TECHNICAL REPORT

External quality assessment scheme for *Bordetella pertussis* serology 2016

As part of the EUPert-LabNet network

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ECDC TECHNICAL REPORT

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This report was commissioned by the European Centre for Disease Prevention and Control (ECDC), as part of the coordination of activities for laboratory surveillance of whooping cough in the EU/EEA Member States (ECDC/FWC/2015/009), coordinated by Dr Sabrina Bacci, Dr Emma Wiltshire and Dr Csaba Ködmön and produced by Dr Kevin Markey and Alex Douglas-Bardsley, National Institute for Biological Standards and Control (NIBSC) (Potters Bar, UK), Dr Norman Fry, Public Health England (PHE) (London, UK), and Alex-Mikael Barkoff and Prof Qiushui He, University of Turku (Turku, Finland), on behalf of the EUPert-LabNet consortium.

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Abbreviations

EEA	European Economic Area
ELISA	Enzyme-linked immunosorbent assay
EQA	External quality assessment
EU	European Union
EUPert-LabNet	Network of European pertussis experts funded by ECDC for this current programme
EUVAC-Net	A former European surveillance network for selected vaccine-preventable diseases hosted at the Statens Serum Institut, Denmark
FDA	US Food and Drug Administration
FHA	Filamentous haemagglutinin
GCV	Geometric coefficient of variation
GM	Geometric mean
IgG	Immunoglobulin G
IHR	In-house reference serum
IS	International Standard
IU	International Units
MHRA	Medicines and Healthcare products Regulatory Agency
MIA	Multiplex immunoassay
NIBSC	National Institute for Biological Standards and Control (UK)
NRL	National reference laboratories
PHE	Public Health England
PT	Pertussis toxin
RVPBRU	Respiratory and Vaccine Preventable Bacteria Reference Unit (of PHE)
WHO RR	WHO Reference Reagent

Executive summary

This external quality assessment (EQA) to assess the ability of national reference laboratories from EU/EEA Member States to correctly perform laboratory serodiagnostic tests for pertussis was a collaborative study organised from February to April 2016. This was achieved by identifying laboratories that produce results significantly different from the values obtained from the majority of participants. Furthermore, the scheme helps to identify methodologies in need of further improvement and areas for training where particular laboratories may improve their methods, procedures and global performance. A blinded panel of seven freeze-dried sera containing different concentrations of anti-pertussis toxin (PT) immunoglobulin G (IgG) (concentrations ranging from no anti-PT IgG to concentrations clinically associated with infection) were sent out by NIBSC to 25 laboratories in 23 EU/EEA Member States to quantify concentrations of anti-PT IgG using their own routine diagnostic enzyme-linked immunosorbent assays (ELISA) or multiplex immunoassays (MIA). Raw data were also returned to NIBSC for secondary analysis.

All 25 laboratories agreed to take part in the study. One laboratory did not return results. Twenty-three of the 24 that returned data only used one method (either an in-house ELISA, an in-house MIA or a commercial ELISA kit) to determine the anti-PT IgG concentrations of the sera panel. One laboratory submitted results obtained using all three methods (an in-house ELISA, a MIA and a commercial kit). A total of 85 data sets from individual assays were collected for 26 assay methods. Of the 23 laboratories that returned their own calculations, 21 ranked the samples in the same order as the calculations by NIBSC using parallel line analysis.

Overall, there is a trend towards more laboratories using commercial kits for the sero-diagnosis of pertussis. The recommendations made in previous ECDC studies are also being followed as a greater number of kits now use purified pertussis toxin as coating antigen and reference sera that are calibrated in IU/ml.
This technical report recommends that only purified PT is used in in-house ELISAs, commercial kits or multiplex immunoassays for serological testing of anti-PT IgG in human sera. Also, a reference serum should be included in each assay and it should be calibrated in IU/ml using one of the international standards (IS).

1. Introduction

The European Centre for Disease Prevention and Control (ECDC) is a European Union (EU) agency with a mandate to identify, assess, and communicate current and emerging threats to human health from communicable diseases. To fulfil its mandate, ECDC operates several disease networks. Within 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 Centre shall maintain and extend such cooperation and support the implementation of quality assurance schemes.'

Whooping cough (pertussis) is an acute respiratory infection most commonly caused by the bacterium *Bordetella pertussis* and to a lesser extent *B. parapertussis*. Pertussis remains endemic worldwide and is an important public health issue. Infants are the most vulnerable group to pertussis infection but older children and adults can display milder symptoms and are also responsible for transmission. In recent years, there has been a marked increase in the number of cases in areas with high vaccination coverage, such as the EU and North America [1-4].

EUVAC-Net was a European surveillance network for selected vaccine-preventable diseases, such as pertussis, hosted at the Staten Serum Institute (SSI), Denmark. The mapping and development of laboratory performance for pertussis was included in EUVAC-Net tasks in 2009 and was contracted to the National Institute for Health and Welfare (THL) based in Turku, Finland. In 2011, the responsibilities of the EUVAC-Net were transferred to ECDC. In 2011-14, pertussis laboratory activities continued to be coordinated by ECDC through a Framework Contract with THL and EUPert-LabNet was created.

In 2015, following an open call for tender (OJ/03/12/2014-PROC/2014/033), a consortium of pertussis experts from nine countries, with leadership from the University of Turku, Finland, was awarded the framework contract 'Coordination of activities for laboratory surveillance of whooping cough in Member States and EEA countries' (ECDC/FWC/2015/009). The aim of this framework service contract is to continue coordination of laboratory activities and support surveillance in the field of pertussis. Work package 2 of the contract comprised EQA schemes for the laboratory diagnostics and molecular typing of pertussis distributed among the NRLs in EU Member States, Iceland, Liechtenstein and Norway.

The EUPert-LabNet consortium and network is led and coordinated by prof. Qiushui He and MSc. Alex-Mikael Barkoff (Turku, Finland).

The UK Public Health England's (PHE) Respiratory and Vaccine Preventable Bacteria Reference Unit (RVPBRU) in Colindale, UK, and the National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK, a centre of the Medicines and Healthcare products Regulatory Agency (MHRA), were contracted to deliver Work package 2: External quality assessments (EQAs) for laboratory diagnostics and molecular typing of pertussis. Work package 2 (coordinated by Dr Norman Fry, PHE – Colindale, Dr Kevin Markey, MHRA –NIBSC) comprises three EQAs for serology, PCR, and strain typing. This report details the first of these EQAs.

EQA schemes are an important part of quality management systems. They use an external agency to evaluate performance of laboratory assays on material that is supplied specifically for the purpose. ECDC's disease networks organise a series of EQAs for EU/EEA countries.

The aim of ECDC EQAs is to identify areas for improvement in laboratory diagnostic capacities relevant to the surveillance of the 52 communicable diseases listed in Decision No. 2119/98/EC 2 (5) and to ensure comparability of results between laboratories from all EU/EEA countries.

The main aims of this specific ECDC pertussis serology EQA scheme are to:

- assess the ability of laboratories to quantify and distinguish concentrations of anti-PT IgG in a panel of serum samples with different concentration of anti-PT IgG
- assess the possibility of setting up assay validity criteria for standardising serological tests using the WHO International Standard or Reference Reagent sera
- assess the general standard of performance, and ensure comparability of results between laboratories from all EU/EEA countries
- assess the effectiveness of analytical procedures (method principle, instruments, reagents, calibration)
- evaluate individual laboratory performance
- identify problem areas
- provide continuing education
- identify training needs.

Laboratory diagnosis of pertussis infection is important for surveillance, treatment and prevention. There have been wide variations in the reporting of laboratory-confirmed pertussis cases globally [6] and one of the reasons could be differences in the methods used for diagnosis. This highlights the importance of harmonisation and standardisation of diagnostic methods in successful laboratory-based surveillance systems.

Serological analysis by ELISA has been widely used for evaluation of antibody responses to pertussis vaccination and diagnosing infection [6]. As part of the EUVAC-Net contract with ECDC (Pertussis Work Area 4), an EQA was organised from July to October 2010 to assess laboratory performance of serological assays for pertussis, to compare in-house references that were being used and to identify any needs for standardisation of serological assays [7]. In that study, participants were requested to use their regular ELISA methods to differentiate two well-defined reference preparations with high (335 IU/ml) and low (106 IU/ml) IgG antibodies to PT. Seventeen national reference laboratories (NRL) in the EU/EEA took part in the study, of which nine used in-house ELISA methods and ten used commercial kits. All ELISA methods which used only purified PT as coating antigen could distinguish the two preparations and gave expected results. Commercial kits from six different manufacturers were used in the study and it was found that those that used a mixture of *B. pertussis* antigens to coat the ELISA plates did not produce the expected results. This indicated that antigen coating could be one of the important factors affecting laboratory performance. It was therefore recommended that purified PT only should be used to coat ELISA plates [7, 8].

In 2011 the members of the EUpertstrain group published the following recommendations for serological diagnosis of pertussis:

- the use of non-detoxified purified PT only as coating antigen [9]
- data points should cover a broad linear range and express results quantitatively in international units per millilitre (IU/ml) [9].

These recommendations were also endorsed in the ECDC Guidance and Protocol for the serological diagnosis of human infection with B. pertussis [8].

A subsequent EQA of pertussis serology in Germany that collected data from 183 German laboratories found that only 69 (38%) were following the EUpertstrain recommendations. The use of mixed coating antigens and lack of international units from the remaining laboratories made it difficult to interpret the data from these laboratories and also compare data between laboratories [10]. More recently, there have been reports that manufacturers producing commercial ELISA kits for serological diagnosis of pertussis are starting to adapt their products to the ECDC recommendations [8, 9, 10].

As part of the previous ECDC contract for the coordination of activities for laboratory surveillance of whooping cough in Member States/EEA countries (ECDC/2011/013), an EQA was organised from July to October 2012. A panel of five freeze-dried sera containing different concentrations of anti-PT IgG was prepared at NIBSC.

The concentrations ranged from no anti-PT IgG to concentrations clinically associated with infection. The panel was blinded and sent to participants who used their own routine diagnostic ELISA assays to determine the anti-PT IgG concentrations. Twenty-one laboratories from twenty countries agreed to take part in the study.

A total of 73 data sets were collected. The majority of the 21 participating laboratories used only one method (in-house ELISA, MIA or commercial kit) to determine the anti-PT IgG concentration of the serum panel. One laboratory submitted results obtained from an in-house ELISA and two different commercial kits. Nine participants used in-house ELISA methods. Thirteen laboratories provided results using six different commercial kits. Sixteen data sets had a sufficient range of dilutions to enable statistical analysis using a four-parameter logistic model and fifteen of these arranged the samples in the same rank of increasing anti-PT IgG concentration. Sample D in this study was the WHO Reference Reagent which has an assigned unitage of 106 IU/ml, and the overall geometric mean was found to be 106 IU/ml, indicating the accuracy of the assays used by most of the participants. The recommendations of this EQA concurred with the previous ECDC recommendations regarding the use of purified PT as the sole coating antigen. In addition, it was recommended that routine in-house reference sera should be calibrated in IU/ml using international reference sera such as WHO International Standard (NIBSC 06/140) or FDA US Lot3 for validation and standardisation of serodiagnostic assays (6).

As part of the current contract with ECDC (ECDC/FWC/2015/009) and to continuously improve the serological diagnosis of pertussis among the EU/EEA Member States, an EQA for pertussis serology was organised from February to April 2016. Details of the countries that took part in this EQA and the two previous studies are given in Annex 1.

This report presents the results of the external quality assessment (EQA) scheme for B. pertussis serology, the analysis of laboratories' performance and provides recommendations.

2. Materials and methods

2.1. Organisation

The EUPert-LabNet *B. pertussis* serology EQA was organised by the NIBSC and was intended for NRLs in EU Member States, Iceland, Liechtenstein and Norway. All NRLs in Europe currently performing serological assays for diagnosis of pertussis infection by measuring serum antibody to PT were invited to participate in the EQA in December 2015. A total of 25 laboratories from 23 countries agreed to participate in this EQA, twenty-four of which were in EU countries and one from Norway (Annex 1a). The majority of those that declined to take part did not perform serodiagnosis.

Throughout this report, where assay results are attributed to the various laboratories, each laboratory is identified only by a randomly assigned code number from 1 to 25, so that they alone can identify which set of assay results they contributed. Separate experiments have been numbered sequentially within laboratories.

2.2 Selection and preparation of sera panel

Human plasma of different concentrations of anti-PT antibodies (IgG) were kindly supplied to NIBSC by Drs Carl-Heinz Wirsing von König and Marion Riffelmann, HELIOS Klinikum, Krefeld, Germany and the Centre for Biological Reference Materials in NIBSC. A panel of seven freeze-dried preparations of human sera containing different concentrations of anti-PT IgG was prepared (Table 1). Samples A and C were prepared previously for the 2012 ECDC organised serology EQA and the method for preparing these samples can be found in the study report [6]. In the previous EQA, these were found to contain approximately 0 and 13-20 IU/ml anti-PT IgG, respectively [6].

Samples B, D, E and G were prepared as follows. Freeze-dried ampoules of NIBSC standard 06/146 which contains 335 IU/ml anti-PT IgG were reconstituted with distilled water and diluted with different volume of a diluting sera containing 6.7 IU/ml anti-PT IgG to obtain to appropriate concentrations of anti-PT IgG in the pooled sera. The diluting sera used were obtained from plasma donated by the Centre for Biological Reference Materials at NIBSC. From this plasma, sera were prepared following re-calcification. In brief, the plasma was thawed at 4ºC. Samples were pooled in sterile glass beakers, 1 M $CaCl_2$ solution (Sigma-Aldrich, Poole, UK) was added at a concentration of 20 µl $CaCl_2$ per 1 ml of plasma. Samples were then incubated at 37°C in a water bath for 30 min and left at 4°C overnight. Sera were extracted from the clot by squeezing the clot with a flat surface and decanting the sera which were then centrifuged at 3,000 x g for 30 min. An Activated Plasma Clotting Time (APCT) test was performed to confirm the absence of any remaining clotting agents. Details of the volumes used for the preparation of each sample in the panel can be found in Table 1.

Each serum sample was filled in 0.5 ml aliquots into 3 ml glass ampoules and then freeze dried on a five-day cycle (starting shelf temperature of -50°C) after which the ampoules were back filled with high purity nitrogen before sealing. The detailed information on plasma samples and resulting serum panel are given in Table 1. Previously freeze-dried ampoules of Pertussis Antiserum (Human) 1st WHO Reference Reagent (NIBSC 06/142) were blinded and relabelled as Sample F.

Table 1. Preparation of the sera panel

Sera panel code	Volume (ml) 06/146 human sera (335 IU/ml)	Volume (ml) human sera (6.7 IU/ml)	Proposed Mean IgG-PT in pooled sample	Filling number after freeze-drying	Number of ampoules produced
Sample A	N/A*	N/A	<2	SS-369	900
Sample B	67	433	~50	14/186	900
Sample C	N/A	N/A	~14-20	SS-366	900
Sample D	97	403	~70	14/188	900
Sample E	127	373	~90	14/190	900
Sample F	Relabelled- Pertussis Antiserum (Human) 1st WHO Reference Reagent (106 IU/ml anti-PT IgG)			06/142	7 800
Sample G	220	280	~150	14/192	900
Reference serum	Pertussis Antiserum (Human) 1st WHO Reference Reagent (106 IU/ml anti-PT IgG)			06/142	7 800

** N/A, not applicable as these samples were prepared previously [6]*

2.3. Sample shipment

Each participating laboratory received two sets of ampoules comprising the panel of seven sera samples of human serum coded by letter together with one ampoule of 06/142 (WHO RR) (Table 1).

The panels were prepared, packed according to local regulations, collected by international courier on 22 February 2016 from NIBSC, South Mimms, Hertfordshire, UK and dispatched to the participating laboratories (Annex 1a). All packages were received in a timely manner allowing laboratories sufficient time to meet the deadline of Friday 22 April 2016.

2.4. Assay methods

The primary aim of the study for each participant was to determine the concentrations of anti-PT-IgG in each sample of the panel of sera using their routine serological methods (i.e. in-house or commercial ELISA or in-house MIA assays).

Laboratories who performed in-house ELISA and MIA used their own methodology, reagents and calculation methods, including their own in-house references and controls. For laboratories that used commercial ELISA kits, all reagents were supplied with the kits. A summary of assay information is given in Annex 4a and 4b.

2.4.1. Antiserum preparations

The following recommendations were provided to the participants:

- ampoules of lyophilised sera be stored at -20°C
- reconstituted samples should be divided into aliquots and stored at -20°C
- the aliquots should be used only once as freeze-thawing was not recommended
- an initial dilution at approximately 1/100 of the reconstituted sera samples followed by ½ dilutions would be suitable.

Information on the volumes to use for reconstituting each sera sample were also included in the shipment package (Annex 3).

In addition, individual laboratories were encouraged to perform a pilot study to choose the suitable dilution for the samples under their own experimental conditions.

2.5. Study design

Participants were requested to complete and return to NIBSC assay sheets describing in detail their assay procedure/conditions together with the raw data for each assay (Annex 3).

2.5.1. For laboratories using their own in-house ELISA and MIA

The participating laboratories were asked to coat ELISA plates using their own in-house sourced PT antigen and perform a minimum of three independent assays on the eight serum preparations (reference serum and seven testing sera). Participants were also asked to include their in-house reference serum (IHR) in all the assays. The three independent assays should be run on three different days. On each assay, dilution curves for each preparation should have at least two or three replicates per assay, and preferably each dilution curve should include at least four doses in the linear region. All preparations should be included in each assay.

Laboratories should use their own methodology, reagents and calculation methods, include their in-house references and controls, and use assay runs which met their internal validity criteria.

2.5.2. For laboratories using commercial ELISA kits

The participating laboratories were asked to perform a minimum of three independent assays on the panel of eight serum preparations provided in this study using the commercial ELISA kit that are routinely used in their laboratory. Participants were requested to include the reference serum provided in the kit in all the assays. The three independent assays should be run on three different days. In each assay, dilution curves for each preparation should have at least two replicates per assay and preferably each dilution curve should include at least four doses in linear region. Laboratories should use the methodology and validity criteria recommended by the kit manufacturer.

2.6. Statistical methods

Raw data were returned to NIBSC for analysis to ensure, as far as possible, consistent calculation of results and to allow inter-laboratory comparison. All analysis was performed using EDQM's CombiStats software (Version 5.0). For all assays, the data for each test preparation were analysed separately against the reference preparation.

Parallel line analysis of log-transformed assay response against log dose was performed, selecting a linear section of the dose-response curve. Linearity was assessed visually, and parallelism assessed by looking at the ratio of slopes between the test and reference samples. Samples were considered non-parallel if the ratio of slopes fell outside of the range of 0.80 to 1.25.

All laboratory mean potency estimates shown are weighted or semi-weighted geometric mean (GM) estimates calculated according to the methods described in Ph. Eur. General Chapter 5.3 [11]. Semi-weighted means have been used where significant heterogeneity of assay estimates was detected ($p<0.05$). Overall mean potencies and confidence intervals are calculated as unweighted geometric mean values. Variability is expressed as a percentage using a geometric coefficient of variation (GCV) (GCV = {10s-1}×100% where s is the standard deviation of the log10 transformed potency estimates).

3. Results

3.1. Characterisation and quality control of freeze-dried samples

The freeze-drying of the samples was successful and each sample generated a homogenous pale yellow robust loose cakes as expected. The mean fill weights for the sera filled for this study ranged from 0.50 to 0.51 g (CV% 0.35 to 3.24), the mean dry weights ranged from 0.0382 to 0.0402 g (CV% 0.49 to 1.70), the residual moisture ranged from 0.26 to 1.21 % w/w (CV% 12.41 to 30.12) and the mean oxygen content was from 0.21 to 0.51 % w/w (CV% 17.86 to 79.00). The freeze-dried sera were retested at NIBSC and University of Turku before shipment to participating countries and the anti-PT titres were found to be close to the proposed values (Table 1).

3.2 Summary of data returned

Of the twenty-five laboratories that received samples, twenty-four completed questionnaires and submitted results by the relevant deadlines. All participants performed either an in-house or commercial ELISA, MIA with exception of Laboratory 16. Laboratory 16 performed the EQA using both in-house and a commercial ELISA as well as MIA. Thus a total of 26 datasets were returned for analysis. Six laboratories used their own in-house ELISA methods; commercial kits from six different manufacturers were used by eighteen laboratories; and two laboratories used MIAs. The different kit manufacturers are referred to as manufacturer A to F. Two different kits from manufacturer C were used and they are referred to as C-1 and C-2. Detailed assay information is presented in Annex 4a and b. The majority of laboratories that performed in-house ELISAs returned assay raw data allowing for recalculation at NIBSC using a common statistical analysis. Only Laboratory 4 submitted data from single point dilutions, which is not applicable for the common statistical analysis. The readings for the reference sera from Laboratory 6 were found to be non-linear and non-parallel. Of the 18 laboratories that performed assays using commercial ELISA kits, eight performed a range of dilutions and returned the raw data meaning the common analysis could be performed by NIBSC.

3.3. Estimates of sera samples anti-pertussis toxin IgG concentrations

The statistical analysis of data supplied by participants using in-house ELISA and MIA methods that could be analysed using parallel line analysis in NIBSC are presented in Table 2. For Laboratories 4 and 6, their calculated results are presented. As expected Sample A was found to be outside the response range of the reference or under detection limits for the majority of the assays and therefore has been omitted.

For the in-house ELISA assays the geometric mean (GM) for Sample B was found to be 43.01 IU/ml, Sample C was 23.71 IU/ml, Sample D 63.18 IU/ml, Sample E 83.85 IU/ml, Sample F 106.12 IU/ml and Sample G 140.13 IU/ml. One participant (Laboratory 14) initially found the titre for Sample D to be approximately twice the expected value, however, when they repeated the analysis using the second ampoule supplied in this study, concentrations closer to the expected values were obtained. The readings from the initial analysis were not used in subsequent calculations.

Results from commercial kits calculated by NIBSC using parallel line analysis (eight laboratories) are shown in Table 3, as well as participants' own calculated results from the remaining ten laboratories that performed single point assays and are therefore not suitable for parallel line analysis. Overall, all assays correctly identified Sample A as the negative control as indicated by the fact that this sample gave a non-parallel response/or less than 5 IU/ml.

Slightly lower GM estimates were found for commercial kits assays with Sample B: 42.52 IU/ml, Sample C: 27.96 IU/ml, Sample D 60.92 IU/ml, Sample E 76.80 IU/ml, Sample F 100.39 IU/ml and Sample G 125.42 IU/ml.

The overall GM of WHO Reference Reagent (Sample F), was found to be 106 IU/ml for in-house methods and 100 IU/ml for commercial kits.

The GCVs for the laboratories using in-house ELISA or MIA assays were 11–18% for the different samples (Table 2). For the laboratories using commercial kits the inter-laboratory GCVs ranged between 12–23% (Table 3) with the exception of Sample C which had a GCV of 70%. This was due to two participants (Laboratories 5 and 17) getting higher than expected readings for this sample.

Histograms of the GM for sera samples B to G from each replicate calculated at NIBSC using parallel line analysis against the WHO Reference Reagent (06/142) are presented in Figure 1. Figure 2 shows the histograms of the geometric means for samples B to G from the participants' own calculated results.

Table 2. Results of in-house ELISA and MIA assays calculated relative to the WHO Reference Reagent at NIBSC[a] or reported by participants

Laboratory	Source of Ag[b]	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Comments
4	In-house		34.18	18.91	66.72	92.32	117.43	136.17	Participant reported results using IHR
			47.2	22.74	64.75	79.9	90.86	151.21	
			51.45	26.96	69.61	114.92	122.26	157.96	
	GM		**43.62**	**22.63**	**67.00**	**94.64**	**109.26**	**148.16**	
6	GlaxoSmith		46	26	63	92	125	157	Participant reported results using IHR
	Kline		34	22	50	66	86	87	
			35	22	51	68	95	95	
			34	20	48	68	80	88	
			58	24	72	91	111	153	
			45	21	61	72	90	124	
	GM		**41**	**22**	**57**	**75**	**97**	**114**	
11	In-house		52.73	31.69	69.08	83.81	96.62	175.96	
			48.56	25.49	47.12	75.67	113.51	119.08	
			51.08	NP	62.41	72.20	132.93	141.91	
			39.40	28.51	63.44	96.12	148.26	199.71	
	GM		**43.98**	**28.84**	**59.29**	**83.44**	**108.48**	**161.68**	
14	In-house or Kaketsuken (Japan)		55.87	25.55	146.21[c]	100.01	98.10	177.54	
			54.09	22.73	151.85[c]	91.21	97.79	172.84	
			55.22	23.90	150.93[c]	95.43	97.63	171.86	
			ND	ND	68.74	ND	ND	ND	
			ND	ND	71.72	ND	ND	ND	
			ND	ND	73.44	ND	ND	ND	
	GM		**54.92**	**24.13**	**71.27**	**96.94**	**98.05**	**177.46**	
16a#	List Biological		35.92	22.60	49.30	63.98	98.42	127.44	
			34.89	22.93	49.82	75.80	111.07	126.43	
			33.79	23.22	49.51	63.87	100.59	117.91	
	GM		**34.58**	**22.77**	**49.51**	**67.50**	**101.74**	**122.94**	
16b	List Biological		31.87	17.24	75.10	102.20	97.96	124.65	
			39.04	20.79	68.74	83.90	101.69	146.59	
			38.05	18.92	59.16	80.46	100.62	125.08	
	GM		**37.25**	**19.32**	**65.48**	**89.91**	**100.79**	**134.59**	
23	GSK		39.33	26.18	59.21	85.55	149.10	141.65	
			44.32	26.43	64.36	75.86	89.25	107.11	
			36.39	26.66	58.89	75.86	78.41	99.38	
	GM		**40.07**	**26.41**	**60.31**	**79.36**	**102.52**	**115.30**	
25	Kaketsuken		49.49	23.19	71.32	83.72	130.12	165.55	Calculated with in-house aliquot of 06/142
	(Japan)		55.22	26.00	*	85.46	138.02	151.57	
			53.67	26.26	92.82	93.68	142.55	160.93	
	GM		**52.53**	**24.78**	**80.49**	**88.33**	**135.68**	**160.81**	
GM of laboratory GMs			43.01	23.71	63.18	83.85	106.12	140.13	
95% limits			(37.67–49.11)	(21.41–26.24)	(55.81–71.53)	(75.66–92.92)	(96.95–116.15)	(121.76–161.27)	
GCV			17%	13%	16%	13%	11%	18%	

[a] See statistic method section;
[b] All participants used purified PT as coating antigen.
[c] Not used in calculations due to technical fault with ampoule
GM Geometric mean
NP: non-parallel at 1% level
ND: not determined
**: removed due variability of test sample.*
 # Laboratory 16 provided two sets of results using ELISA and MIA, the respective results are therefore labelled as 16 a and 16 b

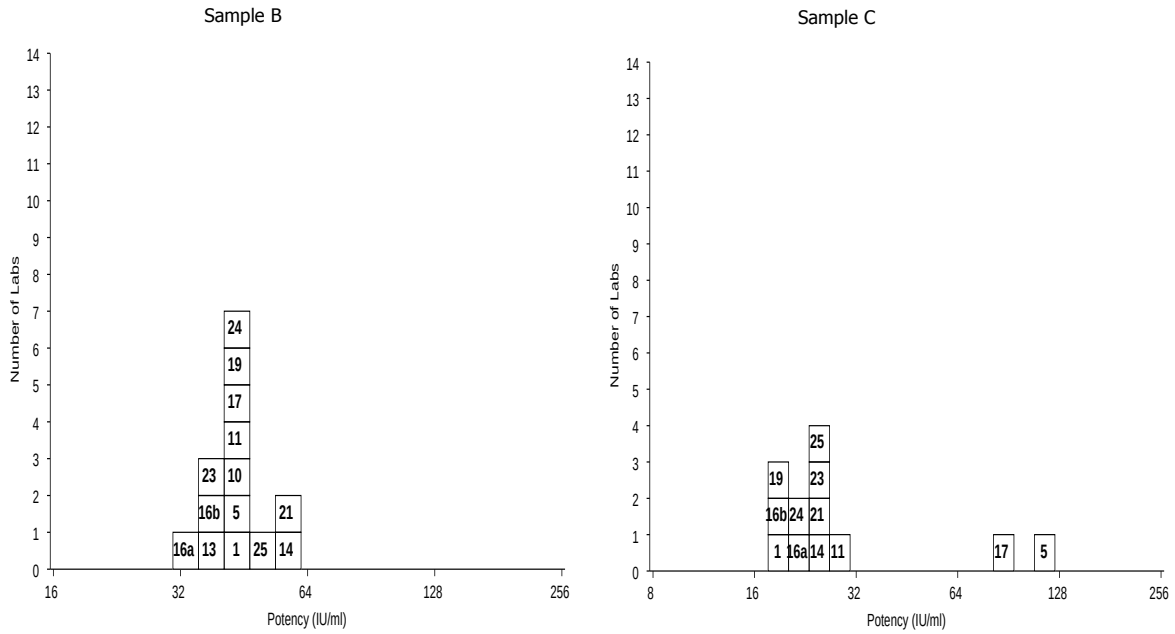
Table 3. Results of commercial kit based assays calculated relative to the WHO Reference Reagent at NIBSC[a] where possible or reported by participants

Laboratory	Kit manufacturer	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Comments
1	Manufacturer A		37.32	RR	53.18	70.36	116.80	137.53	1:100 down 4 wells
			41.81	RR	58.67	74.61	101.63	145.75	
			44.94	17.60	68.54	72.16	111.30	143.14	
	GM		40.77	17.60	59.73	72.27	106.52	143.20	
2	Manufacturer B	1.16	46.41	29.35	59.36	74.75	111.40	123.72	Single point with duplicates Participant reported results
		1.53	45.25	28.88	57.13	72.19	104.25	117.39	
		1.78	45.28	28.99	59.33	73.70	107.39	126.03	
	GM	1.47	45.64	29.07	58.60	73.54	107.64	122.32	
3	Manufacturer A	<5	43.00	17.00	55.00	85.67	91.67	126.00	Single point with triplicates Participant reported results
		<5	32.00	18.33	59.00	78.00	93.00	134.33	
		<5	47.67	16.33	60.00	70.67	87.00	108.67	
	GM		40.33	17.20	57.96	77.87	90.52	122.52	
5	Manufacturer C-1		45.58	110.95	68.59	75.31	106.55	111.08	1:100 down 4 wells in duplicate
			42.58	122.41	69.96	88.65	121.97	121.78	
			52.68	120.15	79.46	97.98	138.85	154.18	
	GM		46.58	117.77	72.18	86.62	121.47	127.48	
7	Manufacturer D	0	30	25	47	65	97	102	Single point with duplicates Participant reported results
		0	36	29	52	71	102	113	
		0	33	26	47	77	103	109	
	GM		32.91	26.61	48.61	70.83	100.63	107.90	
8	Manufacturer C-2	2	57	29	106	142	124	190	Single point Participant reported results
		3	61	32	108	148	133	199	
		3	61	34	107	160	130	200	
	GM	2.62	59.64	31.60	107.00	149.82	128.95	196.28	
9	Manufacturer B	42.0	29	63	76	113	113	141	1:100 down 2 wells, duplicate Participant reported results
		<5	41	31	63	75	93	119	
		<5	44	28	69	76	108	124	
	GM		37.40	37.96	69.13	86.36	104.31	127.66	
10	Manufacturer A		41.25	RR	53.82	69.58	89.01	110.55	1:100 down 4 wells in duplicate
			40.69	RR	54.83	64.86	NL	107.37	
			43.31	RR	57.93	65.94	87.52	109.13	
	GM		42.27		56.18	66.44	87.65	108.81	
12	Manufacturer E	<5	37.0	19.0	55.0	64.5	88.0	105.0	Single point with duplicates Participant reported results
		<5	34.5	18.0	43.0	60.5	81.0	104.5	
		<5	34.0	20.5	56.5	63.0	81.0	110.5	
	GM		35.14	19.14	51.12	62.64	83.27	106.63	
13	Manufacturer A		41.29	RR	60.30	71.45	103.04	134.01	1:100 down 4 wells in duplicate
			39.91	RR	59.25	72.16	90.81	126.29	
			33.77	RR	52.25	65.04	88.20	112.18	
	GM		39.24		58.61	70.31	94.87	126.22	
16c	Manufacturer B	1.07	41.51	26.96	56.98	77.32	103.04	119.04	Single point with duplicates Participant reported results
		1.90	48.03	32.31	60.33	79.44	102.33	144.08	
		1.91	43.79	28.21	55.55	77.34	104.43	116.57	
	GM	1.57	44.36	29.07	57.59	78.03	103.26	125.98	
17	Manufacturer C-1		42.53	86.11	56.51	59.17	101.20	100.40	1:100 down 4 wells in duplicate
			43.50	78.63	47.03	58.87	87.21	102.92	
			41.58	NL	49.40	52.95	89.51	89.06	
	GM		42.58	82.50	51.24	56.74	92.80	96.75	
18	Manufacturer B		43.08	27.53	65.43	75.03	102.58	128.38	Single point in quadruple Participant reported results
			38.10	24.23	52.78	62.78	92.08	99.53	
			47.95	30.53	64.68	77.70	107.55	122.33	
	GM		42.85	27.30	60.67	71.53	100.52	116.05	
19	Manufacturer A		42.72	19.30	49.36	76.21	99.06	121.24	1:100 down 5 wells

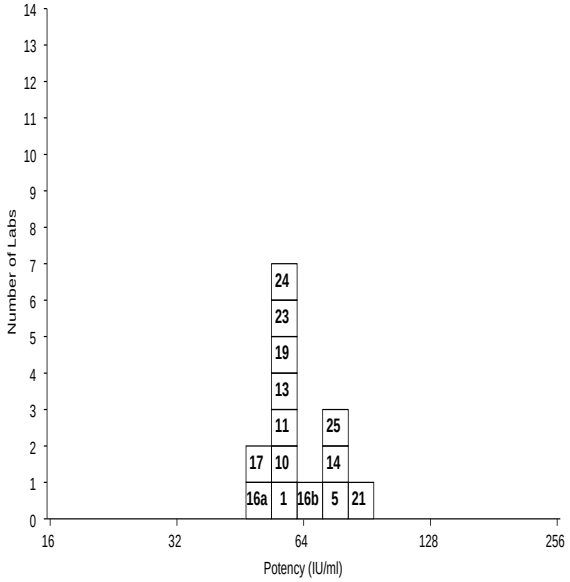
Laboratory	Kit manufacturer	Sample A	Sample B	Sample C	Sample D	Sample E	Sample F	Sample G	Comments
			41.05	16.92	58.38	74.96	99.77	117.42	in duplicate
			40.92	17.78	58.37	61.23	85.87	122.12	
	GM		41.76	18.30	54.98	70.81	96.20	120.93	
20	Manufacturer A	0.03	46.29	21.40	71.50	83.57	116.65	149.63	1:101 down 3 wells Participant reported results
		0.03	65.78	21.24	63.53	83.88	115.87	135.22	
		0.02	60.86	18.72	61.53	82.60	112.86	130.66	
	GM	0.02	57.01	20.42	65.38	83.35	115.12	138.27	
21	Manufacturer F		51.20	22.68	77.44	79.69	81.92	136.25	1:100 down 5 wells
			66.52	27.52	101.63	132.37	127.31	225.88	
			57.20	23.49	72.52	96.43	84.26	154.81	
	GM		57.26	24.04	82.00	99.99	95.05	169.26	
22	Manufacturer B	2.81	41.70	27.50	52.90	55.07	89.33	102.33	Single point with duplicates Participant reported results
		2.57	36.30	24.60	46.37	54.83	84.87	116.33	
		2.35	41.77	25.83	55.07	72.07	113.00	125.67	
	GM	2.57	39.84	25.95	51.31	60.15	94.98	114.37	
24	Manufacturer E		43.88	21.94	RR	74.19	106.41	105.06	1:100 down 6 wells in duplicate
			42.67	20.75	58.78	75.05	NL	122.73	
			39.67	22.85	57.26	85.41	100.44	107.50	
	GM		41.72	22.07	57.47	78.78	101.81	111.08	
GM of lab GMs			42.52	27.96	60.92	76.80	100.39	125.42	
95% limits			(39.18–46.15)	(21.30–36.72)	(55.68–66.65)	(69.39–85.01)	(95.23–105.83)	(115.83–135.82)	
GCV			19%	70%	21%	23%	12%	18%	

[a] see statistic method section
[b] All kits used purified PT as coating antigen except one kit from manufacturer C-1 which used a mixture of PT and FHA as coating antigen (used by Laboratories 5 and 17).
GM: Geometric mean
NL: non-linear at 1% level
NP: non-parallel at 1% level.
RR: outside the response range for the reference

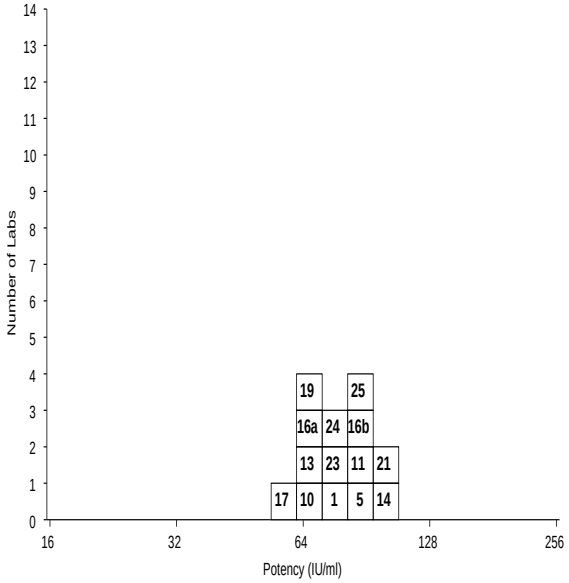
Figure 1. Histograms of geometric mean estimates (IU/ml) for each participant for sera samples B to G calculated relative to the WHO Reference Reagent at NIBSC.



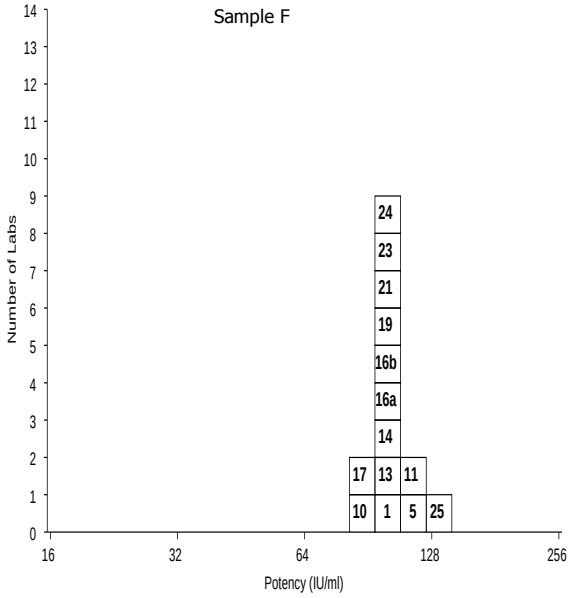
Sample D



Sample E



Sample F



Sample G

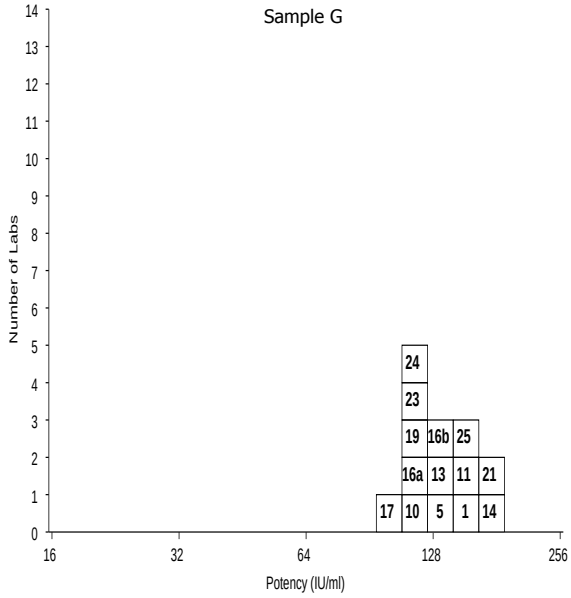
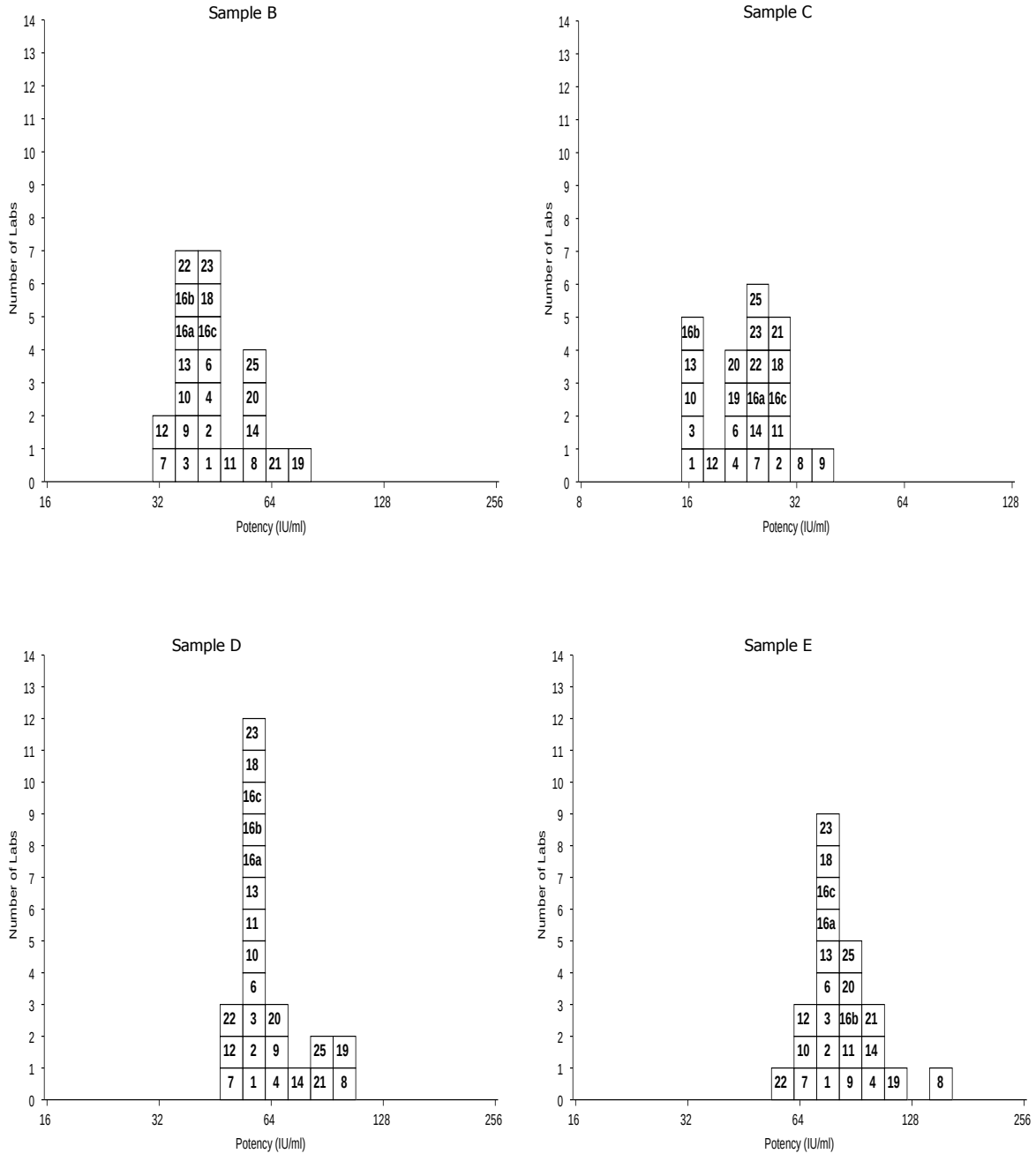
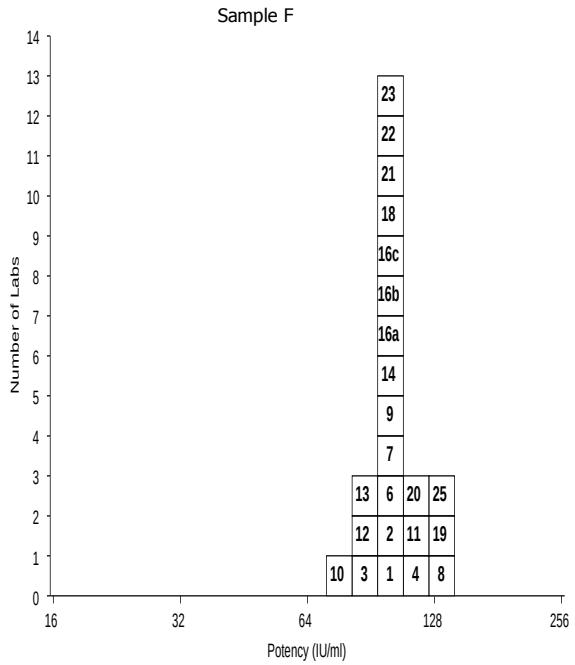
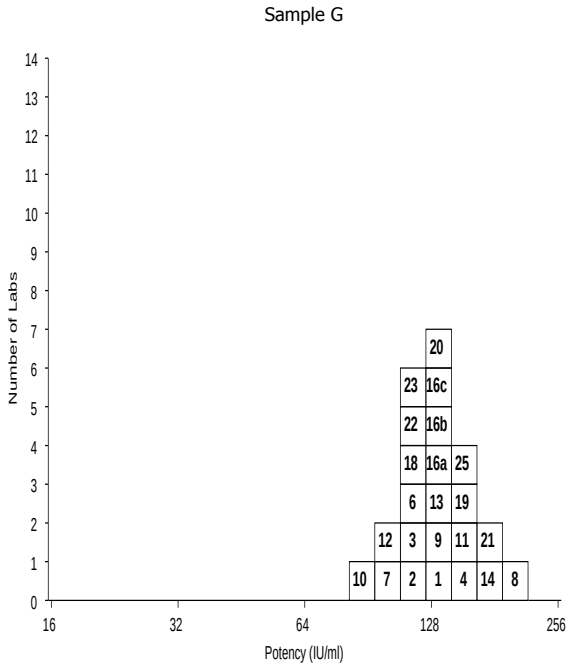


Figure 2. Histograms of geometric mean estimates (IU/ml) for each participant's results for sera samples B to G calculated relative to routine reference sera.







3.4 Ranking of sera samples in increasing IgG concentrations.

Another aim of the present EQA was to assess the ability of the participants to quantify the anti-PT IgG concentration of blinded sera samples and to rank them by increasing concentrations. Table 4 shows the order in which the participants ranked the panel according to increasing anti-PT IgG concentrations from data that could be calculated using parallel line analysis by NIBSC and also participants' own calculations. Of the 26 assay methods retuned to NIBSC, 22 could distinguish the samples in the panel and ranked the samples in the same order. The four participants that generated different ranking orders are highlighted in red. Laboratories 5 and 17 generated results which had Sample C much higher than expected and higher than Samples B, D and E. Laboratories 8 and 21 found Sample E to be higher than Sample F.

Table 4. Sera samples ranked in order of increasing anti-PT IgG concentrations based on both results calculated at NIBSC using parallel line analysis (where applicable) and final concentrations determined by the participants using their routine in-house methods.

		Ranking by increasing anti-PT IgG concentration						
Laboratory	**Method of calculation**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
	Expected rank order	A	C	B	D	E	F	G
1	Both NIBSC and in-house	A	C	B	D	E	F	G
2	Participant's in-house results[2]	A	C	B	D	E	F	G
3	Participant's in-house results[2]	A	C	B	D	E	F	G
4	Participant's in-house results[2]	A	C	B	D	E	F	G
5	Parallel line analysis at NIBSC[1]	A	B	D	E	C	F	G
6	Both NIBSC and in-house	A	C	B	D	E	F	G
7	Participant's in-house results[2]	A	C	B	D	E	F	G
8	Participant's in-house results[2]	A	C	B	D	F	E	G
9	Participant's in-house results[2]	A	C	B	D	E	F	G
10	Both NIBSC and in-house	A	C	B	D	E	F	G
11	Both NIBSC and in-house	A	C	B	D	E	F	G
12	Participant's in-house results[2]	A	C	B	D	E	F	G
13	Both NIBSC and in-house	A	C	B	D	E	F	G

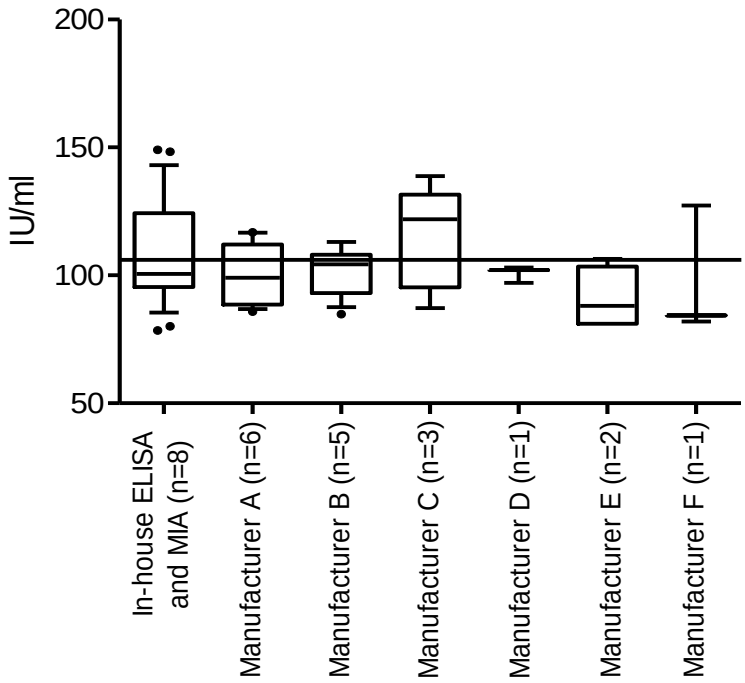
14	Both NIBSC and in-house	A	C	B	D	E	F	G
15	Did not return results							
16a	Both NIBSC and in-house	A	C	B	D	E	F	G
16b	Both NIBSC and in-house	A	C	B	D	E	F	G
16c	Participant's in-house results[2]	A	C	B	D	E	F	G
17	Parallel line analysis at NIBSC[1]	A	B	D	E	C	F	G
18	Participant's in-house results[2]	A	C	B	D	E	F	G
19	Both NIBSC and in-house	A	C	B	D	E	F	G
20	Participant's in-house results[2]	A	C	B	D	E	F	G
21	Both NIBSC and in-house	A	C	B	D	F	E	G
22	Participant's in-house results[2]	A	C	B	D	E	F	G
23	Both NIBSC and in-house	A	C	B	D	E	F	G
24	Parallel line analysis at NIBSC[1]	A	C	B	D	E	F	G
25	Both NIBSC and in-house	A	C	B	D	E	F	G

[1] Participant did not return calculated values for each sera sample or values weren't in IU/ml
[2] Raw data not suitable for parallel line analysis

3.5 Comparison of values obtained for the WHO reference sample, sample F.

Sample F in the present study is the current WHO Reference Reagent Pertussis Antiserum (Human) 1st Reference Reagent (NIBSC code: 06/142) and was blinded in this EQA. A previous collaborative study calibrated the anti-PT IgG for this standard at 106 IU/ml when it was being established [12]. Figure 3 compares the different ELISA and MIA methods that returned raw data that could be analysed at NIBSC, based on type of method used and results calculated by the participants when the model could not be used. The straight line at 106 IU/ml represents the expected value, the box shows the 25–75% interval (where applicable), the whiskers show the 10–90% interval, the dots are outliers and the solid line represents the mean. The means for the in-house methods and all the kits were close to the expected value of 106 IU/ml. Values ranged from 81 IU/ml to 149 IU/ml.

Figure 3. Box plots of the quantitative results of anti PT-IgG ELISAs for Sample F calculated against the Reference Reagent or expressed as international units (IU) by in-house calculation.



The straight line at 106 IU/ml represents the expected value, the box shows the 25-75% interval (where applicable), the whiskers show the 10-90% interval, the dots are outliers and the solid line represents the mean. n= number of laboratories.

4. Discussion

ELISAs for measuring antibodies to pertussis antigens have been widely employed in acellular vaccine clinical trials and in sero-epidemiological studies [3,13]. They have also become more popular for diagnostic purposes [7]. While in-house ELISA methods have been used for years, the number of diagnostic laboratories using different commercial kits has recently increased [6]. The ability of NRLs from EU/EEA Member States to correctly perform laboratory serodiagnostic tests for pertussis is the main focus of this EQA. The scheme helped to identify methodologies in need of improvement and areas for training where particular laboratories could improve their methods, procedures and global performance.

The primary aim of the present exercise was to evaluate seven sera samples to determine concentrations of anti-PT IgG against a reference sera preparation using immunoassays. Through this EQA exercise, it was possible to ascertain how well the assays currently used by different diagnostic laboratories in Europe could differentiate the sera samples and quantify the concentrations of anti-PT IgG. In the present study, unlike the previous EQA, there were no losses in concentration of the sera panel identified after re-calcification and freeze-drying the samples. Once again, all laboratories were able to distinguish sample A (estimated concentration of IgG-PT < 2 IU/ml) from the other samples with higher anti-PT IgG concentrations. The majority of the participants obtained results close to the expected values for the remaining samples in the panel and 23 (88%) of the 26 assay methods ranked the samples in the expected order (Table 4). This is similar to the previous study in which 91% of 21 participating laboratories ranked the samples in the same order, and it does indicate the satisfactory performance of these assays.

In the previous EQA, ten (45%) laboratories used in-house ELISA or MIA methods and twelve used commercial kits (55%). In the present study, eight laboratories used in-house methods, while 18 (69%) laboratories used commercial kits indicating the growing use of commercial kits in diagnostic laboratories. In the previous EQA, kits from six manufacturers were used by the participants. Six commercial kits were also employed in the present study, but kits from one manufacturer used in the previous study were not included in the present study and a kit from manufacturer E was used instead. Previous studies also indicated that the use of commercial kits, which are coated with a mixture of antigens and not just purified PT, had insufficient power to distinguish preparations with high and low anti-PT IgG preparations, and were found to be less accurate than methods that used purified PT [6, 7, 8, 9]. In this EQA, the majority of participants used purified PT as the coating antigen (24 (92%) of the 26 methods). However, one manufacturer, manufacturer C, produces different types of kits with both purified PT (C-2) and a mixture of PT and FHA (C-1) as coating antigens. Two laboratories (laboratories 5 and 17) used the latter kit. Laboratory 5 found that the concentration of sample C was approximately five times the expected value and therefore did not rank the samples in the expected order. However, the other samples were closer to the expected values. Laboratory 17 also found sample C to have a higher concentration than expected with one replicate also being non-linear. The other samples had lower than the expected values and lower values than the overall geometric means. If the high values for sample C are removed, the geometric mean falls from 27.96 IU/ml to 23.15 (17.05–31.44) IU/ml. The values for sample F (the WHO Reference Reagent, 106 IU/ml) calculated for laboratories 5 and 17 were 121 and 93 IU/ml, respectively. This again indicated the disadvantage of using mixed coating antigens.

Six laboratories used kits from manufacturer A, which use purified PT as coating antigen. The results obtained were consistent with the geometric means except for laboratories 10 and 13 where results were lower than the overall geometric means. The results from laboratory 10 for sample C were also found to be outside the response range for the reference. For sample F, they obtained a range from 88 (laboratory 10) to 115 IU/ml (laboratory 20).

The kit produced by manufacturer B also uses purified PT as coating antigen and was used by five laboratories in the present study (laboratories 2, 9, 16c, 18 and 22). Overall, the results obtained from each participant were close to the expected values, but laboratory 9 found sample C to be almost twice the overall mean. Laboratories that used this kit obtained a range of 95 (laboratory 22) to 108 IU/ml (laboratory 2) for sample F.

The kit made by manufacturer E was used by laboratories 12 and 24. The former found the concentrations of the samples to be lower than expected except for A and C. The results from laboratory 24 were close to the overall geometric means for all the samples. For sample F, laboratory 12 found the concentration to be 83 IU/ml while laboratory 24 obtained a value of 102 IU/ml although one replicate for this sample was non-linear.

The kits produced by manufacturer D and manufacturer F were each used by a single participant (laboratories 7 and 21, respectively). The former found that concentrations were slightly lower than expected for samples D and G. The latter's results were a little higher than the overall geometric mean for samples B, D, E and G. The calculated results for sample F were 101 and 107 IU/ml, respectively.

In the present study, 24 (92%) of all assay methods used purified PT as coating antigen while two methods used a mixture of PT and FHA for coating. This is an increase from the previous EQA when 78% of the methods used purified PT as the coating antigen [6]. An even earlier EQA found that 13 (65%) of 20 participants used purified PT while the remaining labs used PT plus FHA (n=5), or PT plus FHA with LPS (n=1) or whole cell lysate (n=1) (7). Taken together, these three studies indicate that more laboratories are beginning to follow the ECDC recommendations [8] if they had not already done so.

There were five different sources of purified PT used by the participants in the present study for in-house ELISAs and MIAs (Table 2), and results were independent of these sources. There was a difference in the variability of in-house methods compared to commercial kits. The GCV for in-house methods ranged from 11–18% while the range for commercial kits was 12–70%. The GCV of 70% in the kit assays was also due to two unexpectedly high readings for sample C (laboratories 5 and 17). Both of these participants used manufacturer C-1 kits which have plates coated with PT and FHA. The previous EQA [6] found that the variability in the kit results was higher than the in-house methods, but such a wide difference was not seen here. This may suggest that standardisation of commercial kits suggested in the previous EQA is taking place leading to improved comparability and harmonisation.

Pertussis Antiserum (human) 1st WHO Reference Reagent (06/142) has been assigned unitage of 106 IU/ampoule of anti-PT IgG in an international collaborative study [12]. Of the 26 assay methods returned, 21 also reported calculated values for the 1st RR using their routine methods and references, of which six were in-house methods. The overall GM of these laboratory in-house methods was found to be 112 IU/ml (Annex 5). The range of values obtained was 81 to 216 IU/ml. The high value was from one assay performed at laboratory 4 while the other two replicates from this participant were 99 IU/ml. When this result is removed, the range is 81 to 127 IU/ml. This may suggest that in-house references used by these laboratories are reasonably calibrated in terms of the 1st IS. The results from the 15 laboratories that used commercial kits (all of which calculated the concentration of 06/142 in IU/ml) were found to range from 84 to 159 IU/ml. The overall geometric mean for kits was 110 IU/ml. In the previous EQA, the results returned in the questionnaires indicated that although all commercial kits are CE marked in terms of quality, the information on reference sera, e.g. their concentration in relation to the international units/or the US Lot 3 were not clearly stated [6]. In the present study, however, the majority of the reference sera in the kits were calibrated in IU/ml using the WHO International Standard (06/140) (Annex 4b), suggesting that standardisation of commercial ELISA kits for pertussis diagnosis is progressing. Since many routine laboratories perform serological diagnosis by commercial kits, guidelines on how to evaluate commercial kits may provide useful information for these diagnostic laboratories.

Overall in comparing the results of this EQA to the previous one, it seems that there is a trend towards more laboratories using commercial kits for the serodiagnosis of pertussis. It also seems that the recommendations made in previous studies are being followed as a greater number of kits now use purified pertussis toxin as coating antigen and reference sera that are calibrated in IU/ml using international reference sera such as the WHO International Standard (06/140) or US Lot 3.

5. Recommendations

The present study indicates that there is an increasing trend towards using commercial kits for serodiagnosis of pertussis. Based on the results of this study, laboratories currently using or considering switching to commercial kits should consider a number of factors. Firstly, the ELISA plates in kits should be coated with purified PT only and reference serum should be calibrated in IU/ml using one of the international standards. The kits should also be CE-marked to conform with appropriate regulations.

In the future, more laboratories may use MIA methods, as antibodies against multiple antigens can be analysed in a shorter timeframe and with less sample than with conventional ELISA. However, the recommendations for MIAs remain the same as those for ELISA in relation to coating antigen and reference sera.

The results of the present EQA were more satisfactory than the previous one [6] indicating the benefits of regular EQA studies with a serum panel of different anti-PT IgG concentrations for the validation and standardisation of serological diagnosis of pertussis in Europe. It is difficult to set a fixed timeframe for when EQAs should be performed, but they should be considered when changes are made to routine tests, such as switching from in-house ELISA to using commercial kits or implementing a MIA. The serum panel used in the present study will be available from NIBSC and can be used to determine the performance of new or existing assay methods.

Annexes 5a and 5b highlight that there are a large number of cut-off values used to determine the presence of recent infection. It may be useful for the pertussis diagnostic community to decide on uniform cut-off values in IU/ml. Work package 5 ('Propose the reference method of anti-PT ELISA and MIA and the possible cut-off for serological diagnosis of pertussis in Europe, in order to standardise and harmonise the methods in use') of the current ECDC contract is tasked with achieving this.

Overall, the present EQA study found that using a range of dilutions and comparing sera samples to the Pertussis Antiserum (human) 1st WHO Reference Reagent (06/142) on plates only coated with purified pertussis toxin facilitated accurate determination of anti-PT IgG concentrations in IU/ml. This corresponds to observations made previously. Therefore, the previous recommendation should be reinforced that only purified PT is used in immunoassays whether for in-house ELISA, commercial kits or multiplex immunoassays for serological testing of anti-Pt IgG in human sera. Also, a reference serum should be included in each assay and it should be calibrated in IU/ml using one of the international standards.

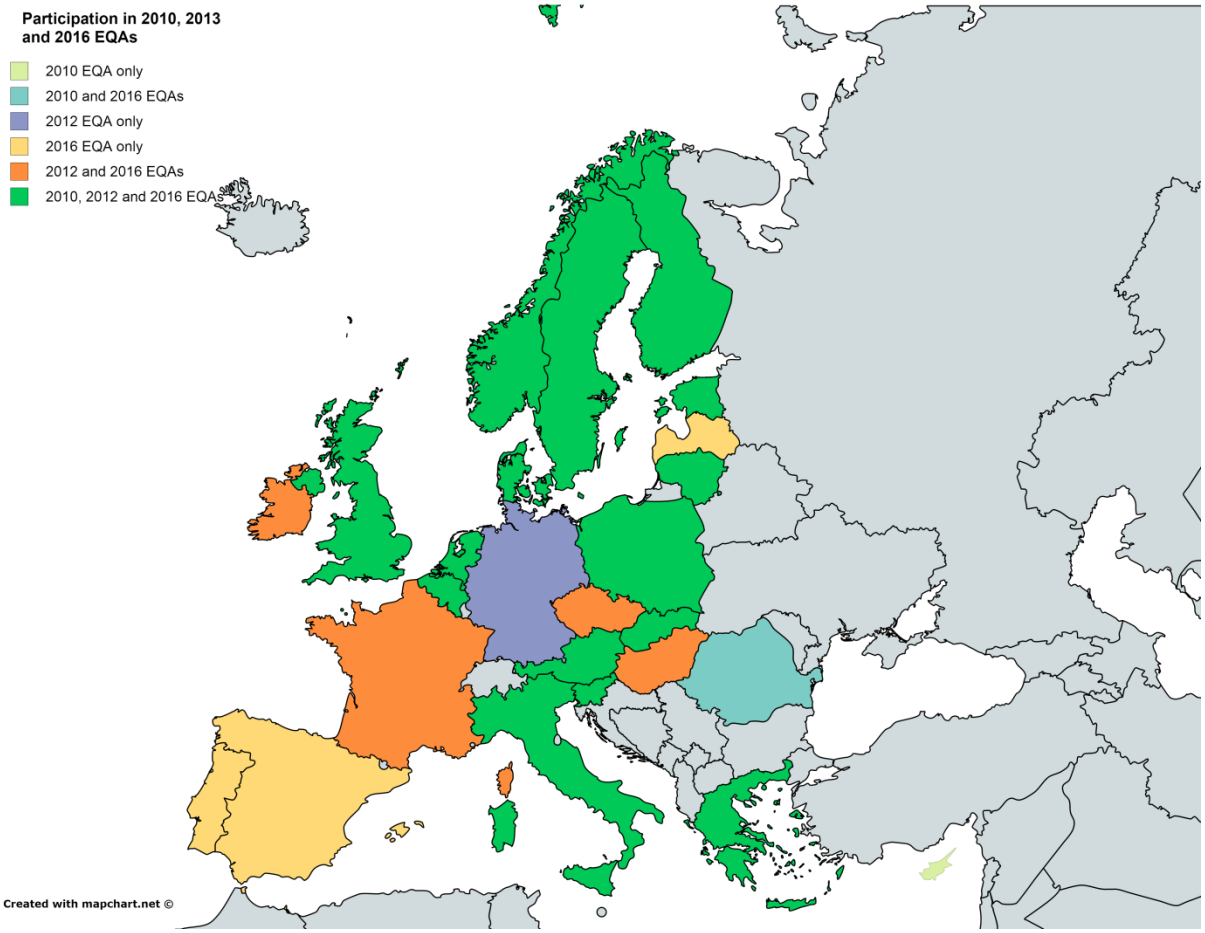
References

1. Gabutti G, Rota MC. Pertussis: A review of disease epidemiology worldwide and in Italy. Int J Environ Res Public Health. 2012 Dec;9(12):4626-38.
2. Clark TA. Changing pertussis epidemiology: everything old is new again. J Infect Dis 2014;209:978-81.
3. Barkoff AM, Gröndahl-Yli-Hannuksela K, He Q. Seroprevalence studies of pertussis: what we learned in different immunized populations. Pathog Dis 2015;73(7). pii: ftv050.
4. Esposito S, Principi N; European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Vaccine Study Group (EVASG). Immunization against pertussis in adolescents and adults. Clin Microbiol Infect. 2016 Apr 26. pii: S1198-743X(16)00010-0. doi: 10.1016/j.cmi.2016.01.003. [Epub ahead of print]
5. European Parliament and of the Council. Decision No 2119/98/EC of the European Parliament and of the Council of 24 September 1998 setting up a network for the epidemiological surveillance and control of communicable diseases in the Community. 1998. Available here: http://eur-lex.europa.eu/eli/dec/1998/2119/oj
6. European Centre for Disease Prevention and Control. External quality assurance scheme for *Bordetella pertussis* serology 2013. Stockholm: ECDC; 2014
7. Xing D, Newland P, Rigsby P, Hockley J, Markey K, He Q. EUVAC-Net collaborative study evaluation and standardization of serology for diagnosis of pertussis. J Immunol Methods. 2011; 372:137–145.
8. European Centre for Disease Prevention and Control. Guidance and Protocol for the serological diagnosis of human infection with *Bordetella pertussis*. Stockholm: ECDC; 2012.
9. Guiso N, Berbers G, Fry NK, He Q, Riffelmann M, Wirsing von König CH. What to do and what not to do in serological diagnosis of pertussis: recommendations from EU reference laboratories. Eur J Clin Microbiol Infect Dis.2011;30:307–12.
10. Riffelmann M, Hunfeld KP, Müller I, Xing D, Kennerknecht N, Wirsing von König CH. External quality assessment of pertussis serology in Germany. Eur J Clin Microbiol Infect Dis. 2013 Mar;32(3):421-3.
11. European Directorate for the Quality of Medicines and Healthcare. Chapter 5.3. Statistical analysis of results of biological assays and tests. In: European Pharmacopoeia, 2015
12. Xing D, Wirsing von König CH, Newland P, Riffelmann M, Meade B, Corbel M and Gaines-Das R. International collaborative study: Evaluation of proposed International Standard for pertussis antiserum (human)" WHO/BS/08.2083
13. Gustafsson L, Hallander H, Olin P, Reizenstein E and Storsaetter J. A controlled trial of a two-component acellular, a five-component acellular and a whole-cell pertussis vaccine. NEJM. 1996; 334 (6): 349-355.

Annex 1a. List of participants in this EQA

Country	Contact person	Laboratory/Institution
Austria	Joanna Jasinska	Institut fur Spezifische Prophylaxe und Tropenmedizin, Medical University of Vienna
Belgium	Kris Huygen	National Reference Centres for *Bordetella* and Toxigenic *Corynebacteria*, Service Immunology, Scientific Institute of Public Health (WIV-ISP)
Czech Republic	Jana Zavadilova	National Reference Laboratory for Pertussis and Diphtheria, National Institute of Public Health
Denmark	Tine Dalby Charlotte Sværke Jørgensen Tina Ceper	Dept. of Microbiological Surveillance and Research and Dept. of Microbiological Diagnostics and Virologi, Statens Serum Institut
Estonia	Liidia Dotsenko Svetlana Rudõka	Laboratory of Communicable Diseases, Health Board, Tallinn
Finland	Qiushui He Alex-Mikael Barkoff	Department of Medical Microbiology and Immunology, University of Turku
France	Sophie Guillot Benoit Garin	Centre National de Reference de la Coqueluche et autres bordetelloses, Institut Pasteur de Paris
Greece	Petridou Evangelia	Serology - Microbiology Department, "Aghia Sophia" Athens Children's Hospital
Hungary	Ildiko Paluska Ferencz	Legionella - Bordetella Laboratory, Hungarian National Center for Epidemiology
Ireland	Adele Habington	Molecular Laboratory, Microbiology Department, Our Lady's Children's Hospital, Crumlin
Italy	Clara Maria Ausiello Giorgio Fedele Pasqualina Leone	Department of Infectious, Parasitic, and Immune-mediated Diseases, Istituto Superiore di Sanità
Latvia	Jelena Galajeva	Riga East University Hospital, Latvian Centre of Infectious Diseases, National Microbiology Reference Laboratory
Lithuania	Vilnele Lipnickiene Algirdas Griškevicius	National Public Health Surveillance Laboratory
Netherlands	Guy Berbers Pieter van Gageldonk Kristin Kremer	Centre for Infectious Disease Control, National Institute for Public Health and the Environment (RIVM).
Norway	Tove Herstad Aase Audun	Norwegian Institute of Public Health
Poland	Waldemar Rastawicki	Bacteriology Department , National Public Health Institute-National Institute of Hygiene
Portugal	Maria Augusta Santos	LNR Bordetella-National Institute Health Dr. Ricardo Jorge
Romania	Georgeta Cristina Oprea	"Cantacuzino" National Institute for Research (Romania) – Vaccine Preventable Diseases Laboratory (*Bordetella pertussis* National Reference Center)
Slovakia	Lucia Mad'arová	Regional Authority of Public Health Banská Bystrica
Slovenia	Tamara Kastrin Karmen Cerne	National Laboratory of Health, Environment and Food
Spain	Fernando de Ory Mª Del Pilar Balfagon Sierra	Centro Nacional de Microbiología, Insituto de Salud Carlos III
Sweden	Lena Wehlin Margaretha Ljungman	The Public Health Agency of Sweden
United Kingdom	Diane Lindsay Alistair Brown	Scottish Microbiology Reference Laboratory
	Norman K. Fry John Duncan David Litt	Bacteriology Reference Department (RVPBRU), Public Health England

Annex 1b. Map of participants in previous EQAs



Annex 1c. Summary of EQAs on pertussis serology organised through ECDC

Year	Organising laboratory	Number of participating countries	Published reference
2010	NIBSC, UK	17	Xing et al 2011 (7)
2012	NIBSC, UK	20	ECDC 2014 (6)
2016	NIBSC, UK	23	In press

Annex 2. The EUPert-LabNet *Bordetella pertussis* serology EQA questionnaire

Reply Form

(Please return the form before **20 Nov 2015)**

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 serological diagnostic assay(s) for pertussis?

Yes □ No □

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

** tick 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

ELISA for IgG: anti-PT Yes □ No □

Others Yes □ No □ (if yes, please specify)__IgM, IgA_____

How is the assay validated? In-house □

Other □

Please specify:_commercial assay

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 X□

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 advised to take note of the disclaimers in the 'Instructions for Use' which accompany the samples and of the prohibitions against (i) use in humans (ii) further transfer and (iii) use for commercial purposes. They are also requested not to publish or circulate information concerning this study without the prior agreement of the consortium.*

Annex 3. The EUPert-LabNet *Bordetella pertussis* serology EQA: study protocol and results submission form

EQA Scheme for the Laboratory Diagnostics of Pertussis

Study protocol for laboratories:

Participants

Laboratories currently performing serological assays for diagnosis of pertussis by measuring antibodies to pertussis toxin

The aims of the study are:

- to assess current laboratory performance of serological assays for pertussis
- to compare in-house reference preparations that are currently used by participants for the serological assay/ or reference preparations provided in commercial kits
- to identify needs for standardisation and training in serological assays

Materials

The set of materials received by participants contains a panel of eight sera preparations containing seven defined but blinded concentrations of anti-pertussis toxin IgG (coded by letter). Also included is the Pertussis Antiserum (Human) 1st WHO Reference Reagent (06/142) for use as a reference to determine the anti-PT IgG concentrations.

Antiserum preparations:

Vials of freeze dried sera should be stored at -20°C.

The panel should be reconstituted with the following volumes of sterile distilled water.

Sample	Volume dH_2O (mL)
Pertussis Antiserum (Human) 1st WHO Reference Reagent (06/142)	1.0
Sample A	0.5
Sample B	0.5
Sample C	0.5
Sample D	0.5
Sample E	0.5
Sample F	1.0
Sample G	0.5

Samples should be divided in to aliquots and stored at -20°C. The aliquots should be used only once as freeze-thawing is not recommended.

In our study, we found an initial dilution at approximately 1/100 of the reconstituted Pertussis Antiserum (Human) 1st WHO Reference Reagent (06/142) and Samples A to G in the first row in IgG ELISA assays followed by ½ dilutions to be satisfactory.

However, individual laboratories may perform a pilot study to choose the suitable dilution for theses samples under their own experimental conditions.

Others:

Participants will be encouraged to include their in-house reference serum and antigen /or reference serum/antigen provided by commercial kits in the assays. (See details in the Methods section).

Methods

The primary goal of the study is to determine the anti-PT IgG concentrations of the sera preparations provided by ELISA.

For laboratories using their own in-house ELISA:

The participating laboratories are asked to coat ELISA plates using their own in-house sourced pertussis toxin antigen and perform a minimum of three independent assays on the eight serum preparations. Participants are also

asked to include their in-house reference serum (IHR) in the assay. The three independent assays should be run on three different days. In each assay, dilution curves for each preparation should have at least 2 or 3 replicates per assay and preferably each dilution curve should include at least four doses in the linear region. All preparations should be included in each assay. As far as possible each plate should also include the appropriate IHR if applicable.

Laboratories should use their own methodology, reagents and calculation methods, include their in-house references and controls, and use assay runs that meet their internal validity criteria.

For laboratories using commercial ELISA kits:

The participating laboratories are asked to perform a minimum of three independent assays on the panel of eight sera preparations provided in this study using the commercial ELISA kit that is routinely used in their laboratory. Participants should include the reference serum provided in the kit in the assay. The three independent assays should be run on three different days. In each assay, dilution curves for each preparation should have at least two replicates per assay and preferably each dilution curve should include at least four doses in the linear region.

For the assays using commercial kits, laboratories should use the methodology and validity criteria recommended by the kit manufacturer.

Collection and analysis of data:

NIBSC will be responsible for the collection and analysis of data submitted by the participating laboratories. Samples are available for shipment on 22 Feb 2016. Laboratories are requested to schedule sample analysis so that all results are ready for submission by 22 April 2016. Participants are required to supply all raw data to NIBSC for analysis using common methods. Participants are also encouraged to submit their results calculated by their own methods. At the NIBSC, data will be collected, coded and entered into a database for analysis. Laboratories will be identified by a code to maintain confidentiality.

In order that data can be efficiently analysed and that transcription errors are minimised, it is requested that data are returned in an **electronic format,** such as Excel. We recognise that participants will have different plate layouts and plate readers which they use routinely, and that these may not be readily reconfigured. To accommodate this, the data can be accepted in a variety of formats.

It is very important that the data submitted are clearly annotated and that the plate layout, initial dilutions and dilution factors are clearly indicated.

We have attached an Excel file that may be used to return data if it is suitable for your data format however it is not compulsory to use it or it can be modified to suit. Data as text files, in Excel spreadsheets, or incorporated into Word documents can be readily used. Laboratories are also asked to fill in Tables 1 and 2 describing their routine set up of the ELISAs.

Please also use Table 3 to show your plate layouts, please do this in addition to annotating your raw data. Table 4 is for use only by participants who score their results as positive and negative.

Please complete and return all relevant tables.

Legal/ethical factors

Participants are responsible for their own legal/ethical issues according to the regulations in their country for handling of human samples.

All laboratories participating in the study will be fully acknowledged. In the report, where assay results are attributed to the various laboratories, each laboratory will be identified only by a code number. Each participant will be told the identity of their own code number alone, so that they alone can identify which set of assay results they contributed.

To be completed for each assay

EQA scheme for the laboratory diagnostics of pertussis

Table 1: Information Form (a)

For laboratories who use own in-house ELISA

Name of Laboratory:-

Antigen information:	
Pertussis toxin antigen (e.g. mixture with other antigen(s) /or purified PT) If is mixture, please specify Sources e.g. in-house or/commercial If it has a commercial source, please specify	
Is any stabiliser used in the antigen solution (if yes, please specify)	
Diluent and pH used for coating antigen	
Conjugate information	
Type of conjugates for **IgG** assay, please specify the source of the conjugates)	
In-house reference antiserum	
Source	
Calibration against and unitage/concentration assigned	
Diluent used for anti-sera and pH of the diluent	
Validity criteria used	

To be completed for each assay

EQA scheme for the laboratory diagnostics of pertussis

Table 1: Information Form (b)

For laboratories who use commercial ELISA kits

Name of Laboratory:-

Commercial kit information:	
Manufacturer name	
Product code number	
Antigen information	
Coating antigen information e.g. whole cell suspension, purified PT, PT+FHA, other mixture	
Reference antiserum provided by the kit	
Is there any unitage for the reference anti-serum? If yes, please specify	
Calibration against which standard?	
If positive control and negative control included in the kit?	
Validity criteria stated in the kit? **If yes, please specify**	
If the Kit provides quantitative results?	

To be completed for each assay

EQA scheme for the laboratory diagnostics of pertussis

Table 2. Assay Sheet for laboratories

Name of Laboratory

Antigen: Own sourced/commercial kit PT *

Sample Code	Assay Number (or assay date)								
	1			2			3		
	Reconstitution volume per Ampoule if applicable	Initial Dilution in 1st row	Dilution factor through the plate	Reconstitution volume per Ampoule if applicable	Initial Dilution in 1st row	Dilution factor through the plate	Reconstitution volume per Ampoule if applicable	Initial Dilution in 1st row	Dilution factor through the plate
06/142									
A									
B									
C									
D									
E									
F									
G									
IHR/Kit ref.									
Please add more rows if required									

**Please delete as appropriate*
IHR= In-house reference

If more than 3 assays performed, please photocopy this sheet and change the assay number accordingly.

To be completed for each assay^

EQA scheme for the laboratory diagnostics of pertussis

Table 3: Plate layout for laboratories

(Please use this sheet to show sample layout and dilutions made down the plate.)

Name of Laboratory:

Antigen:-Own sourced/commercial kit PT *

Assay Number/Date :

Please input sample name and dilution into each row and column if applicable.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

^Please make copies of this form and use to show the layout for all plates used in each independent assay.

To be completed for each assay^

EQA scheme for the laboratory diagnostics of pertussis

Table 4: Assay Sheet for positive and negative results

Name of Laboratory:-

Antigen:-Own sourced/commercial kit PT *

Please input sample name and dilution into each row and column if applicable.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

^Please copy this sheet and fill in for each assay.

Annex 4a. The EUPert-LabNet *Bordetella pertussis* serology EQA: Information from participants that used in-house ELISA and MIA methods.

Lab Code	Number of Assays	Information (in house antigen)	Antigen stabiliser	Diluent	Conjugate	In-house reference serum	Diluent used	In-house Ref serum calibrated against	In-house reference unitage
4	3	Saturated solution of active pertussis toxin 288 µg/ml SSI Diagnostica	Ammonium sulfate	Carbonate buffer pH 9.60	IgG; Rabbit a-human IgG, HRP, P0214, Dako Denmark A/S	In-house	PBS pH 7.40 containing 0.1% Tween 20, 0.1% milk powder and 0,05% phenol red	1st WHO IS Reference Reagent (06/140).	440 IU/ml
6	6	Purified PT GlaxoSmithKline Rixensart Belgium	Glycerol	Phosphate buffered saline pH 7.4	Goat anti human IgG (phosphatase) Art 075-1002 Kirkegaard and Perry Labs, USA	06/140 NIBSC, Potters bar, England	1% Bovine Serum Albumin (BSA) – PBS	NIBSC 06/142	106 IU/ml anti-PT IgG
11	4	purified dPT stock (Novartis)= 100 µg/ml	Glycerol 50%	PBS pH 7.4	ReserveAP Goat anti-human IgG (gamma) Antibody, Phosphatase Labelled (Kirkegaard & Perry Laboratories Inc. Gaithersburg, MD, USA)	1) Pertussis Antiserum internal reference from the lab sera collection (14 IU/ml) 2) NIBSC 06/140 (335 IU/ml).	BSA fraction V, 1g/L PBS 1x + Tween-20 (0.5 ml/L PBS)	1st WHO IS Reference Reagent (06/140).	14 IU/ml
14	3	Purified PT Former Netherlands Vaccine Institute or Kaketsuken (Kumamoto, Japan)	Glycerol 50%	N/A PT is coupled to activated carboxylated microspheres	Goat anti-Human IgG (γ chain specific) R-phycoerythrin conjugated, Jackson Immunoresearch, #109-116-098	Pool of diagnostic sera submitted to the RIVM for suspected whooping cough for which the diagnosis was confirmed with high IgG PT concentrations	PBS pH 7.2 containing 0.1% (v/v) Tween-20 and 3% (w/v) BSA	Pertussis Antiserum (Human) 1st IS 06/140	607 IU/ml
16a	3	PT #179B List Biological Laboratories Inc.	Glycerol	PBS w/0,02% Sodium azide pH 7.0	A3187 from Sigma Anti-human IgG (gamma chain specific)- ALP Lot#066K6001	NIBSC 06/140	PBSw/0,02% Sodium azide/0,05% Tween20/0,1% BSA pH 7.0		335 IU/ml
16b	3	PT #181 List Biological Laboratories Inc.	NK	Bio-plex amine couplings kit	Anti-human IgG R-phycoerythrin conjugate P8047 Sigma, lot SLBH5768V	In-house pool	PBSw/0,05% Tween20/3% BSApH 7.0	NIBSC 06/142	760 IU/ml
23	3	Purified PT TOH 15 from Glaxo SmithKlein Beecham, Belgium	NK	PBS pH 7.4	Lyophilised conjugate: Goat anti human IgG Reserve AP. #KIRK0751-1002 from Kirkegaard & Perry Laboratories Inc. Gaithersburg, MD, USA	IgG 42, an immunoglobulin from Statens Bakteriologiska Laboratorium, Sweden	0.1% BSA in PBS and 0.05% Tween20 pH 7.4	NIBSC WHO IS Pertussis Antiserum 06/142	840 IU/ml
25	3	Purified PT Kaketsuken, Japan	Glycerol	Carbonate coating buffer (Clin-tech Ltd, Guildford) 0.05M sodium bicarbonate pH 9.7	Rabbit anti-human horse radish peroxidase (Sigma A8792)	IHR 1: Pertussis Antiserum (Human) 1st Reference Reagent (NIBSC, 06/142) IHR 2: Bordetella pertussis antiserum, human (NIBSC, 89/530)	Serum Diluent (Clin-Tech) casein solution in PBS/Tween	N/A	

Annex 4b. The EUPert-LabNet *Bordetella pertussis* serology EQA: Information from participants that used commercial kit ELISA methods

Lab code	Number of Assays	Kit manufacturer and catalogue reference	Coating Antigen	Reference serum Unitage	Ref serum calibrated against	Positive and Negative controls	Validity criteria	Quantitative results
1	3	Manufacturer A	PT	IU/ml	WHO 06/140	Yes	Calibrators' ODs must be in the given valid range. Positive and negative controls must be within the range given in the enclosed lot sheet	Yes
2	3	Manufacturer B	Purified PT	No unitage	N/A	Yes	Blanco O.D. <0.25; 0.45< Std < 1.	Yes, IU/ml
3	3	Manufacturer A	Purified PT	Calibrator 1 200 IU/ml Calibrator 2 100 IU/ml Calibrator 3 25 IU/ml Calibrator 4 5 IU/ml	The controls were calibrated using the first International WHO Standard (WHO International Standard Pertussis Antiserum, human, 1st IS NIBSC Code 06/140).	Yes	For every group of tests performed, the extinction values of the calibration sera and the international units determined for the positive and negative controls must lie within the limits stated for the relevant test kit lot. A quality control certificate containing these reference values is included. If the values specified for the controls are not achieved, the test results may be inaccurate and the test should be repeated.	Yes in IU/ml
5	3	Manufacturer C-1	PT + FHA	No unitage	N/A	Yes	k- < 0.200 and < cut-off; 0.150 < cut-off < 1.300; K+ > cut-off Results in kit specific units Cut-off: 10 U Grey zone: 8.5 – 11.5 U Negative: < 8.5 U Positive: > 11.5 U	Semi -quantitative
7	3	Manufacturer D	Purified PT	Concentrations of antibodies *to B. pertussis* antigens should be quantitatively expressed in UI/ml as reference preparations are available	C30 Calibrator: A ready to use calibrator containing 30 UI/ml (arbitrary international units) of human specific IgG antibodies to B. pertussis Toxin. C60 Calibrator: A ready to use calibrator containing 60 UI/ml C120 Calibrator: A ready to use calibrator containing 120 UI/ml	Yes	The following criteria must be met for the test to be valid. If these criteria are not met, the test should be considered invalid and should be repeated 1. C120: OD≥ 1.3 C60: OD≥ 0.9 C30: OD≥ 0.5 2. Ratio OD C120/ OD C60: > 1.3 3. Ratio OD C60/ OD C30: >1.3 4. Optional Positive Control > 100 IU/ml 5. Negative Control < 15 IU/ml CUT-OFF: <40 IU/ml: Negative Result ≥40 IU/ml and <100 IU/ml: Intermediate Result ≥100 IU/ml: Positive Result	Yes
8	3	Manufacturer C-2	PT	Standard A: 0 IU/ml; Standard B: 25 IU/ml; Standard C: 50 IU/ml; Standard D: 100 IU/ml; Standard F: 200 IU/ml	WHO antiserum 06/140	There are IgG Control Low: range 0-20; IgG Control High range 67.6-178.1	Substrate blank: absorbance value<0.1; Standard A: absorbance value<0.2; Standard B: absorbance value> Standard A; Standard C: absorbance value> Standard B; Standard D: absorbance value> Standard C; Standard E: absorbance value> 1.000	Yes

Lab code	Number of Assays	Kit manufacturer and catalogue reference	Coating Antigen	Reference serum Unitage	Ref serum calibrated against	Positive and Negative controls	Validity criteria	Quantitative results
9	3	Manufacturer B	Purified PT	Yes, the antibody activities are expressed in IU/ml and refer to the WHO 1st standard serum	WHO International Standard Pertussis Antiserum 1st NIBSC code:06/140	Yes	Substrate blank<0,25 OD. Negative control must be negative The mean OD of the Standard serum must be within the validity range as given in the lot specific quality control certificate of the kit. The variation of the OD values of the standard serum must not be higher than 20%	Yes
10	3	Manufacturer A	Inactivated *Bordetella pertussis* toxin antigen	Calibrator 1 200 IU/ml Calibrator 2 100 IU/ml Calibrator 3 25 IU/ml Calibrator 4 5 IU/ml	1st IS NIBSC code 06/140	Positive control and negative control provided with defined IU/ml and validity range stated.	Validity range for positive and negative control – in the case of the lot number used positive control range 50-92 IU/ml, and negative control range 0-18 IU/ml. Also OD of 200 IU/ml calibrator (calibrator 1) >1.0, and OD of 25 IU/ml calibrator (calibrator 3) >0.140. Also OD values of calibrator1>OD calibrator2>OD calibrator 3>OD calibrator 4	Yes
12	3	Manufacturer E	Pertussis Toxin	IU/ml	WHO 1st IS 06/140	Cut-off control, Negative control, Positive control	Neg.control - Absorbance value 450/620nm <0.15 Cut off control - Absorbance value 450/620nm >0.25 Positive control - Absorbance value 450/620nm >0.58 Call - Absorbance value 450/620nm 0.436-1.742 LRC 10-40 IU/ml HRC 83-326 IU/ml	Yes
13	3	Manufacturer A	Purified PT	Calibrator 1 200 IU/ml Calibrator 2 100 IU/ml Calibrator 3 25 IU/ml Calibrator 4 5 IU/ml	1st IS NIBSC 06/140	Yes.	OD Cal1>OD Cal2>OD Cal3>OD Cal4 OD Cal1 >1.0; OD Cal3>0.140; PC 50-92 IU/ml; NC 0-18 IU/ml	Yes
16c	3	Manufacturer B	Purified PT	IU/ml	US Lot 3	Negative control	Substrate blank<0.25 OD. Negative control must be negative The mean OD of the Standard serum must be within the validity range as given in the lot specific quality control certificate of the kit (OD 0.48-1.62). The variation of the OD values of the standard serum must not be higher than 20%	Yes
17	3	Manufacturer C-1	PT + FHA mixture	Kit specific units	NK	Negative control, Cut-off control, Positive control	Substrate blank: Absorbance value < 0,1 Negative control:(absorbance value <0,2 and < cut-off. Cut-off control: Absorbance value 0.150-1.30. Positive control: Absorbance value > cut-off	Patient (mean) absorbance value x 10 / cut-off = NTU Cut-off: 10 NTU Grey zone: 9-11 NTU Negative: <9 NTU Positive: >11 NTU

Annex 5. The EUPert-LabNet *Bordetella pertussis* serology EQA: calculation of geometric means of each participants' assays performed by NIBSC and individual participants in IU/ml.

	Methods	Results calculated by Combistats v 06/142 using raw data by NIBSC							Geometric mean of multiple assays results calculated by participants								
Lab		**A**	**B**	**C**	**D**	**E**	**F**	**G**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**06/142**	**Units**
1	Manufacturer A		40.77	17.60	59.73	72.27	106.52	143.20	< 5	41.81	17.21	59.70	71.97	104.59	138.05	102.55	IU/ml
2	Manufacturer B	No raw data returned							1.47	45.64	29.07	58.60	73.54	107.64	122.32	99.10	IU/ml
3	Manufacturer A	No raw data returned							< 5	40.33	17.20	57.96	77.87	90.52	122.52	117.48	IU/ml
4	In-house		-	-	-	-	-	-	2.54	43.62	22.63	67.00	94.64	109.26	148.16	128.54	IU/ml
5	Manufacturer C-1		46.58	117.77	72.18	86.62	121.47	127.48	Participant calculated results returned in kit specific units								
6	In-house		-	-	-	-	-	-		41.15	22.42	56.88	75.41	96.67	113.75	101.12	IU/ml
7	Manufacturer D		-	-	-	-	-	-		32.91	26.61	48.61	70.83	100.63	107.90	96.28	IU/ml
8	Manufacturer C-2		-	-	-	-	-	-	2.62	59.64	31.60	107.00	149.82	128.95	196.28	2.62	IU/ml
9	Manufacturer B		-	-	-	-	-	-	< 5	37.40	37.96	69.13	86.36	104.31	127.66	111.17	IU/ml
10	Manufacturer A		42.27	RR	56.18	66.44	87.65	108.81	0.17	38.52	16.50	54.37	64.09	77.88	90.24	92.78	IU/ml
11	In-house		43.98	28.84	59.29	83.44	108.48	161.68	1.21	47.75	29.64	61.53	84.59	122.98	162.63	100.19	IU/ml
12	Manufacturer E		-	-	-	-	-	-	< 5	35.14	19.14	51.12	62.64	83.27	106.63	94.41	IU/ml
13	Manufacturer A		39.24	RR	58.61	70.31	94.87	126.22	< 5	38.41	17.27	53.77	71.97	86.81	125.25	94.18	IU/ml
14	MIA		54.92	24.13	71.27	96.94	98.05	177.46	0.28	54.93	23.66	147.78	99.33	101.97	172.59	N/A	IU/ml
15																	
16a	In-house		34.58	22.77	49.51	67.50	101.74	122.94	0.50	38.65	25.66	58.66	73.97	107.54	128.22	109.13	IU/ml
16b	MIA		37.25	19.32	65.48	89.91	100.79	134.59	0.16	35.59	17.31	59.43	85.24	99.22	131.52	110.85	IU/ml
16c	Manufacturer B		-	-	-	-	-	-	1.57	44.36	29.07	57.59	78.03	103.26	125.98	107.05	IU/ml
17	Manufacturer C-1		42.58	82.50	51.24	56.74	92.80	96.75	N/A								
18	Manufacturer B		-	-	-	-	-	-	0.93	42.85	27.30	60.67	71.53	100.52	116.05	112.38	IU/ml
19	Manufacturer A		41.76	18.30	54.98	70.81	96.20	120.93	<2	75.60	22.27	98.93	112.96	135.93	151.30	134.88	IU/ml
20	Manufacturer A		-	-	-	-	-	-	0.02	57.01	20.42	65.38	83.35	115.12	138.27	128.53	IU/ml
21	Manufacturer F		57.26	24.04	82.00	99.99	95.05	169.26	1.84	64.85	29.02	88.47	106.91	103.69	169.25	112.40	IU/ml
22	Manufacturer B		-	-	-	-	-	-	2.57	39.84	25.95	51.31	60.15	94.98	114.37	112.01	IU/ml
23	In-house		40.07	26.41	60.31	79.36	102.52	115.30	-	41.32	25.96	60.89	79.32	104.71	116.59		IU/ml
24	Manufacturer E		41.72	22.07	57.47	78.78	101.81	111.08	Participant calculated results returned in kit specific units								
25	In-house		52.53	24.78	80.49	88.33	135.68	160.81	<7	53.85	26.14	83.73	83.90	130.89	158.32	126.09	IU/ml

N/A: not provided;
-: not able to calculate

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