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TECHNICAL REPORT

External quality assessment scheme for *Bordetella pertussis* vaccine antigen expression, 2021

On behalf of EUPert-LabNet network

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External quality assessment scheme for *Bordetella pertussis* vaccine antigen expression, 2021

On behalf of the ERLNPert-Net network

This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), coordinated by Dr Sabrina Bacci and Dr Marlena Kaczmarek, and produced by Dr Norman Fry, UK Health Security Agency (UKHSA) (London, UK), Dr David Litt, Mr Alex-Mikael Barkoff and Prof Qiushui He, University of Turku (UTU) (Turku, Finland), on behalf of the European Reference Laboratory Network for Pertussis (ERLNPert-Net) consortium, as part of the coordination of activities for laboratory surveillance of whooping cough in Member States/EEA countries (referring to Specific Contract ECDC/2019/023).

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Abbreviations

ACV	Acellular vaccine
COVID-19	Coronavirus infectious disease 2019
DSN	Disease Specific Network
DTaP	Diphtheria toxoid, Tetanus Toxoid and acellular Pertussis vaccine
DTwP	Diphtheria toxoid, Tetanus Toxoid and whole-cell Pertussis vaccine
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
ELISA	Enzyme-linked immunosorbent assay
EQA	External Quality Assessment
ERLNPert-Net	European Reference Laboratory Network for Pertussis (Consortium of European pertussis experts funded by ECDC)
EU	European Union
EUPert-LabNet	Name of former consortium of European Pertussis Laboratory Surveillance Network funded by ECDC
FIM	Fimbrial protein
FHA	Filamentous haemagglutinin
mAb(s)	Monoclonal antibody (antibodies)
NCTC	National Collection of Type Cultures
NIBSC	National Institute for Biological Standards and Control (UK)
NRL	National Reference Laboratory
PCR	Polymerase chain reaction
PRN	Pertactin
PT	Pertussis toxin
QMS	Quality management system
RIVM	National Institute for Public Health and the Environment, Ministry of Health, Welfare and Sport (the Netherlands)
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
UK	United Kingdom
UK HSA	United Kingdom Health Security Agency
UTU	University of Turku (Finland)
VPBS	Vaccine Preventable Bacteria Section
WCV	Whole-Cell Vaccine
WGS	Whole Genome Sequencing

Executive summary

Main findings

- This report presents the results of the first external quality assessment (EQA) scheme for *Bordetella pertussis* vaccine antigen expression, pertactin (PRN), pertussis toxin (PT) and filamentous haemagglutinin (FHA) by the European Centre for Disease Prevention and Control (ECDC) as part of the European Reference Laboratory Network for Pertussis (ERLNPert-Net) activities.
- Antigen expression testing of *B. pertussis* isolates is not routinely performed by all Member State reference laboratories. As part of the ERLNPert-Net activities, capacity-building and training was provided to 13 Member States[1], while a total of 15 Member States were considered to have experience in this technique.
- The EQA was conducted during 2021 and only 12 laboratories from 12 EU/EEA countries participated in the scheme. This was due to a combination of factors, including the active involvement of many laboratories and personnel with their response to COVD-19 pandemic. Of the 12 Member States that participated in this EQA, eight performed at least one of the antigen expression components. Of these eight, seven had prior experience of the technique.
- The test panel included eight strains of *B. pertussis* together with three control strains. The panel included *B. pertussis* strains belonging to the serotypes/fimbrial serotypes 1,0,0 (FIM- [minus]); 1,2 (FIM2); 1,3 (FIM3) and 1,2,3 (FIM2,3) together with those expressing and not expressing pertactin (PRN+/-), pertussis toxin (PT+/-) and filamentous haemagglutinin (FHA+/-).
- The EQA scheme was divided into three sections: (1) Fimbrial serotyping: to assess the ability of each laboratory to correctly report the expression/non-expression of fimbriae in the panel and report the fimbrial serotype for each strain; (2) pertactin/pertussis toxin expression: to assess the ability of each laboratory to correctly report the expression/non-expression status of pertactin/pertussis toxin for each strain and (3) filamentous haemagglutinin expression (optional): to assess the ability of each laboratory to correctly report the expression/non-expression status of FHA for each strain.
- For serotyping, the two reagents recommended were the WHO International Standard monoclonal antibody for serotyping *B. pertussis* fimbrial antigen 2 (1st WHO International Standard), National Institute for Biological Standards and Control (NIBSC) code: 06/124, and WHO International Standard monoclonal antibody for serotyping *B. pertussis* fimbrial antigen 3 (1st WHO IS) NIBSC code: 06/128. For vaccine antigen (PRN, PT, FHA) expression, the following reagents were recommended: PeM4 monoclonal antibody, which targets the pertactin (PRN) region 1 (from National Institute for Public Health and the Environment, Ministry of Health, Welfare and Sport in the Netherlands (RIVM), supplied by the UK Health Security Agency (UK HSA) for this EQA scheme on request); non-WHO reference material anti-PT S1 subunit monoclonal antibody 10D6 NIBSC code: 99/512; non-WHO reference material anti-PT S3 subunit monoclonal antibody 7E10 NIBSC code: 99/542 and non-WHO reference material anti-filamentous haemagglutinin monoclonal antibody 2E9 NIBSC code: 99/572 (available from National Institute of Biological Standards and Control (NIBSC), UK).
- Only 2 of 11 (18%) laboratories scored 8/8 (100%, intended results) in section (1) fimbrial serotyping, compared to 7/14 (50%) in a previous fimbrial serotyping EQA which comprised four strains [1]. In this EQA all 11 laboratories used monoclonal antibodies (mAbs). Ten of 11 used 06/124 mAb Fim2 and 06/128 mAb Fim3 from NIBSC and one used in-house mAbs. Analysis was performed by ELISA for 5/10 and slide agglutination by 6/10 laboratories. Explanations for the low overall scores for fimbrial serotyping in this EQA include potential variation in phenotypic expression due to delay in transport and differing pre-test culture conditions; autoagglutination; differences in experience of operators in technique used; lack of availability of additional control material and differences in interpretation and cut-offs used for the ELISA.
- Eight of 10 (80%) laboratories scored 8/8 (100%, intended results) in section (2) for the ability to distinguish PRN expression/non-expression in *B. pertussis*. Most laboratories (7/10) used the recommended ELISA protocol, with five using the PeM4 pertactin mAbs, two using the non-WHO reference material *B. pertussis* polyclonal anti-69kD serum 97/558 (NIBSC) and one using in-house pertactin polyclonal antibodies and Western blot.
- Four of seven (57%) laboratories scored 8/8 (100%, intended results) in section (2) for the ability to distinguish PT expression/non-expression; three of these laboratories used the recommended ELISA protocol with the anti-pertussis toxin monoclonal antibodies 99/512 (S1) and 99/542 (S3) (NIBSC) and one used Western blot with an in-house polyclonal pertussis toxin antibody.
- Six of seven (86%) laboratories scored 100%, in section (3) for the ability to distinguish FHA expression/non-expression, four using the recommended ELISA protocol with the non-WHO reference material anti-filamentous haemagglutinin monoclonal antibody 2E9 (99/572, NIBSC), one using Western blot with an in-house polyclonal FHA antibody and two using whole genome sequencing (WGS) to predict FHA expression.

[1] The UK also participated in training as it was an EU Member State at the time.

- Three laboratories used analyses from WGS to predict expression of PRN, PT and/or FHA. One laboratory did not submit results for the PRN expression as these methodologies were not available. One of the other two laboratories correctly predicted PRN expression/non-expression by identifying the presence of silent mutations or premature stops/insertions within the coding region, respectively. Only one laboratory performed PT prediction and incorrectly predicted expression for the PT- strain (EQA06), scoring 7/8. Only one of two laboratories performing FHA prediction correctly predicted FHA expression/non-expression for the FHA- strain (EQA07), scoring 8/8.
- Results for the first EQA on vaccine antigen (PRN, PT, FHA) expression were encouraging, especially for pertactin which was the priority target due to the rise in circulating PRN non-expressing strains.
- Overall, WGS performed by three countries and its analysis was successful in identifying known mutations and previously described reasons for non-expression, but in some cases it incorrectly predicted the phenotype.
- Pertussis National Reference Laboratories (NRLs) should be able to correctly perform fimbrial serotyping and vaccine antigen expression assays for surveillance of circulating *B. pertussis* isolates.
- This EQA identified training needs in fimbrial serotyping and the detection of vaccine antigen expression to help achieve the above.

1. Background

ECDC is a European Union (EU) agency with a mandate to operate dedicated surveillance networks (DSNs) and identify, assess and communicate current and emerging threats to human health from communicable diseases. As part of its mission, ECDC shall '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 ECDC shall maintain and extend such cooperation and support the implementation of quality assurance schemes' (Article 5.3, EC 851/2004).

External quality assessment (EQA), an integral part of a quality management system (QMS), evaluates performance of laboratories by an outside agency on material that is supplied specifically for this purpose. ECDC organises a series of EQAs for EU/EEA countries. A list of completed EQAs is provided in Annex 1 [1-7]. The aims of these EQAs are to identify areas for improvement in laboratory diagnostic and reference capacities relevant to the surveillance of the diseases listed in Decision No. 2119/98/EC 2, and to ensure comparability of results between laboratories from all EU/EEA countries.

The main purposes of EQA schemes are:

- to assess the general standard of performance ('state of the art');
- to assess the effects of analytical procedures (method principle, instruments, reagents, calibration);
- to evaluate individual laboratory performance;
- to identify and analyse problem areas;
- to provide continuing education;
- to identify needs for training activities.

Pertussis (whooping cough) is an acute bacterial infection caused by *Bordetella pertussis*, which can affect people of all ages. Similar illnesses may be caused by *Bordetella parapertussis* and *Bordetella holmesii*, but vaccination with the current pertussis vaccines does not protect against these other two *Bordetella* species. Infants are the most vulnerable group with the highest rates of morbidity and mortality, while older children and adults usually display milder symptoms. However, the older age group is an important reservoir and source of infection for infants. Increases in awareness of pertussis infections and reported cases in many countries have highlighted the need for good laboratory methods for the detection, identification and characterisation of clinical infections caused by *B. pertussis* and other *Bordetella* species.

Whole-cell pertussis vaccines (WCVs) were originally developed from suspensions of killed whole organisms and first licensed in the USA in 1914 [8]. In 1948, the pertussis vaccine was combined with diphtheria and tetanus toxoids to become a diphtheria and tetanus toxoids, and whole-cell pertussis (DTwP) vaccine [9] and these became widely available from the 1950s [10]. Combined DTwP vaccines have been used in the Expanded Programme on Immunisation since 1974 [10]. The widespread use of pertussis WCVs led to a significant decline in reported cases, hospitalisation rates and deaths due to pertussis worldwide [11]. Concern regarding the reactogenicity of the pertussis WCV led to the development of less reactive vaccines using one or more *B. pertussis* components (termed acellular vaccines, ACVs) that induced protective immune responses with fewer local and systemic reactions [12]. Many countries replaced their pertussis WCVs with these pertussis ACVs (diphtheria and tetanus toxoids, and acellular pertussis, DTaP) for primary immunisations and/or boosters in the 1990s–2000s [13-16]. These ACVs contain different combinations of pertussis toxin (PT), pertactin (PRN), filamentous hemagglutinin (FHA), and fimbriae 2 and 3 (FIM2,3).

The antigenic variation between *B. pertussis* vaccines (both WCV and ACV) and circulating strains has been described in detail [17]. Following the introduction of ACVs, *B. pertussis* isolates not expressing the vaccine antigen PRN have been widely reported but these *B. pertussis* PRN-deficient isolates can still cause typical symptoms of pertussis. In addition, *B. pertussis* strains not expressing PT or FHA have been reported in countries including Australia, France, Sweden and the USA [18-24].

While the non-expression of vaccine antigen targets is a cause for concern, the clinical implications remain unclear. The isolation of vaccine antigen deficient isolates of *B. pertussis* from clinical cases of pertussis clearly means that they are still capable of infection. A combination of laboratory, epidemiological and clinical data will allow better assessment of their significance.

Due to the widespread occurrence of vaccine antigen deficient *B. pertussis* strains, it is important that countries monitor these changes as part of their national surveillance activities. ECDC has previously provided training and organised EQAs to improve and assess laboratory techniques for surveillance in the Member States. In a previous ECDC-funded (ECDC/2015/009) European Pertussis Laboratory Surveillance Network (EUPert-LabNet) project, training for vaccine antigen expression detection using ELISA was provided to reference laboratories in a number of European countries. ECDC has organised this first EQA in antigen expression to assess the competency of the Member States in performing this technique.

External Quality Assessment (EQA) is a critical component in assessing laboratory performance. As part of the Coordination of the European Reference Laboratory Network for Pertussis (ERLNPert-Net), Framework Contract (FWC), ECDC/2019/023, the UK Health Security Agency's Vaccine Preventable Bacteria Section, Colindale, UK was contracted to deliver Work Package 2: EQA scheme for detection of vaccine antigen expression of *B. pertussis* isolates among the NRLs in Member States, Iceland, Liechtenstein and Norway. This report describes the EQA.

2. Introduction

Pertussis is endemic worldwide and is an important cause of morbidity and mortality, particularly among infants. Despite current prevention strategies pertussis remains an important public health issue. To inform national control strategies, high-quality laboratory methodology and epidemiological information are required for treatment, prevention, vaccine effectiveness, management and surveillance.

Previous ECDC *Bordetella* EQA schemes have addressed laboratory methods for *Bordetella* species identification and *B. pertussis* typing using phenotypic and genotypic methods [6]; *B. pertussis* serology [2, 4] and detection of *B. pertussis* by PCR [3, 7].

B. pertussis isolates not expressing the vaccine antigen pertactin (PRN) have been widely reported, but to date, isolates not expressing pertussis toxin (PT) or filamentous haemagglutinin (FHA) remain rare [17, 19, 21, 25]. The ability of laboratories to detect both vaccine antigen expression and lack of expression in *B. pertussis* isolates is therefore important for surveillance activities.

EQA schemes are important for accurate identification and assessment of laboratory performance. This EQA scheme was designed to assist the assessment, development and standardisation of techniques for the characterisation of the expression and non-expression of vaccine antigen targets for surveillance. The design of the EQA was intended to cover the differing capacity and access to technology across the participating laboratories. From previous EQAs it was known that most laboratories are familiar with fimbrial serotyping and ELISA methodologies. Since some laboratories now have the access to whole genome sequencing technology, this was also included as an option.

EQA schemes enable the performance of the NRLs from each EU/EEA Member State to be independently assessed. Furthermore, through the scheme, recommendations can be made for improvements to methodologies and areas identified for further training.

The specific aims of this *B. pertussis* vaccine antigen expression EQA scheme were:

- to evaluate the ability of participants to determine expression/non-expression of fimbriae (fimbrial serotyping);
- to evaluate the ability of participants to determine expression/non-expression of pertactin (PRN);
- to evaluate the ability of participants to determine expression/non-expression of pertussis toxin (PT);
- to evaluate the ability of participants to determine expression/non-expression of filamentous haemagglutinin (FHA);
- to assess differences in methodology, interpretation and reporting of results;
- to identify training needs;
- to assist with the establishment of 'best practice' in current assays, interpretation and reporting.

3. Materials and methods

3.1 Organisation

The ERLNPert-*Net B. pertussis* vaccine antigen expression EQA, which was organised by the UK Health Security Agency's Vaccine Preventable Bacteria Section (UKHSA – VPBS), Colindale, UK, was intended for NRLs in EU Member States and EEA countries (Iceland, Liechtenstein and Norway). Invitations were initially sent to ECDC-designated pertussis laboratory experts in 29 countries in May 2021. In total, 12 laboratories participated, 11 from EU countries and one from Norway. Eight of these 12 laboratories performed at least one of the antigen expression components and seven of these eight had prior experience of the technique.

3.2 Selection of the panel

Clinical isolates of *B. pertussis* were selected from those kindly provided by the Institut Pasteur, National Reference Centre for Whooping Cough and other *Bordetella* infections, Paris, France and the UK Health Security Agency (UK HSA)– VPBS culture collection (see Table 1). The panel was designed to include *B. pertussis* strains belonging to the serotypes 1,0,0 (FIM- [minus]); 1,2 (FIM2); 1,3 (FIM3) and 1,2,3 (FIM2,3) together with those expressing and not expressing pertactin (PRN+/-), pertussis toxin (PT+/-) and filamentous haemagglutinin (FHA+/-).

3.3 Distribution of panels and instructions

Detailed instructions and reply forms were emailed to participants (Annex 2). The panels of test and control strains, together with PRN monoclonal antibody PeM4 (if requested) were prepared, packed according to local regulations and collected on 23 August 2021 by courier from UK HSA London for shipment on dry ice to the 12 laboratories. Six arrived within three days but unfortunately the remaining packages were delayed in customs, taking from four to ten days to arrive. In some cases the samples arrived thawed (due to delay and/or the fact that the dry ice could not be refilled), but in all cases the strains were successfully recovered.

Table 1. Characteristics of the strains in the ERLNPert-Net *B. pertussis* vaccine antigen expression EQA panel, including eight test strains and three control strains (August 2021)

Original reference number	Strain	Description
S1	*B. pertussis* serotype 1,2	FIM2 strain [26]
FR3693	*B. pertussis* serotype 1,3	Clinical isolate from three-month-old infant, France; PRN negative due to insertion of an IS*481* in the PRN gene [25, 27]
FR3708	*B. pertussis* serotype 1,3	Clinical isolate from five-month-old; PRN negative due to insertion of an IS*481* in the PRN gene [25, 27]
H121560437	*B. pertussis* serotype 1,2,3	Clinical isolate from three-month-old, isolated 2012, UK
H122660366	*B. pertussis* serotype 1,0,0	Clinical isolate from three-month-old, June 2012, UK
FR3469	*B. pertussis* serotype 1,3	Clinical isolate from two-month-old, isolated 2005, France; PT negative [28]
FR4624	*B. pertussis* serotype 1,3	Clinical isolate from two-month-old, isolated 2009, France; PRN negative and FHA negative [28]
FR3793	*B. pertussis* serotype 1,3	Clinical isolate from 2.5-month-old, isolated 2007, France; PRN negative due to deletion of part of the PRN gene [25]
Original reference number	**Control strains**	**Description**
FR3749	*B. pertussis* serotype 1,3	PT negative control strain*
FR4624	*B. pertussis* serotype 1,3	PRN and FHA negative control strain
FR3469	*B. pertussis* serotype 1,3	PRN negative control strain

**Due to a transcription error this strain was erroneously described as a positive control strain for PRN, PT and FHA. Participants were notified of this finding in a timely manner.*

Table 2. Recommended antibody reagents for testing of *B. pertussis* EQA panel and source

Antigen and target if specified	Antibody reagent	Source	Notes
Fimbrial antigen 2 (FIM2)	06/124	National Institute for Biological Standards and Control (NIBSC), United Kingdom. https://www.nibsc.org	
Fimbrial antigen 3 (FIM3)	06/128		
Pertussis toxin (PT) S1 subunit	99/512		
Pertussis toxin (PT) S3 subunit	99/542		
Filamentous haemagglutinin (FHA)	99/572		
Pertactin (PRN) region 1	PeM4	National Institute for Public Health and the Environment (RIVM) Bilthoven, the Netherlands https://www.rivm.nl/	Supplied by UK HSA to participants on request for this EQA.

3.4 Testing

The EQA was designed in three parts:

1. Fimbrial serotyping: to assess the ability of each laboratory to correctly report the expression/non-expression of fimbriae in the panel and report the fimbrial serotype for each strain.
2. PRN/PT expression: to assess the ability of each laboratory to correctly report the expression/non-expression status of PRN/PT for each strain.
3. FHA expression (optional): to assess the ability of each laboratory to correctly report the expression/non-expression status of FHA for each strain.

Participants were instructed to perform serotyping according to their standard methods using monoclonal or polyclonal antibodies from NIBSC, or other commercial sources, as previously described [8].

Participants were asked to determine the expression of the three vaccine antigens using previously published ELISA methods and/or by prediction from PCR and/or DNA sequencing. A protocol for the ELISA by Alex-Mikael Barkoff, from the University of Turku (UTU), Finland was provided together with recommended references from the literature [22,25,28] (Annex 2).

A reporting sheet for the results was included with the instructions and sent to each participant by e-mail (MS Word document) to be returned by the deadline.

3.5 Data analysis

The intended results from the organising laboratory (Table 3) were used as a basis for the scoring.

Participants were expected to:

- perform serotyping of the *B. pertussis* isolates;
- determine the vaccine antigen expression status of *B. pertussis* isolates using ELISA or genotypic methods;
- accurately interpret and report results depending on the methods used and results obtained.

Table 3. Intended results for ERLNPert-Net *B. pertussis* vaccine antigen expression EQA panel (August 2021)

EQA panel number	Original reference no.	Serotype		PRN	PT	FHA
EQA01	S1	1,2	FIM2	PRN+	PT+	FHA+
EQA02	FR3693	1,3	FIM3	PRN-	PT+	FHA+
EQA03	FR3708	1,3	FIM3	PRN-	PT+	FHA+
EQA04	H130940400	1,2,3	FIM2,3	PRN+	PT+	FHA+
EQA05	H122660366	1,0,0	FIM-	PRN+	PT+	FHA+
EQA06	FR3469	1,3	FIM3	PRN+	PT-	FHA+
EQA07	FR4624	1,3	FIM3	PRN-	PT+	FHA-
EQA08	FR3793	1,3	FIM3	PRN-	PT+	FHA+

4. Results

The EQA consisted of three sections: fimbrial serotyping (Part 1); expression of PRN and PT (Part 2) and expression of FHA (Part 3). Fimbrial serotyping was performed using slide agglutination or ELISA. Expression (or prediction of expression) was performed by ELISA, Western blot or WGS, depending on each participant's selection and capacity.

Participation was as follows:

- Part 1: Eleven of 12 laboratories (92%) performed fimbrial serotyping.
- Part 2: Ten of 12 (83%) performed PRN expression (seven using ELISA, 1 by Western blot and two by WGS analysis), and seven of 12 (58%) performed PT expression (four using ELISA, one by Western blot and two by WGS analysis).
- Part 3: Seven of 12 (58%) laboratories analysed FHA expression (six using ELISA and one using DNA sequence analysis).

In Tables 4 to 8 below, results in red indicate a discrepancy between the reported result and the intended result.

4.1 Serotyping

Eleven of the 12 participating laboratories submitted fimbrial serotyping results (Table 4). Laboratory 9 did not perform this section, which is why it does not appear in Table 4. All 11 laboratories used monoclonal antibodies. Ten of 11 used 06/124 mAb Fim2 and 06/128 mAb Fim3 from NIBSC and one used in-house mAbs. Analysis was performed by ELISA for 5/10 and slide agglutination by 6/10 laboratories. The EQA05 strain (FIM-) was scored as FIM- by 5/10 laboratories and FIM2 by 5/10 laboratories.

Table 4. Results of *B. pertussis* fimbrial serotyping

EQA panel number	Intended result	Results by laboratory											
		Laboratory no:	1	2	3	4	5	6	7	8	10	11	12
		Antibodies:	mAb	mAb	mAb	mAb	mAb	mAb	mAb	mAb	mAb	mAb	mAb
		Source:	NIBSC	NIBSC	NIBSC	inhouse	NIBSC	NIBSC	NIBSC	NIBSC	NIBSC	NIBSC	NIBSC
		Method:	ELISA	slide	ELISA	slide	slide	slide	ELISA	slide	ELISA	ELISA	slide
EQA01	**FIM2**	-	FIM2	FIM3	FIM2	FIM2	FIM2	FIM2	FIM2	FIM-	FIM2	FIM2	FIM2
EQA02	**FIM3**	-	FIM3	FIM3	FIM3	FIM3	FIM3	FIM3	FIM3	FIM3	FIM3	FIM3	FIM3
EQA03	**FIM3**	-	FIM3	FIM2	FIM3	FIM3	FIM3	FIM2,3	FIM3	FIM-	FIM3	FIM3	FIM3
EQA04	**FIM2,3**	-	FIM2,3	FIM2,3	FIM2,3	FIM2,3	FIM-	FIM2,3	FIM2,3	FIM2	FIM2	FIM2,3	FIM2,3
EQA05	**FIM-**	-	FIM-	FIM2	FIM2*	FIM2	FIM-	FIM2	FIM2	FIM-	FIM-	FIM2	FIM-
EQA06	**FIM3**	-	FIM3	FIM3	FIM3	FIM3	FIM3	FIM3	FIM3	FIM-	FIM2,3	FIM3	FIM3
EQA07	**FIM3**	-	FIM3	FIM3	FIM3	FIM3	FIM3	FIM3	FIM3	FIM-	FIM3	FIM3	FIM3
EQA08	**FIM3**	-	FIM3	FIM3	FIM3	FIM3	FIM2,3	FIM2	FIM3	FIM-	FIM3	FIM3	FIM3
Overall score			8/8	5/8	7/8	7/8	6/8	5/8	7/8	2/8	6/8	7/8	8/8

**Reported as low optical density*

4.2 PRN and PT expression

Ten of the 12 participating laboratories submitted PRN expression results (Table 5). Laboratories 2 and 9 did not perform this activity and therefore do not appear in Table 5. Seven of 10 used ELISA, five with PeM4 monoclonal antibodies and two with *B. pertussis* anti-69kD polyclonal antibody serum (sheep) catalogue no. 97/558 (NIBSC); one performed analysis by Western blot with an in-house polyclonal antibody. Two laboratories used WGS to predict PRN expression, one of which (Laboratory 8) provided an analysis of the mutations identified and the predicted effect at the protein level (Table 6). Eight of 10 laboratories scored 8/8 (100%). Of the two laboratories which scored less than 100%, one scored 7/8 (using WGS) and one 6/8 using ELISA.

Table 5. Results of *B. pertussis* PRN expression

EQA panel number	Intended result	Results by laboratory										
		Laboratory no:	1	3	4	5	6	7	8	10	11	12
		Antibody	NIBSC 97/558	PeM4	Inhouse polyclonal	PeM4	NIBSC 97/558	PeM4	N/A	PeM4	PeM4	N/A
		Method	ELISA	ELISA	Western blot	ELISA	ELISA	ELISA	WGS*	ELISA	ELISA	WGS*
EQA01	**PRN+**	-	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+	PRN-
EQA02	**PRN-**	-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-
EQA03	**PRN-**	-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-
EQA04	**PRN+**	-	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+	PRN-	PRN+	PRN+
EQA05	**PRN+**	-	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+	PRN-	PRN+	PRN+
EQA06	**PRN+**	-	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+	PRN+
EQA07	**PRN-**	-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-
EQA08	**PRN-**	-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-	PRN-
Overall score			8/8	8/8	8/8	8/8	8/8	8/8	8/8	6/8	8/8	7/8

N/A, not applicable
**expression predicted from analysis of WGS data.*

Table 6. Results of *B. pertussis* PRN expression prediction from WGS (Laboratory 8)

EQA panel number	Intended result	Mutation identified	Effect of mutation at protein level
EQA01	**PRN+**	Position 2007: G → A	Silent mutation
EQA02	**PRN-**	Position 1614-1682: replacement with 15 bp (variant 1)	Introduces premature stop codon (TAG)
EQA03	**PRN-**	Position 1614-1682: replacement with 15 bp (variant 2)	Introduces premature stop codon (TAG)
EQA04	**PRN+**	No	N/A
EQA05	**PRN+**	No	N/A
EQA06	**PRN+**	No	N/A
EQA07	**PRN-**	Position 1614-1682: replacement with 15 bp (variant 2)	Introduces premature stop codon (TAG)
EQA08	**PRN-**	Position 10-562: 3210 bp insertion	Insertion of *dacB* and *queA* genes into *prn* gene

N/A, not applicable (i.e. having no effect at the PRN protein level).
dacB codes for the protein d-ala-d-ala carboxypeptidase (DacB).
queA codes for the enzyme S-adenosylmethionine: tRNA ribosyltransferase-isomerase (QueA).

Seven laboratories submitted PT expression results (Table 7). Four used ELISA with the anti-PT monoclonal antibodies 99/512 (S1) and 99/542 (S3) (NIBSC). One laboratory performed analysis by Western blot with an in-house polyclonal antibody and two laboratories used WGS to predict PT expression. Overall, four of seven laboratories correctly identified the PT expression in eight of eight strains, while the other three laboratories all incorrectly identified only one strain in the panel (EQA06).

Table 7. Results of *B. pertussis* PT expression

EQA panel number	Intended result	Result by laboratory							
		Laboratory no.	3	4	7	8	10	11	12
		Antibodies	99/512 (S1) 99/542 (S3)	Polyclonal guinea pig serum (in-house)	99/512 (S1) 99/542 (S3)	N/A	99/512 (S1) 99/542 (S3)	99/512 (S1) 99/542 (S3)	N/A
		Method	ELISA	Western blot	ELISA	WGS*	ELISA	ELISA	WGS*
EQA01	**PT+**	-	PT+	PT+	PT+	PT+	PT+	PT+	PT+
EQA02	**PT+**	-	PT+	PT+	PT+	PT+	PT+	PT+	PT+
EQA03	**PT+**	-	PT+	PT+	PT+	PT+	PT+	PT+	PT+
EQA04	**PT+**	-	PT+	PT+	PT+	PT+	PT+	PT+	PT+
EQA05	**PT+**	-	PT+	PT+	PT+	PT+	PT+	PT+	PT+
EQA06	**PT-**	-	PT-	PT-	PT-	PT+	PT+	PT-	PT+
EQA07	**PT+**	-	PT+	PT+	PT+	PT+	PT+	PT+	PT+
EQA08	**PT+**	-	PT+	PT+	PT+	PT+	PT+	PT+	PT+
Overall score:			8/8	8/8	8/8	7/8	7/8	8/8	7/8

N/A, not applicable;
**expression predicted from analysis of whole genome sequencing (WGS) data.*

4.3 FHA expression

Seven laboratories submitted FHA expression results (Table 8). Four used ELISA with the anti-FHA monoclonal antibody 2E9, 99/572 (NIBSC). One laboratory performed analysis by Western blot with an in-house polyclonal antibody and two laboratories used WGS to predict FHA expression. Laboratory 9 effectively reported typing results (i.e. allele numbers), the organisers took the lack of any reported result for *fhaB* 2400_5550 for EQA07 as a prediction of FHA non-expression and a reported result as a prediction of expression (reported as allele 1 for EQA01-06 and EQA08).

Table 8. Results of *B. pertussis* FHA expression

EQA panel number	Intended result	Result by laboratory							
		Laboratory no:	3	4	7	9	10	11	12
		Antibody:	99/572	Polyclonal mouse serum (in-house)	99/572	N/A	99/572	99/572	N/A
		Method:	ELISA	Western blot	ELISA	WGS*	ELISA	ELISA	WGS*
EQA01	**FHA+**	-	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+	FHA-
EQA02	**FHA+**	-	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+
EQA03	**FHA+**	-	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+
EQA04	**FHA+**	-	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+
EQA05	**FHA+**	-	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+
EQA06	**FHA+**	-	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+
EQA07	**FHA-**	-	FHA-	FHA-	FHA-	FHA-	FHA-	FHA-	FHA-
EQA08	**FHA+**	-	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+	FHA+
Overall score			8/8	8/8	8/8	8/8	8/8	8/8	7/8

N/A, not applicable
**expression predicted from analysis of WGS data.*

5. Discussion

B. pertussis vaccine antigen expression

Fimbriae and the fimbrial proteins FIM2 and FIM3 are considered important vaccine components for both whole-cell and acellular pertussis vaccines [29]. The traditional serotyping scheme for *B. pertussis* was dependent on the presence/absence of three major agglutinogens (including FIM2 and FIM3), and several minor ones. Hence the historic descriptions of strains: NCTC 101911 as serotype 1.2.3.4.0.0 and NCTC 10908 as serotype 1.2.0.0.5.6[2].

Robinson et al [30] proposed a simplification of this scheme to include just three agglutinogens, agglutinogen 1 and FIM2 and FIM3. In this scheme, NCTC 101911 is serotype 1,2,3 and NCTC 10908 is serotype 1,2. Due to agglutinogen 1 always being present, this has since been further reduced by others to use only the fimbrial notation, i.e. serotype 1,2,3 (FIM2, FIM3); serotype 1,2 (FIM2); serotype 1,3 (FIM3) and serotype 1,0,0 (FIM-). The traditional methodology for serotyping was slide agglutination using polyclonal antibodies. WHO international standards for fimbrial serotyping *B. pertussis* (monoclonal antibodies) are available from NIBSC and can be applied using slide agglutination or ELISA. Both of these techniques were used in this EQA.

The emergence of circulating strains of *B. pertussis* not expressing acellular vaccine antigen components, including PRN, PT and FHA, requires appropriate laboratory techniques for monitoring and surveillance purposes. Western blotting is a classic technique in molecular biology to identify specific proteins from a complex mixture of proteins extracted from cells. The technique comprises separation by size, transfer to a solid support, and detection and visualisation of the target protein using primary and secondary antibodies. Western blotting has been applied to the analysis of PRN expression in *B. pertussis* [25, 31]. Barkoff and colleagues [27] described an indirect ELISA for PRN expression based on that used for fimbrial expression [32]. This methodology was further expanded to include PT and FHA [27].

Serotyping of *Bordetella pertussis* isolates

A previous EQA [6] evaluated the ability of participants to serotype *B. pertussis* isolates using monoclonal antibodies. Only 7/14 (50%) laboratories correctly identified the serotype of the four *B. pertussis* strains in the panel (using monoclonal antibodies). In this EQA, 12/14 laboratories performed slide agglutination (for which a protocol was supplied) and 2/14 used ELISA (for which a protocol was supplied). Reasons given for this low score included auto-agglutination using the slide agglutination protocol and inexperience with this technique. The two centres using ELISA both correctly identified all four serotypes [6].

In the EQA described here, only two of 11 laboratories scored 100% (intended results), one using slide agglutination and one using ELISA. However, prior to dispatch, the panel was only tested using polyclonal antibodies not monclonal antibodies. One strain in the panel EQA05, designated as serotype 1,0,0 / FIM-, was reported variously as FIM-, FIM2 or FIM2 (low) by participants. This may be indicative of cross-reactivity, a low level of FIM2 expression or possible mixed expression of FIM- and FIM2. If this strain was excluded from the analysis, an additional four laboratories would have scored 100% (one using slide agglutination, three using ELISA).

Explanations for the lower overall score in this EQA include potential variation in phenotypic expression due to delay in transport and differing pre-test culture conditions; autoagglutiniation; differences in experience of operators in technique used; lack of availability of additional control material and differences in interpretation and cut-offs used for the ELISA.

As previously noted, satisfactory results are possible with the slide agglutination technique, but experience is required and more consistent results may be achieved using the ELISA protocol. In general, the ELISA is considered more appropriate for processing larger numbers of strains at one time. Given the decrease in *B. pertussis* isolates referred to NRLs since the COVID-19 pandemic, these techniques may not have been used on a routine basis.

[2] National Collection of Type Cultures, UK Health Security Agency, Porton Down, Salisbury, UK; https://www.culturecollections.org.uk/collections/nctc.aspx

Expression of PRN, PT and FHA

This is the first ECDC EQA to assess the ability of laboratories to detect expression/non-expression of PRN, PT and FHA in *B. pertussis*.

PRN expression

The overall results for the ability to distinguish PRN expression/non-expression in *B. pertussis* were very good, with eight of 10 laboratories (80%) scoring 8/8 (100%). Most laboratories (7/10) used the recommended ELISA protocol, with five using the PeM4 PRN mAbs, two using the non-WHO reference material *B. pertussis* anti-69kD serum 97/558 (NIBSC) and one using in-house PRN polyclonal antibodies. Of these PRN antibodies, currently only 97/558 polyclonal is available commercially. One of the aims of the ERLNPert-Net consortium is to make the PeM4 monoclonal product commercially available, although this has not happened to date.

The reason for the discrepancy in the two results for EQA04 and EQA05 (scored as PRN- vs. PRN+ intended) by Laboratory 10 is unknown.

Two laboratories (8 and 12) performed WGS to predict expression of PRN. One predicted all 8/8 correctly, while the other scored 7/8 due to the incorrect prediction of EQA01 as PRN- (vs. PRN+ expected result). The reason for this incorrect prediction is unknown.

For each strain, Laboratory 8 reported information on the mutation identified (if present) in the *prn* gene and the predicted effect of these mutations at the protein level (Table 6). For example, for EQA01 a single base change from G → A was reported as a synonymous, therefore silent mutation (PRN+). No mutations were found in EQA04, EQA05 and EQA06, which were correctly scored as 'not applicable' (i.e. no effect at the protein level (PRN+)). On the other hand, mutations were found in EQA02, EQA03 and EQA07 which were predicted to result in premature stop codons (i.e. non-expression (PRN-)) which was the expected result for each of these strains. The result for EQA08 was reported as a large insertion of the two genes *dacB* and *queA* into the *prn* gene, resulting in non-expression (PRN-). The gene *dacB* codes for the protein d-ala-d-ala carboxypeptidase (DacB) and *queA* codes for the enzyme S-adenosylmethionine: tRNA ribosyltransferase-isomerase (QueA). While this result (PRN-) is the expected correct result, the original description of this strain EQA08/FR3793 is that of PRN negative due to deletion of part of the *prn* gene. No insertion is described by Bouchez et al [25] in the *prn* gene. Moreover, sequencing of region 1 of the *prn* revealed a deletion of the BP1053 gene coding for a copy of the insertion element IS*1663*, located upstream of *prn* and the first part of the *prn* gene (GenBank accession no. FJ480200) [25]. A possible explanation for the description of *dacB* and *queA* gene insertion could be in the alignment and analysis of the assemblies from this strain to the reference sequence.

PT expression

Seven laboratories performed the PT expression part of the EQA (Table 7). Only one PT negative strain was included in the test panel, EQA06. Four of seven (57%) laboratories scored 100%, three of these laboratories used the recommended ELISA protocol with the anti-PT monoclonal antibodies 99/512 (S1) and 99/542 (S3) (NIBSC) and one used Western blot with an in-house polyclonal PT antibody. The other three laboratories scored 7/8 (88%), all three scoring EQA06 as PRN+ (vs. PRN- expected result). Two of these laboratories (Laboratory 8 and Laboratory 12) used WGS to predict PT expression. The remaining laboratory used the recommended ELISA and mAb.

The original description of EQA06/FR3469 describes this strain of *B. pertussis* as PT negative by Western blot analysis, but the reason for non-expression was stated as unknown [28]. A possible explanation for the incorrect prediction from WGS analysis could be that the reason for non-expression lies outside the coding region of the PT.

FHA expression

Seven laboratories performed the FHA expression part of the EQA (Table 8). Results for this section were better than those obtained for PRN expression. However, it should be noted that only one FHA negative strain was included in the test panel. Six of seven (86%) laboratories scored 100%, four using the recommended ELISA protocol with the non-WHO reference material anti-filamentous haemagglutinin monoclonal antibody 2E9 (99/572, NIBSC), one using Western blot with an in-house polyclonal FHA antibody and one using WGS to predict FHA expression. The remaining laboratory scored 7/8 (88%) and incorrectly predicted that EQA01 did not express FHA (FHA- versus expected score FHA+). The reason for this incorrect assignment is unknown.

Identification of training needs

The poor overall score for serotyping indicates that training is required in this technique, specifically ELISA and/or slide agglutination using monoclonal antibodies. It is noted that a greater number of discrepancies was reported for slide agglutination than for ELISA.

Although a number of the participating laboratories have received training on vaccine antigen (PRN, PT, FHA) expression using ELISA and monoclonal antibodies, it is apparent that further training is required for selected laboratories.

Invitations were sent to 29 EU/EAA countries, however only 12 laboratories participated in this EQA: eleven in the serotyping section; ten in the PRN expression (ELISA, seven; Western blot, one; WGS, two); seven in the PT expression (ELISA, four; Western blot, one; WGS, two) and seven in the FHA expression (ELISA, four; Western blot, one; WGS, two) sections.

It is important to note that this EQA was carried out during the COVID-19 pandemic, and staff from many pertussis NRLs were involved in SARS-CoV-2 diagnostic and surveillance activities. The relatively low participation rate in this EQA (compared to previous EQA schemes) may be due to laboratory staff being too busy with SARS-CoV-2 activities to have capacity to participate in EQAs. Furthermore, some laboratories may have chosen not to participate because they do not use these techniques routinely, or are not able to perform fimbrial typing and vaccine antigen expression. This would suggest that wider training may be required by pertussis reference laboratories in other EU/EAA countries that did not participate in this EQA.

While 15 laboratories had previous experience of vaccine antigen (PRN, PT, FHA) expression analysis, only seven of these participated in this element of this EQA.

Although overall the expression results were encouraging, not all laboratories that had received training achieved 100% of expected results. The reasons for this require further investigation. Possible explanations include technical issues, changes in personnel and considerable elapse of time since initial training was received.

Limitations

The panel and control strains were not tested using all the methods applied in the EQA prior to shipment.

The reason for non-expression was not known for all strains included in the EQA.

There were issues with several shipments containing the strains and antibodies, resulting in delays and the thawing of dry ice (although all of the strains were revived successfully).

Not all participating laboratories had received training in the expression ELISA technique.

The number of participants were reduced compared to similar previous pertussis EQAs, possibly due to time constraints in microbiology laboratories during the COVID-19 pandemic.

Of the 12 participating laboratories, not all were able to perform all sections of the EQA.

Although the inclusion of laboratories performing sequencing/WGS was welcomed, there was no clear protocol for the analysis, other than seeking previously described mutations/reasons for non-expression.

6. Recommendations

Pertussis NRLs should be able to perform fimbrial serotyping of *B. pertussis* isolates using ELISA or slide agglutination.

Due to the increase in circulating strains of *B. pertussis* which do not express vaccine antigen components, including PRN, PT and FHA, NRLs should be able to determine presence or absence of expression by ELISA or Western blotting.

As access to WGS increases, agreed criteria for analysis and interpretation are required.

The complementary nature of phenotypic and genotypic investigations for *B. pertussis* should be recognised, while clearly acknowledging the limitations of each methodology.

Hospital laboratories in EU/EEA countries should be actively encouraged to attempt isolation of *B. pertussis* from clinical pertussis cases and, if successful, to refer such strains to the NRLs for further investigation and for surveillance purposes.

The availability of standardised protocols and recommendations for the laboratory diagnosis and molecular surveillance of *B. pertussis* by ECDC and the ERLNPert-Net consortium [33], together with future training workshops and EQA schemes, will support national surveillance activities for this important vaccine-preventable disease.

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Annex 1. List of EQAs performed under the ERLNPert-Net External quality assessment scheme for *Bordetella pertussis*

Year conducted	Topic	Number of participating laboratories	Reference
2022	Antimicrobial susceptibility testing	17	European Centre for Disease Prevention and Control. External quality assessment scheme for *Bordetella pertussis* antimicrobial susceptibility testing, 2022. Stockholm: ECDC; 2023. [in press]
2021	Vaccine antigen expression	12	European Centre for Disease Prevention and Control. External quality assessment scheme for *Bordetella pertussis* vaccine antigen expression, 2021. Stockholm: ECDC; 2023 [this study].
2020	Serology	17	European Centre for Disease Prevention and Control. External quality assessment scheme for *Bordetella pertussis* serology – 2020 data. Stockholm: ECDC; 2022.
2018	PCR	28	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.
2016	Serology	25	European Centre for Disease Prevention and Control. External quality assessment scheme for *Bordetella pertussis* serology 2016. Stockholm: ECDC; 2018.
2013	Serology	21	European Centre for Disease Prevention and Control. External quality assessment scheme for *Bordetella pertussis* serology 2013. Stockholm: ECDC; 2014.
2013	*Bordetella* identification and *B. pertussis* typing	16	European Centre for Disease Prevention and Control. EQA scheme for *Bordetella* identification and *B. pertussis* typing. Stockholm: ECDC; 2014.
2012	PCR	21	European Centre for Disease Prevention and Control. External quality assessment scheme on PCR for *Bordetella pertussis*, 2012. On behalf of the EUPert-LabNet network Stockholm: ECDC; 2012.

Annex 2. The ERLNPert-Net External quality assessment scheme for *Bordetella pertussis* vaccine antigen expression

2021 Specific Contract ECDC/2019/023 - Work package 2

Instructions and reply form

23 August 2021

Coordination of the European Reference Laboratory Network for Pertussis (ERLNPert-Net)

Contract reference: OJ/2019/OCS/10548 and regarding the Framework Contract (FWC), ECDC/2019/023. Work package 2: EQA scheme for detection of vaccine antigen expression of *Bordetella pertussis* isolates among the National Reference Laboratories in Member States, Iceland, Liechtenstein and Norway.

Instructions

The study is in three parts:

1. Fimbrial serotyping: to assess the ability of each laboratory to correctly report the expression/ non-expression of fimbriae in the panel and report the fimbrial serotype for each strain.
2. Pertactin/pertussis toxin expression: to assess the ability of each laboratory to correctly report the expression/non-expression of pertactin/pertussis toxin for each strain.
3. Filamentous haemagglutinin expression (optional): to assess the ability of each laboratory to correctly report the expression/non-expression of FHA for each strain.

1. Fimbrial serotyping

Fimbrial serotyping of *Bordetella pertussis* using monoclonal antibodies to *B. pertussis* fimbriae type 2 and 3 or polyclonal antibody Anti-agglutinogen 2 and Polyclonal antibody Anti-agglutinogen 3.

Methods:

i) Protocol from UTU 2019 Pertactin (Prn), Filamentous Haemagglutinin (FHA), Pertussis Toxin (PT) and Fimbrial 2 & 3 (Fim2&3) expression – ELISA

ii) European Centre for Disease Prevention and Control. EQA scheme for *Bordetella* identification and *B. pertussis* typing. Stockholm: ECDC; 2014. Annex 2. https://www.ecdc.europa.eu/en/publications-data/external-quality-assurance-scheme-bordetella-identification-and-b-pertussis.

Please enter your details and results below and return by Friday 26 November 2021.

1.	Fimbrial serotyping					
Name:						
Laboratory:						
Country:						
Method:	Slide agglutination	Y/N				
	ELISA	Y/N				
	Other		Please describe			
Antibodies	Monoclonal	Y/N				
	Polyclonal	Y/N				
Antibody	06/124 mAb Fim2	06/128 mAb Fim3	89/598 Polyclonal Antiagg2	89/600 Polyclonal Antiagg3	Other	Final result for fimbrial serotype e.g. FIM2, FIM3, FIM2/3, FIM^{-}
Strain						
EQA01						
EQA02						
EQA03						
EQA04						
EQA05						
EQA06						
EQA07						
EQA08						
Control strains						

If performing slide agglutination please enter results as +++, ++, + and – to indicate degree of agglutination.

Please list control strains used. Please enter final fimbrial serotype result for each panel strain in shaded column

2&3. Pertactin/pertussis toxin/FHA expression

Determination of status of expression/non-expression of pertactin/pertussis toxin/FHA for each strain by PCR/sequencing or ELISA

Methods:

i) Protocol from UTU 2019 Pertactin (Prn), Filamentous Haemagglutinin (FHA), Pertussis Toxin (PT) and Fimbrial 2 & 3 (Fim2&3) expression – ELISA

ii) Barkoff AM, Mertsola J, Pierard D, Dalby T, Hoegh SV, Guillot S, Stefanelli P, van Gent M, Berbers G, Vestrheim D, Greve-Isdahl M, Wehlin L, Ljungman M, Fry NK, Markey K, He Q. Pertactin-deficient *Bordetella pertussis* isolates: evidence of increased circulation in Europe, 1998 to 2015. Euro Surveill. 2019 Feb;24(7):1700832. doi: 10.2807/1560-7917.ES.2019.24.7.1700832.

iii) Barkoff AM, Guiso N, Guillot S, Xing D, Markey K, Berbers G, Mertsola J, He Q. A rapid ELISA-based method for screening *Bordetella pertussis* strain production of antigens included in current acellular pertussis vaccines. J Immunol Methods. 2014 Jun;408:142-8. doi: 10.1016/j.jim.2014.06.001.

iv) Pawloski LC, Queenan AM, Cassiday PK, Lynch AS, Harrison MJ, Shang W, Williams MM, Bowden KE, Burgos-Rivera B, Qin X, Messonnier N, Tondella ML. Prevalence and molecular characterization of pertactin-deficient *Bordetella pertussis* in the United States. Clin Vaccine Immunol. 2014 Feb;21(2):119-25. doi: 10.1128/CVI.00717-13.

Please enter your details and results below and return by Friday 26 November 2021.

2 and 3.	**Pertactin expression/PT expression/FHA expression**				
Name:					
Laboratory:					
Country:					
Method:	ELISA	Y/N			
	PCR	Y/N			
	Sequencing	Y/N			
	Other	Please describe			
Antibodies	PM	Y/N			
	Other	Please describe			
Antibody					
Pertactin	PeM4	Y/N			
	Other	Please describe			
PT	99/512 (S1) 99/542 (S3)	Y/N Y/N			
FHA	99/572	Y/N			
			Final result for pertactin expression i.e. PRN^+, PRN^-	Final result for pertussis toxin expression i.e. PT^+, PT^-	Final result for FHA expression i.e. FHA^+, FHA^-
Strain					
EQA01					
EQA02					
EQA03					
EQA04					
EQA05					
EQA06					
EQA07					
EQA08					
Control strains					
C1 Positive control for PRN, PT, FHA					
C2 Negative control for PRN & FHA					
C3 Negative control for PT					

Annex 3. List of participating laboratories in alphabetical order

Country	Laboratory/institution
Belgium	Laboratorium Microbiologie, UZ Brussel, Brussel
Czechia	NRL for Pertussis and Diphtheria National Institute of Public Health, Prague
Finland	University of Turku, Institute of Biomedicine, Research Center for Infections and Immunity, Turku
France	Institut Pasteur, Paris
Hungary	National Public Health Center *Legionella-Bordetella* Laboratory, Budapest
Ireland	Microbiology Laboratory, Children's Health Ireland at Crumlin, Dublin
Italy	Dept. of Infectious Diseases, Istituto Superiore di Sanità, Rome
Norway	Folkehelseinstituttet, Prøvemottak, Oslo
Romania	Cantacuzino' MMNIRD, Bucharest
Slovenia	National Laboratory of Health, Environment and Food, Department for Public Health Microbiology, Ljubljana
The Netherlands	RIVM, Centrum Infectieziekteonderzoek, Diagnostiek en Screening, Bilthoven
Spain	*Neisseria*, *Listeria* and *Bordetella* Unit (Unidad de *Neisseria, Listeria* y *Bordetella*), Reference and Research Laboratory for Vaccine Preventable Bacterial Diseases (Laboratorio de Referencia e Investigación en Enfermedades bacterianas prevenibles por vacunación), National Centre for Microbiology (Centro Nacional de Microbiología), Instituto de Salud Carlos III, Madrid.

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