Journal of Immunological Methods 510 (2022) 113361

Contents lists available at ScienceDirect

Journal of Immunological Methods

journal homepage: www.elsevier.com/locate/jim

Technical Note

Whole blood based point-of-care assay for the detection of anti-pertussis toxin IgG antibodies

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ARTICLE INFO

Keywords:
Pertussis
In vitro –diagnostics
Pertussis toxin
Point-of-care
Serology
Lateral flow

ABSTRACT

Current serological diagnosis of pertussis is usually done by ELISA to determine serum specific anti-pertussis toxin (PT) IgG antibodies. However, the ELISAs are often central-laboratory based, require trained staff, and have long turnaround times. A rapid point-of-care (POC) assay for pertussis serology would aid in both diagnosis and surveillance of the disease. In this study, a quantitative lateral flow assay (LFA) with fluorescent Eu-nanoparticle reporters was used for the detection of anti-PT antibodies from whole blood. The assay was evaluated by testing overall 141 samples including 25 before and 116 one month after acellular pertussis booster vaccination. LFA results were compared to those obtained with standardized anti-PT IgG ELISAs with paired serum samples. Correlation between the assays was high (Pearson $R = 0.832$), and the achieved analytical sensitivity of the LFA was 29 IU/mL, which would be sufficient for clinically relevant cutoffs for determining recent infections. The paired samples, collected pre- and post-booster, demonstrated a significant increase in anti-PT IgG antibodies similar to that detected by ELISA. The developed LFA opens up several alternatives for a suitable POC test also in middle- and low-income countries.

1. Introduction

Despite a high childhood vaccination rate, pertussis remains endemic worldwide (World Health Organization, 2010). Furthermore, the number of pertussis cases in many countries with high vaccination coverage has increased in the last decade (Nieves and Heininger, 2016). Currently, the diagnosis in the early stages of pertussis infections is based on culture and PCR, and in late-stage on serology through ELISA, multiplex immunoassay assay or immunoblotting. Although serology is not recommended for diagnosing pertussis in infants, it is particularly useful for providing valuable information about the level of pertussis circulation among children, adolescents and adults. In ELISAs, use of purified pertussis toxin (PT), the causative agent of pertussis, is recommended, since it is solely produced by *Bordetella pertussis* (Leber, 2014).

Clinical symptoms of pertussis in adolescents and adults are often atypical, which leads to missed cases and misdiagnoses (Guiso, 2013). Furthermore, pertussis is highly contagious and tends to occur in epidemics at 2- to 5-year intervals (Nieves and Heininger, 2016). Together with the difficulty of clinical diagnosis, there is a need for epidemiological studies and constant vigilance about pertussis prevalence. This task would be aided by simple serological assays. The current serology-based diagnosis methods have some weaknesses as the ELISAs are often central-laboratory-based, require trained staff, and have long turnaround times (Table 1). Lateral flow assays (LFA), also known as immunochromatographic assays, provide a simple platform for point-of-care (POC) diagnostics. In this study, an earlier developed LFA platform for the detection of anti-PT IgG antibodies from serum was transformed to be used with whole blood samples (Salminen et al., 2018). The LFA assay was then compared to a standardized ELISA with paired serum samples.

2. Material and methods

A total of 141 whole blood (in heparin) and their paired serum samples were collected for this study, out of which 25 were collected before, and 116 samples one month after a booster dose of acellular vaccination (Tdap3-IPV vaccine, Boostrix™-IPV - GlaxoSmithKline

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https://doi.org/10.1016/j.jim.2022.113361
Received 9 April 2022; Received in revised form 30 August 2022; Accepted 21 September 2022
Available online 26 September 2022

Table 1
Overview of practical differences, advantages and disadvantages between standardized pertussis serological ELISA (Barkoff et al., 2012) and lateral flow assay.

	Standardized ELISA	Lateral flow
Sensitivity	81–93%[a]	78%[b]
Specificity	96–99%[a]	99%[b]
Amount of PT (ng)	400	100
Amount of sample	5 μl (serum)	1 μl (blood)
Washing steps	Two times four	None
Assay time (hours)[c]	> 6	< 2
Limit of detection (IU/ml)	2	20[d]
Hands on time per sample (minutes)	10	4

[a] de Melker et al., 2000.
[b] Knuutila et al., 2021.
[c] For 20 samples (one ELISA plate).
[d] Salminen et al., 2018.

(GSK), Wavre, Belgium) from Finnish study participants of different ages in 2018–2020 (Versteegen et al., 2021). Both blood and serum samples were stored in −20 °C. Anti-PT IgG antibodies were measured from the serum samples with the standardized ELISA (Barkoff et al., 2012) at the Finnish National Reference Laboratory for Pertussis and Diphtheria (University of Turku). Before inclusion into the study, all subject data, except for age, sex, and previous vaccination history were anonymized for the study samples.

The samples were tested with lateral flow test strips as previously described (Salminen et al., 2018) with slight modifications: 0.1 μg of native PT (GlaxoSmithKline, Belgium) per test was used as the test line, and the test strips were cut 0.23 cm wide. The assay was performed using two steps. First, 5 μl of whole blood was diluted in 300 μl of 10 mM phosphate buffer (135 mM NaCl, 0.5% Tween-20, 1% BSA, 0.06% bovine γ -globulin, pH 8.0). The LF test strips were then dipped into 96-plates which contained 60 μl of the diluted whole blood, with two replicate strips for each sample. In order to have a standard curve for different levels of anti-PT IgG antibodies a dilution series of WHO pertussis reference sera 06/142 (106 anti-PT IgG IU/ml) and 06/140 (335 anti-PT IgG IU/ml) (NIBSC, Potters Bar, UK) were tested as described above. However, they were first spiked in a 1:3-ratio with whole blood from a healthy individual without a recent (> 10 years) vaccination which had been stored in −20 °C in several aliquots to be used in all assays. A pooled in-house anti-PT IgG negative control serum sample was prepared and tested similarly, in a single dilution. Once the samples were absorbed, 10^7 europium nanoparticles conjugated with goat anti-human IgG in 60 μL of buffer were added. The entire sample panel was tested twice. Time resolved fluorescence of the test and control lines were measured as previously described (Salminen et al., 2018). The highest reading from the location of both the test and control lines was obtained, and the average ratio of the maximum signal of the test line divided by the maximum signal of the control line was calculated from the duplicate runs, defined as test-to-control line ratio (t/c). The correlation between LFA and ELISA results was calculated as Pearson's correlation coefficient with IBM SPSS statistics 28.0 software for windows (IBM Corp., Armonk, NY, USA).

3. Results and discussion

Lateral flow POC assay was utilized for the detection of anti-PT IgG antibodies from whole blood. The intra-assay coefficient of variation in two test replicates was 14.6%, and the inter-assay variation between LFAs was 14.1%. The linear correlation of the spiked dilutions of WHO serum standards in whole blood provided a linear regression coefficient of 0.99 (Fig. 1). Thereafter, it could be demonstrated that the assay was quantitative and linear at the relevant analytic concentrations, and that whole blood in itself is not interfering with the assay. The comparison of anti-PT IgG results obtained before and after vaccination between the

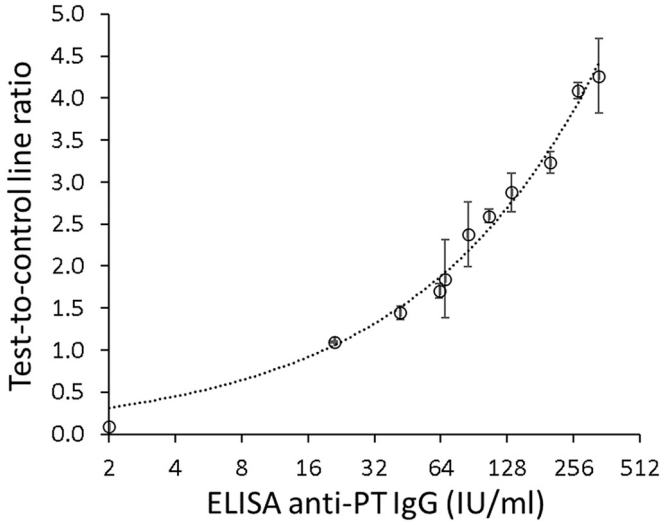

Fig. 1. Comparison of LFA signal readout of WHO standard serum samples (06/140 and 06/142) spiked in whole blood in comparison to the expected ELISA anti-PT IgG values (IU/ml). A separate anti-PT IgG negative control serum sample is marked as 2 IU/ml. Results from the lateral flow assay are presented as the signal ratio of the fluorescence signals between the test line (containing PT) and the control line. Standard deviation of replicate tests is presented as error bars. The regression coefficient of the line of best fit was 0.99.

LFA and the ELISA generated a Pearson correlation coefficient $R = 0.832$ (95% confidence interval 0.726–0.907, $p < 0.001$) (Fig. 2). Highly deviating outliers between the results, mainly a lower LFA result, may occur from weak antibody avidity (Barkoff et al., 2012) due to a limited reaction time between antigen and antibody in the LFA, or from different levels and types of matrix interferences between serum and whole blood samples (Juntunen et al., 2017).

Based on the average signal level plus three times the standard deviation of the two negative samples (1 anti-PT IgG IU/ml), the analytical sensitivity of the LFA was 28.7 IU/mL, which is sufficient for the

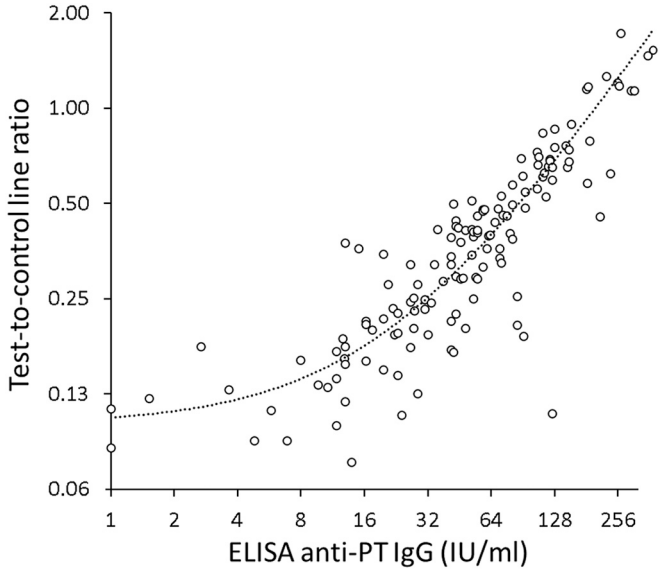

Fig. 2. Comparison of the whole blood samples' ($N = 141$) LFA results with the serum based standardized ELISA. Results from lateral flow assay are presented as the average signal ratio between the test line and the control line from two duplicate runs and two parallel tests. The intra-assay coefficient of variation in two test replicates was 14.6% on average, and the inter-assay variation between LFAs was 14.1%. Pearson correlation coefficient between the LFA and ELISA results was 0.832.

clinically relevant cutoffs: higher than 100 IU/ml indicating recent infection within a year, or alternatively between 50 and 100 IU/ml indicating a recent infection within the past few years. Similar analytical sensitivities and linear correlation were reported earlier for LFAs measuring anti-PT IgG from serum (Salminen et al., 2018) and oral fluid samples (Knuutila A et al. Unpublished data). The increase in anti-PT IgG between pre- and post-vaccine samples was well correlated between the assays (Pearson $R = 0.73$ (95% confidence interval 0.471–0.873, $p < 0.001$) (Fig. 3). Out of the paired 25 samples pre and post-booster, in 20 samples at least a two-fold increase in test-to-control line ratio was noted with the LFA after vaccination (samples with an increment of >0.2 t/c-ratio) (Fig. 3). In four out of the five samples without an increase by LF, there was either no significant increase by ELISA or the overall antibody concentrations post-vaccination were lower than the analytical sensitivity of the LFA (< 30 IU/ml). Thus, the LFA can be used reliably to demonstrate increases of anti-PT IgG concentrations between paired blood samples.

WHO has developed ASSURED criteria (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users) for evaluating POC devices (Naseri et al., 2022). The use of whole blood would remove the technical requirements for serum separation, such as centrifuges, or the long waiting time for the serum to separate with gravity. Thereafter, whole blood would be an easier and faster sample to be used for POC-testing. Hypothetically, only one μl of blood was required for testing. The buffer dilution step was found to be critical when bigger amounts of fresh blood (higher than 5 μl of the whole blood per test strip) were used straight after the blood acquisition, as a lot of red blood cells would bind to the PT test line and interfere with the fluorescence readout. A simple freezing step was found to lyse most of the cells. Alternatively, in practical use, physical or buffer-based whole blood filtering and lysing would be required before adding the sample to the strip, which is already a common approach for many developed tests (Songjaroen et al., 2012). This in the end adds only a layer of complexity for the test design, which is not visible to the end-user, and may remain essential also to decrease negative matrix interference effects of whole blood. The used LFA is simple to perform with minimal training, and the only external equipment needed are portable fluorescence readers, which already exist for research and commercial use (Liang et al., 2015, Rundstrom et al., 2007, Shao et al., 2017, Song and Knotts, 2008). Alternatively, other label platforms could be considered for further development of test, such as semi-quantitative absorbance-based labels (Haglund et al., 2022), or upconverting nanophosphors which provide well-correlated readout in comparison to Europium nanoparticles (Juntunen et al., 2016). Since serological diagnosis of pertussis is not usually performed in countries with limited resources, this type of test could be more useful for the diagnostics of this disease in middle- or low-income countries.

4. Conclusions

The correlation between LFA and standardized ELISA was proven to be high when whole blood samples were tested before and after acellular pertussis booster vaccination. Paired blood samples from pre- and post-booster demonstrated a reliably detectable increase in anti-PT IgG antibodies similar as with ELISA. Further testing with clinical patient samples would be needed to demonstrate the practical use of the assay for diagnostics. Since the incidence of pertussis has increased worldwide, there is a need for an easily performed alternative to recommended conventional ELISA assays for both diagnosis and surveillance, even for countries with high vaccination coverage. A POC test is usually performed near the site of patient care, has a rapid time from sampling to result, and provides actionable information about the patient. This reduces the need for multiple patient visits, enables timely treatment, and provides quick epidemiological information (Kozel and Burnham-Marusich, 2017). The potential effect of POC tests is at its greatest in low-resources countries, where difficult logistics and sparse laboratory infrastructure make traditional testing for infectious diseases challenging.

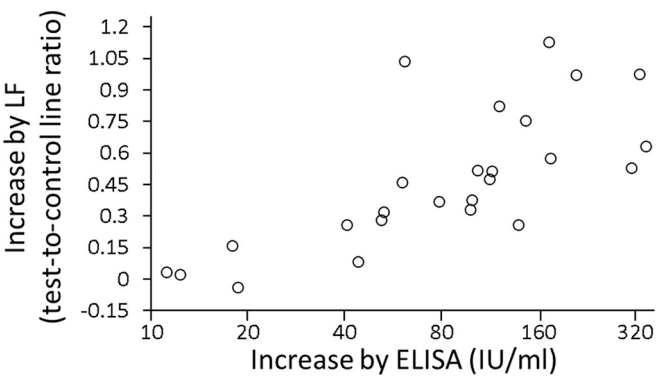

Fig. 3. Comparison between a noted increase of the anti-PT IgG concentrations in the LFA and ELISA with paired serum samples ($N = 25$), counted as signal or concentration difference at one month post-booster and pre-booster anti-PT IgG antibodies. Pearson correlation efficient between the increments between the assays was 0.73.

Funding

This work was supported by grants from Päivikki and Sakari Sohlberg Foundation, Tampere Tuberculosis Foundation, and Finnish Culture Foundation. Results reflect the authors' view; the funders or Innovative Medicines Initiative 2 Joint Undertaking is not responsible for any use that may be made of the information it contains. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Institutional review board statement

Whole blood samples were collected during the IMI2 PERISCOPE Consortium initiated booster vaccination trial. This human clinical study was designed and conducted in accordance with the provisions of the World Medical Association (1996) and the International Conference on Harmonisation Guidelines for Good Clinical Practice. The trial was registered at the EU Clinical Trial database (EudraCT number 2016–003678-42) and was approved by the Medical Research Ethics Committees in University of Turku (ETMK Dnro: 129/1800/2017) in Finland. Written informed consent was obtained from all adult participants, and parents or legal guardian of minors, at the start of the study.

Declaration of Competing Interest

None.

Acknowledgments

We thank Kaisu Kaistinen and Raakel Luoto for their assistance at the study clinic for the collection of whole blood samples. Members involved with the overall clinical study are acknowledged as earlier described (Versteegen et al., 2021). This work was supported by the PERISCOPE (pertussis correlates of protection Europe) project. The PERISCOPE project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement number 115910. This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation program; the European Federation of Pharmaceutical Industries and Associations; and Bill & Melinda Gates Foundation.)

GlaxoSmithKline, Belgium, kindly provided the purified PT antigen. The Department of Life Technologies, University of Turku, provided the

lateral flow testing-related materials, equipment, facilities, and fluorescent labels for the tests.

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