: 10 to 10,000 (mean, 913; mode, 200; median, 200)
Bordetella PCR assay in-house	22 (79)
Bordetella PCR assay commercial	10 (36)
Commercial kit used	
AmpliSens *Bordetella* multi-FRT PCR kit (AmpliSens)	1 (4)
R-DiaBorM (Diagenode) and *Bordetella pertussis* R-gene (Argene)	1 (4)
Fast Track Diagnostics Respiratory Pathogens 33 (Fast Track Diagnostics Ltd)	1 (4)
Fluoro-type *Bordetella* (Hain Lifescience GmbH)	1 (4)
Ridagene *Bordetella* (R-Biopharm AG)*	2 (7)
Bordetella pertussis, B. parapertussis or *B. bronchiseptica* Real TM (Sacace Biotechnologies, ref B84-100FRT)	1 (4)
Seeplex PneumoBacter ACE Detection Kit (Seegene)	1 (4)
Allplex (Bacterial) Respiratory Panel 4, (Seegene)	1 (4)
Bordetella pertussis and parapertussis TibMolbiol LightMix (Roche)	1 (4)
Publications cited for in-house protocols (by 28 laboratories)	**Number out of n=28 laboratories (%)**
ECDC Guidance and protocol for the use of real-time PCR in laboratory diagnosis of human infection with *Bordetella pertussis* or *Bordetella parapertussis*, 2012 [12]	6 (21)
WHO Laboratory Manual for the Diagnosis of Whooping Cough caused by *Bordetella pertussis*| *Bordetella parapertussis*, 2013 [13]	3 (11)
Antila et al 2006 [14]	2 (7)
Birkebæk et al, 1994 [15]	1 (4)
Dragsted et al 2004 [16]*	2 (7)
Farell et al 1999 [17]	1 (4)
Fry et al 2009 [18]	3 (11)
Fry et al 2004 [19]	1 (4)
Glare et al 1990 [20]	1 (4)
Grogan et al [21]	2 (7)
Guthrie et al 2010 [22]	1 (4)
Hasan et al [23]	1 (4)
He et al 1993 [24]	1 (4)
Hozbor et al 1999 [25]	1 (4)
Knorr et al 2006 [26]*	1 (4)
Kösters et al 2001 [27]	3 (11)
Kösters et al 2002 [28]*	1 (4)
Martini et al 2017 [29]	1 (4)
Njamkepo et al 2011 [30]	1 (4)
Reischl et al, 2001a [31]	2 (7)
Reischl et al, 2001b [32]	1 (4)
Reischl et al, 2002 [33]	1 (4)
Roorda et al 2011 [34]	5 (18)
Tatti et al 2011 [35]	2 (7)
van der Zee et al 1993 [36]	1 (4)
van Doornum et al 2003 [37]	1 (4)
Xu et al 2010 [38]	2 (7)
Gene targets cited by 28 laboratories for 29 datasets	Number. out of n=29 (%)
IS*481* only	1 (3)
IS*481*+IS1001	5 (17)
IS*481*+ptxP	2 (7)
IS*481*+ IS1001+BP485	1 (3)
IS*481*+IS1001+ptxP	4 (14)
IS*481*+IS1001+IS1002	2 (7)
IS*481*+IS1001+recA	1 (3)
IS*481*+IS1001+ptxP+recA	1 (3)
IS*481*+IS1002+recA	1 (3)
IS*481*+IS1001+IS1002+ptxP+recA+fla	1 (3)
IS*481*+IS1001+IS1002+ptxP+recA	1 (3)
IS*481*+IS1001+ptxP+recA+pilT	1 (3)
IS*481*+IS1001+ptxP+hIS1001	1 (3)
IS*481*+IS1001+ptxP+hIS1001+fla	1 (3)
IS*481*+IS1001+ptxP+ptxA	1 (3)
IS*481*+IS1001+ptxP+recA+fla	1 (3)
ptxP+IS1001+recA+commercial kits targets not given	1 (3)
IS*481*+IS1001+ptxP+por+hIS1001	1 (3)
Gene targets not specified in kit	3 (10)
Real-time (qPCR) only	24 (83)
Conventional (block-based PCR) only	3 (10)
Both	2 (7)
Thermocyclers used by 28 laboratories to report 29 datasets	**Number of thermocyclers out of n=29 (%)**
Real-time thermocyclers	
FluoroCycler 12 (Hain Lifescience)	1 (3)
Pico Real 96 (Thermo Scientific)	1 (3)
ABI 7500 real-time (Applied Biosystems).	3 (10)

ABI Prism 7500 Fast (Applied Biosystems)	1 (3)
ABI QuantStudio 3 (Applied Biosystems).	1 (3)
ABI QuantStudio 5 (Applied Biosystems).	1 (3)
LightCycler 2.0 (Roche)	7 (24)
LightCycler 480 Instrument II (Roche Molecular Diagnostics)	3 (10)
RotorGene Q (Qiagen)	2 (7)
StepOne (ABI)	1 (3)
Mx3005P (Stratagene)	1 (3)
CFX96 Real-Time PCR Detection System (Bio-Rad)	1 (3)
Conventional thermocylers	
ABI 7900/7500 Applied Biosystems)	1 (3)
ABI9700 (Applied Biosystems)	1 (3)
ABI 2720 Thermal Cycler (Applied Biosystems)	1 (3)
PCR - Mastercycler (Eppendorf)	2 (7)
Labcycler (SensoQuest)	1 (3)
Volume of DNA extract added/Final volume of PCR mix	
5/15	3 (10)
6/15	1 (3)
6/16	1 (3)
3/20	1 (3)
5/20	10 (34)
4/21	1 (3)
2/25	1 (3)
2.5/25	1 (3)
5/25	6 (21)
8/25	1 (3)
10/25	2 (7)
5/50	1 (3)
10/100	1 (3)
Controls included in PCR run	
Positive	28 (97)
Negative	29 (100)
Extraction	10 (34)
Inhibition	19 (66)
Comments	
'For this panel, no inhibition/extraction controls were included since samples were DNA extracts.'	
'For IS*1001*, our specific probe had expired, so we needed to run the product on a gel. Therefore, we do not know the exact Cq value. However, the band was clearly for positive IS*1001* for both samples, so it could be around 29-35 cycles.'	
'Unfortunately, we have a shortage of *B. holmesii* identification kit of TibMolBiol, so we are not able, for the moment, to differentiate species.'	
'Inhibition control: sample at a 1:10 dilution.'	
'We have also tested samples with commercial Multiplex PCR system Seeplex PneumoBacter ACE detection (Seegene) and the results are positive for *Bordetella pertussis* only for 3 samples– No.1, 2, 5. No.3 is negative. This information is not included in our formal result submission, but for our own internal system checking in the laboratory.'	
'Positive controls: *B. pertussis* DSM 5571 (type strain) *B. parapertussis* DSM 13415 (type strain).'	
'Positive strains for *B. pertussis* (S1, S3), *B. parapertussis* (75456*), B. bronchiseptica* (75457), *B. holmesii* (75458).'	
'Clinical samples extracted with an internal control to check for inhibition. Because these samples were already extracted no internal control was added in this case.'	
'Although we include these 4 controls (positive, negative, extraction and internal) in our routine PCR assay, for this 'Bordetella PCR EQA', we have only included positive and negative controls.'	

IS: insertion element
hIS1001: Bordetella holmesii*-specific IS1001 target*
ptxP: pertussis toxin promoter
BP485: reported as specific target sequence for B. pertussis [39]
recA: housekeeping gene
fla: flagellin gene
pilT: B. bronchiseptica twitching mobility gene.
**: laboratory reporting two datasets used (i) commercial kit Ridagene Bordetella (R-Biopharm AG) and (ii) in-house assay with references indicated with asterisk.*

4 Discussion

Sensitivity, specificity and reproducibility

All datasets reported the successful detection of all three concentrations of *B. pertussis* and reported them as *B. pertussis* (n=28) or *Bordetella* spp. (n=1). All 29 datasets from all 28 laboratories achieved the intended results for the four samples in the panel containing *B. pertussis*.

The composition and concentrations of DNA in the second EUPert-LabNet EQA were essentially the same as those used in 2012 to allow measurable comparison of performance. The difference between the two panels was that in the first panel, the 'medium' *B. pertussis* concentration was included in duplicate, while in this second panel, the 'high' *B. pertussis* concentration was included in duplicate. A significant improvement in the second EUPert-LabNet study was demonstrated compared with the first EUPert-LabNet *Bordetella pertussis* PCR EQA from 2012, with 22 (100%) laboratories detecting the high and 21 (95.5%) the medium, but only 15 (68.2%) the low concentration of *B. pertussis* [7].

The number of laboratories using qPCR compared to conventional PCR increased from 13 of 21 (62%) in the first EQA to 26 of 28 (93%) in the second EUPert-LabNet EQA. However, no difference was seen in the sensitivity of real-time compared to conventional PCR, as illustrated by the detection of *B. pertussis* DNA at all three dilutions with both platforms.

Once again, interpretation and reporting affected the perceived specificity. It is well known that IS*481* is not completely specific for *B. pertussis* and may be found in *B. holmesii* and certain strains of *B. bronchiseptica*, so IS*481* positive-only results should not be reported as definitive confirmation of *B. pertussis*. IS*481*-only results are best reported as *Bordetella* spp. with an explanatory statement [35,40]. Six laboratories using IS*481* and no specific target for *B. pertussis* incorrectly reported the *B. holmesii* sample as *B. pertussis*. By simply changing the reporting of IS*481*-only results to the recommended interpretation, these laboratories could improve their accuracy in reporting.

Similarly, the insertion element IS*1001* is not completely specific for *B. parapertussis*, but can also be found in *B. bronchiseptica.* While clinical infection by *B. bronchiseptica* is rare, IS*1001* positive-only results should not be reported as definitive confirmation of *B. parapertussis*. Thus, IS1001-only results are best reported as *B. parapertussis* or *B. bronchiseptica* [35].

Commercial kits claiming to specifically detect *B. pertussis* and *B. parapertussis* using only these targets (IS*481* and IS*1001*) should also update their interpretation.

Differentiation between *Bordetella pertussis* and other *Bordetella* species associated with respiratory disease, *B. parapertussis, B. holmesii and B. bronchiseptica*

Bordetella pertussis gene targets

Gene targets used by participants for detecting *B. pertussis* included the IS*481* (known to show cross-reactivity with *B. holmseii* and certain *B. bronchiseptica*) and IS*1002* (also known to show cross-reactivity with *B. parapertussis*).

Species-specific gene targets used for *B. pertussis* included the pertussis toxin promoter region [16,18], *B. pertussis* BP485 [39] and the porin protein gene [23].

The pertussis toxin subunit A gene is known to show cross-reactivity with *B. parapertussis* and *B. bronchiseptica* [35], but is used in a commercial kit (Amplisens) together with two additional targets described as specific for *B. parapertussis* and *B. bronchiseptica* respectively. Results of PCR analysis using this kit for detection and differentiation of these three species are based on combinations of amplification results interpreted in accordance with the manufacturer's instructions.

Bordetella parapertussis gene targets

Gene targets for *B. parapertussis* included IS*1001* (known to show cross-reactivity with *B. bronchiseptica*).

Other *Bordetella* species targeted

Gene targets for *Bordetella holmesii* included IS*481* (due to cross-reactivity) and hIS*1001*, designed to be *Bordetella holmesii*-specific, and *recA*, the housekeeping gene [14,29,35].

Specific gene targets for *Bordetella bronchiseptica* included the flagellin gene (*fla)* and *B. bronchiseptica*-twitching mobility gene *pilT* [21,25].

Evidence of good laboratory practice of PCR technique by checking for evidence of contamination

All but two laboratories demonstrated good laboratory practices of PCR technique, providing no false-positive reporting of the two 'negative' samples. The reporting of the detection of *B. pertussis* or *Bordetella* spp. in sample 4 by two laboratories (albeit with late Cqs) highlights the problems of potential cross-contamination when using a highly sensitive assay.

Strengths

By including a similar panel composition as the previous one from 2012, a direct comparison on performance could be made over time.

Limitations

The strains included were clinical isolates and the whole genome sequence for these was not available in time for this report. As previously described, the samples included in the panel were purified high molecular size genomic DNA and more realistic 'simulated' clinical samples could in addition include human DNA.

5 Conclusions

The participation rate was very high, with 28 national reference laboratories from 27 EU/EEA countries. There was also a significant improvement in the sensitivity of assays used by participants in achieving the intended results compared with the first EUPert-LabNet Bordetella pertussis PCR EQA (2012), as demonstrated by all participants detecting all three dilutions of *B. pertussis,* including the lowest one, which was intended to be more challenging.

While the vast majority of laboratories used qPCR, no difference was seen this time in the sensitivity of real-time compared to conventional PCR, which was previously shown to be less sensitive.

Results interpretation and reporting on species identified was problematic with certain PCR assays. Although the cross-reactivity of certain gene targets used in these assays, particularly IS*481* and IS*1001*, is well recognised, several participants (6 to 14 laboratories) reported results on detected *Bordetella* spp. from these targets incorrectly.

6 Recommendations

Interpretative comments accompanying results using only IS*481* or IS*1001* targets should be revised. In addition, results that are PCR-positive and IS*481*-only should be more accurately described as *Bordetella* spp. together with an explanatory statement similar to the following:

- 'This specimen was positive for the insertion element IS*481*, which is present in multiple copies in *B. pertussis*. This element can also occur in some other Bordetella spp., i.e., *B. holmesii* and some (but not all) strains of *B. bronchiseptica'*.

Moreover, results that are PCR-positive and IS*1001*-only should be more accurately described as *Bordetella* spp. together with an explanatory statement similar to the following:

- 'This specimen was positive for the insertion element IS*1001*, which is present in *B. parapertussis* and some (but not all) strains of *B. bronchiseptica'*.

The interpretation of results using commercial kits with unspecified targets for the detection of *B. pertussis* and/or *B. parapertussis* should also be carefully reviewed in light of the above statements.

The current EQA results suggest the need for a revision of current guidelines and protocols for the use of qPCR in laboratory diagnosis of human infection with *Bordetella pertussis* or *Bordetella parapertussis*.

The inclusion of *B. pertussis*-specific targets to allow the definitive reporting of detection of *B. pertussis* is suggested.

References

1. Nieves DJ, Heininger U. *Bordetella pertussis*. Microbiol Spectr. 2016 Jun;4(3).
2. Martinón-Torres F, Heininger U, Thomson A, Wirsing von König CH. Controlling pertussis: how can we do it? A focus on immunization. Expert Rev Vaccines. 2018 Apr;17(4):289-297.
3. Zlamy M. Rediscovering Pertussis. Front Pediatr. 2016 Jun 8;4:52.
4. Lapidot R, Gill CJ. The Pertussis resurgence: putting together the pieces of the puzzle. Trop Dis Travel Med Vaccines. 2016 Dec 12;2:26.
5. European Centre for Disease Prevention and Control. Expert consultation on pertussis – Barcelona, 20 November 2012. Stockholm: ECDC; 2014. Available from: http://ecdc.europa.eu/publications-data/expert-consultation-pertussis.
6. European Centre for Disease Prevention and Control. Pertussis. In: ECDC. Annual epidemiological report for 2015. Stockholm: ECDC; 2017. Available from: http://ecdc.europa.eu/publications-data/pertussis-annual-epidemiological-report-2015.
7. European Centre for Disease Prevention and Control. External quality assurance scheme on PCR for *Bordetella pertussis*, 2012. On behalf of the EUpert-labnet network. Stockholm: ECDC; 2012. Available from: http://ecdc.europa.eu/publications-data/external-quality-assessment-eqa-scheme-pcr-bordetella-pertussis-2012-behalf.
8. Public Health England. Guidelines for the Public Health Management of Pertussis in England. London: PHE; 2018. Available from: http://www.gov.uk/government/publications/pertussis-guidelines-for-public-health-management.
9. Muyldermans G, Soetens O, Antoine M, Bruisten S, Vincart B, Doucet-Populaire F, et al. External Quality Assessment for Molecular detection of *Bordetella pertussis* in European Laboratories. J Clin Microbiol. 2005 Jan;43:30–35.
10. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, et al. The MIQE Guidelines: *M*inimum *I*nformation for Publication of *Q*uantitative Real-Time PCR *E*xperiments. Clin Chem. 2009 Apr;55:611-622.
11. Bustin SA, Beaulieu J-F, Huggett J, Jaggi R, Kibenge FSB, Olsvik PA, et al. MIQE précis: Practical implementation of minimum standard guidelines for fluorescence-based quantitative real-time PCR experiments. BMC Mol. Biol. 2010 Sep 21;11:74.
12. European Centre for Disease Prevention and Control. Guidance and protocol for the use of real-time PCR in laboratory diagnosis of human infection with *Bordetella pertussis* or *Bordetella parapertussis*. Stockholm: ECDC; 2012. Available from: http://ecdc.europa.eu/publications-data/guidance-and-protocol-use-real-time-pcr-laboratory-diagnosis-human-infection.
13. World Health Organization. Laboratory Manual for the Diagnosis of Whooping Cough caused by *Bordetella pertussis*/*Bordetella parapertussis*. Update 2014. Geneva: WHO; 2014. Available from: http://www.who.int/immunization/documents/diseases/who_ivb_14.03.
14. Antila M, He Q, de Jong C, Aarts I, Verbakel H, Bruisten S, et al. *Bordetella holmesii* DNA is not detected in nasopharyngeal swabs from Finnish and Dutch patients with suspected pertussis. J Med Microbiol. 2006 Aug;55(Pt 8):1043–1051.
15. Birkebæk NH, Heron I, Skjødt K. *Bordetella pertussis* diagnosed by polymerase chain reaction. APMIS. 1994 Apr;102(4):291-294.
16. Dragsted DM, Dohn B, Madsen J, Jensen JS. Comparison of culture and PCR for detection of *Bordetella pertussis* and *Bordetella parapertussis* under routine laboratory conditions. J Med Microbiol. 2004 Aug;53:749-754.
17. Farell DJ, Dagard G, Mukkur TK. Nested Duplex PCR to Detect *Bordetella pertussis* and *Bordetella parapertussis* and Its Application in Diagnosis of Pertussis in Nonmetropolitan Southeast Queensland, Australia. J Clin Microbiol. 1999 Mar;37(3):606-610.
18. Fry NK, Duncan J, Wagner K, Tzivra O, Doshi N, Litt DJ, et al. Role of PCR in the diagnosis of pertussis infection in infants: 5 years' experience of provision of a same-day real-time PCR service in England and Wales from 2002 to 2007. J Med Microbiol. 2009 Aug;58(Pt 8):1023-1029.
19. Fry NK, Tzivra O, Li YT, McNiff A, Doshi N, Maple PA, et al. Laboratory diagnosis of pertussis infections: the role of PCR and serology. J Med Microbiol. 2004 Jun;53(Pt 6):519-525.
20. Glare EM, Paton JC, Premier RR, Lawrence AJ, Nisbet IT. Analysis of a repetitive DNA sequence from *Bordetella pertussis* and its application to the diagnosis of pertussis using the polymerase chain reaction. J Clin Microbiol. 1990 Sep;28:1982-1987.
21. Grogan JA, Logan C, O'Leary J, Rush R, O'Sullivan N. Real-time PCR-based detection of *Bordetella pertussis* and *Bordetella parapertussis* in an Irish paediatric population. J Med Microbiol. 2011 Jun;60(Pt 6):722-729.

22. Guthrie JL, Robertson AV, Tang P, Jamieson F, Drews SJ. Novel Duplex Real-Time PCR Assay Detects *Bordetella holmesii* in Specimens from Patients with Pertussis-Like Symptoms in Ontario, Canada. J Clin Microbiol. 2010 Apr;48(4):1435–1437.
23. Hasan MR, Tan R, Al-Rawahi GN, Thomas E, Tilley P. Evaluation of amplification targets for the specific detection of *Bordetella pertussis* using real-time polymerase chain reaction. Can J Infect Dis Med Microbiol. 2014 Jul;25(4):217-221.
24. He Q, Mertsola J, Soini H, Skurnik M, Ruuskanen O, Viljanen MK. Comparison of polymerase chain reaction with culture and enzyme immunoassay for diagnosis of pertussis. J Clin Microbiol. 1993 Mar;31(3):642-645.
25. Hozbor D, Fouque F, Guiso N. Detection of *Bordetella bronchiseptica* by the polymerase chain reaction. Res Microbiol. 1999 Jun;150(5):333−341.
26. Knorr L, Fox JD, Tilley PA, Ahmed-Bentley J. Evaluation of real-time PCR for diagnosis of *Bordetella pertussis* infection. BMC Infect Dis. 2006 Mar 23;6:62.
27. Kösters K, Riffelmann M, Wirsing von König CH. Evaluation of a real-time PCR assay for detection of *Bordetella pertussis* and *B. parapertussis* in clinical samples. J Med Microbiol. 2001 May;50(5):436-440.
28. Kösters K, Reischl U, Schmetz J, Riffelmann M, Wirsing von König CH. Real-Time LightCycler PCR for Detection and Discrimination of *Bordetella pertussis* and *Bordetella parapertussis*. J Clin Microbiol. 2002 May;40(5):1719-1722.
29. Martini H, Detemmerman L, Soetens O, Yusuf E, Piérard D. Improving specificity of *Bordetella pertussis* detection using a four target real-time PCR. PLoS ONE. 2017 Apr 12:12(4):e0175587.
30. Njamkepo E, Bonacorsi S, Debruyne M, Gibaud SA, Guillot S, Guiso N. Significant Finding of *Bordetella holmesii* DNA in Nasopharyngeal Samples from French Patients with Suspected Pertussis. J Clin Microbiol. 2011 Dec;49(12):4347-4348.
31. Rapid and Specific Detection of *Bordetella pertussis* in Clinical Specimens by LightCycler PCR. In: Meuer S, Wittwer C, Nakagawara KI, editors. Rapid Cycle Real-Time PCR: Methods and Applications. Berlin: Springer; 2001. pp. 313-321.
32. Reischl U, Lehn, N, Sanden GN, Loeffelholz MJ. Real-Time PCR Assay Targeting IS*481* of *Bordetella pertussis* and Molecular Basis for Detecting *Bordetella holmesii*. J Clin Microbiol. 2001 May;39(5):1963-1966.
33. Reischl U, Kösters K, Leppmeier B, Linde HJ, Lehn N. Rapid Detection and Simultaneous Differentiation of *Bordetella pertussis* and *Bordetella parapertussis* in Clinical Specimens by LightCycler PCR. In: Reischl U, Wittwer C, Cockerill F, editors. Rapid Cycle Real-Time PCR — Methods and Applications: Microbiology and Food Analysis. Berlin Springer; 2002. pp. 31-43.
34. Roorda L, Buitenwerf J, Ossewaarde JM, van der Zee A. A real-time PCR assay with improved specificity for detection and discrimination of all clinically relevant *Bordetella* species by the presence and distribution of three Insertion Sequence elements. BMC Res Notes. 2011 Jan 21;4:11.
35. Tatti KM, Sparks KN, Boney KO, Tondella ML. Novel Multitarget Real-Time PCR Assay for Rapid Detection of *Bordetella* Species in Clinical Specimens. J Clin Microbiol. 2011 Dec;49(12):4059-4066.
36. van der Zee A, Agterberg C, Peeters M, Schellekens J, Mooi FR. Polymerase chain reaction assay for pertussis: Simultaneous detection and discrimination of *Bordetella pertussis* and *Bordetella parapertussis*. J Clin Microbiol. 1993 Aug;31(8):2134-2140.
37. van Doornum GJ, Guldemeester J, Osterhaus AD, Niesters HG. Diagnosing Herpesvirus Infections by Real-Time Amplification and Rapid Culture. J Clin Microbiol. 2003 Feb;41(2):576–580.
38. Xu Y, Xu Y, Hou Q, Yang R, Zhang S. Triplex real-time PCR assay for detection and differentiation of *Bordetella pertussis* and *Bordetella parapertussis*. APMIS. 2010 Sep 1;118(9):685–691.
39. Probert WS, Ely J, Schrader K, Atwell J, Nossoff A, Kwan S. Identification and Evaluation of New Target Sequences for pecific Detection of *Bordetella pertussis* by Real-Time PCR. J Clin Microbiol. 2008 Oct;46(10):3228-3231.
40. Public Health England. Guidelines for the Public Health Management of Pertussis in England. London: PHE; 2018. Available from: https://www.gov.uk/government/publications/pertussis-guidelines-for-public-health-management.

Annex 1. Invitation letter to participants for *Bordetella pertussis* PCR EQA program, reply form and short questionnaire (2018)

01/09/2017

Coordination of activities for laboratory surveillance of whooping cough in Member States and EEA countries - As part of the coordination of activities (OJ/03/12/2014-PROC/2014/033), EUpert-labnet network

Work package 2: EQA scheme for the laboratory diagnostics and molecular typing of pertussis distributed among the National Reference Laboratories in Member States, Iceland, Liechtenstein and Norway.

Dear Colleagues,

As you are aware, laboratory diagnosis of pertussis is important for treatment, prevention and surveillance. One reason for the variation in reporting of laboratory-confirmed pertussis cases globally is due to the variety of methods used (see Euro Surveill. 2012;17(32). pii: 20239).

External Quality Assessment (EQA) is critical for accurate diagnosis and assessment of laboratory performance. As part of a previous contract with ECDC (Coordination of activities for laboratory surveillance of whooping cough in Member States and EEA countries), the first EUpert-labnet *B. pertussis* PCR EQA study was organised by the UK's Health Protection Agency for National Reference Laboratories in EU Member States, Iceland, Liechtenstein and Norway in 2012. In total, 21 laboratories participated. The aims of this PCR EQA included the evaluation of the sensitivity, specificity and reproducibility of current assays to detect *B. pertussis* DNA.

http://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/20120906-TER-EQA-pertusis.pdf

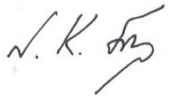
We are now organizing a further EQA in 2017 to evaluate *Bordetella* PCR assays (focussing on *B. pertussis*) used for diagnosis of pertussis by the national reference laboratories from each EU/EEA Member State.

This forthcoming study will comprise a panel of up to 10 samples containing various concentrations of *Bordetella*/non-*Bordetella* DNA representing the levels seen following extraction of clinical specimens. Participating laboratories are asked to use their usual *B. pertussis* PCR method(s) (commercial kit or in-house) that are routinely used for the diagnosis of pertussis to analyse the panel. Full instructions will be distributed together with the sample shipment.

The aim of this study is to assess PCR assays ability to detect/identify/correctly report the presence of genomic DNA from clinically relevant *Bordetella* species. Following this further recommendations for standardisation, improvements to methodologies and training needs will be made to ECDC and participants. It is anticipated that these results will be published by ECDC and/or in an international journal. We warmly invite you to participate in this study.

We would very much appreciate you returning the 'Reply form' to us and your agreement to participate in this study.

Yours sincerely,



Dr Norman Fry[1], Dr David Litt[1], Dr Kevin Markey[2] and Alex Barkoff[3]

[1] Public Health England – National Infection Service.
[2] Division of Bacteriology – National Institute for Biological Standards and Control.
[3] University of Turku/National Institute for Health and Welfare Pertussis group.

Bordetella pertussis EQA Reply Form

(Please return the form to norman.fry@phe.gov.uk before 20 September 2017)

_____

Person for correspondence:

Shipment Address:

Tel:

Fax:

E-mail:_____

Please tick the boxes which apply to your laboratory:

Do you agree to participate in this collaborative study and agree to submit all raw data as requested?

Yes □ No □

If the answer is "yes", please give the following information:

Do you routinely perform molecular (DNA-based) diagnostic assay(s) for pertussis?

Yes □ No □

Typical number of assays per week*/month*/year* ____

* delete as appropriate

Please specify the following assays which are carried out routinely in your laboratory and for which you are prepared to contribute data for this study

Bordetella pertussis PCR Yes □ No □

Is the assay in-house? Yes □ No □

Please give publication if possible: _____

or commercial? Yes □ No □

Please give details of kit/manufacturer: _____

Shipping regulation for human samples and 'legal' requirements

Does your organisation need to obtain an import permit to receive the human samples in this study? Yes □ No □

If yes, please let us know when you are able to send your import licence to us, which is essential to enable us to make the shipment to you: _____

**Participants in the study are requested not to publish or circulate information concerning this study without the prior agreement of the consortium.*

_____

Annex 2. List of participants

Country	Contact person	Laboratory/Institution
Austria	Alexander Indra Ernst Amtmann	Austrian Agency for Health and Food Safety (AGES)
Belgium	Denis Pierard Oriane Soetens	Laboratory of Microbiology, UZ Brussel Hospital
Bulgaria	Nadia Brankova	Department of Microbiology, National Center of Infectious and Parasitic Diseases
Croatia	Andrea Babić-Erceg	Croatian National Public Health Institute
Czech Republic	Jana Zavadilová	National Reference Laboratory for Pertussis and Diphtheria, National Institute of Public Health
Denmark	Randi Føns Petersen Tine Dalby	Department of Microbiological Surveillance and Research, Statens Serum Institut
England	Norman Fry David Litt	Respiratory and vaccine preventable bacteria reference unit, Public Health England – National Infection Service
Estonia	Grethel Simonlatser Grete-Katariine Kuum	Health Board Laboratory for Communicable Diseases
Finland	Qiushui He Alex-Mikael Barkoff	Finnish Reference Laboratory for Pertussis and Diphtheria, Department of Medical Microbiology and Immunology, University of Turku
Germany	Marion Riffelmann Nicole Kennerknecht	HELIOS Klinikum Krefeld, Institut für Hygiene und Labormedizin
Greece	Evangelia Petridou	Athens Children's Hospital Agia Sofia
Hungary	Ildiko Paluska Ferencz	Legionella - *Bordetella* Laboratory, Hungarian National Center for Epidemiology
Iceland	Freyja Valsdóttir Lena Ros Asmundsdottir	Department of Clinical Microbiology Landspitali University hospital
Ireland	Adele Habington Suzanne Cotter	Molecular Laboratory, Microbiology Department, Our Lady's Children's Hospital, Crumlin
Italy	Paola Stefanelli Gabriele Buttinelli	Istituto Superiore di Sanità
Latvia	Jelena Storozenko Tatjana Kolupajeva Oksana Savicka	Infectology Center of Latvia
Lithuania	Algirdas Griškevicius Ana Steponkiene	Molecular Biology Testing Subdivision, National Public Health Surveillance Laboratory
Luxembourg	Frédéric Decruyenaere Monique Perrin	Laboratoire national de sante, Département de microbiologie Service de bactériologie, mycologie, antibiorésistance et hygiène hospitalière
Malta	Paul Caruana Graziella Zahra	Microbiology Labs, Pathology Department, Mater Dei Hospital
Norway	Didrik Vestrheim Martha Bjørnstad	Department of Bacteriology, Norwegian Institute of Public Health
Poland	Katarzyna Piekarska M. Rzeczkowska	Department of Bacteriology, National Institute of Public Health
Portugal	Paula Palminta	National Institute of Health
Romania	Georgeta Cristina Oprea Sorin Dinu	Splaiul Independentei
Scotland	Rory Gunson Alasdair MacLean	West of Scotland Specialist Virology Centre, Glasgow Royal Infirmary
Slovakia	Lucia Mad'arová	Regionálny úrad verejného zdravotníctva Banská Bystrica NRC pre pertussis a parapertussis
Slovenia	Tamara Kastrin	National Laboratory of Health, Environment and Food
Spain	Raquel Abad Torreblanca	*Neisseria*, *Listeria* and *Bordetella* Unit Reference and Research Laboratory for Vaccine-Preventable Bacterial Diseases National Centre for Microbiology Instituto de Salud Carlos III
Sweden	Malin Grabbe Therese Nilsson	Karolinska Universitetslaboratoriet Klinisk mikrobiologi

Annex 3. EUPert-LabNet *Bordetella pertussis* PCR EQA reply and result submission forms

Bordetella pertussis EQA reply form

Please return the form to norman.fry@phe.gov.uk 17:00 on Friday 16 February 2018.

Person completing form/results:

Country:

E-mail:

Date:

Is the Bordetella/*B. pertussis* PCR assay used

in-house? Yes □ No □

Please give publication if possible: _____

or commercial? Yes □ No □

Please give details of kit/manufacturer/catalogue number: _____

Please give details of gene targets if known

Target(s)	IS*481*	IS*481*+IS*1001*	IS*481*+ ptxA-Pr	IS*481*+other	IS*481*+IS*1001*+ ptxA-Pr	IS*481*+IS*1001*+other	Other
	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N	Y/N
If 'other', please give details:							

What platform/manufacturer/model of thermocycler do you use?

What is the final volume of the PCR reaction mix?

What volume (in microliters, µL) of the DNA extract was added to your PCR mix?

What controls are included in the PCR run?

Positive/Negative/Extraction/Inhibition etc.

Any comments?

EUPert-LabNet – Bordetella PCR EQA result submission form

Return by 17:00 on Friday 16 February 2018 to norman.fry@phe.gov.uk.

EQA#	Qualitative Result		Quantitative Result				Comments (e.g. inhibition, etc.)
	B. pertussis (Pos/Neg/NT*)	*Bordetella* spp. (Pos/Neg/NT*)	Crossing Point (Cq values)		Calculated Amount (pg or GU) or Concentration (pg/µl or GU/ml), of *Bordetella* DNA**		
			B. pertussis	*Bordetella*			
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

**Pos: positive (detected)*
Neg: negative (not detected)
NT: not tested
Cq: crossing threshold (for real-time PCR)
***:Calculated from standard curve (real-time PCR)*
Pg = pictogram
GU = genome units.

Person completing form/results:

Country:

E-mail:

Date:

European Centre for Disease Prevention and Control (ECDC)

Address:
Gustav III:s boulevard 40, SE-169 73 Solna, Sweden

Tel. +46 858601000
Fax +46 858601001
www.ecdc.europa.eu

An agency of the European Union
www.europa.eu

Subscribe to our publications
www.ecdc.europa.eu/en/publications

Contact us
publications@ecdc.europa.eu

Follow us on Twitter
@ECDC_EU

Like our Facebook page
www.facebook.com/ECDC.EU

ECDC is committed to ensuring the transparency and independence of its work

In accordance with the Staff Regulations for Officials and Conditions of Employment of Other Servants of the European Union and the ECDC Independence Policy, ECDC staff members shall not, in the performance of their duties, deal with a matter in which, directly or indirectly, they have any personal interest such as to impair their independence. Declarations of interest must be received from any prospective contractor(s) before any contract can be awarded.
www.ecdc.europa.eu/en/aboutus/transparency

HOW TO OBTAIN EU PUBLICATIONS

Free publications:

- one copy:
  via EU Bookshop (http://bookshop.europa.eu);
- more than one copy or posters/maps:
  from the European Union's representations (http://ec.europa.eu/represent_en.htm);
  from the delegations in non-EU countries (http://eeas.europa.eu/delegations/index_en.htm);
  by contacting the Europe Direct service (http://europa.eu/europedirect/index_en.htm) or calling 00 800 6 7 8 9 10 11 (freephone number from anywhere in the EU) (*).

  (*) The information given is free, as are most calls (though some operators, phone boxes or hotels may charge you).

Priced publications:

- via EU Bookshop (http://bookshop.europa.eu).

Publications Office