Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

A rapid lateral flow immunoassay for serological diagnosis of pertussis

Teppo Salminen [a,1], Aapo Knuutila [a,b,1], Alex-Mikael Barkoff [b], Jussi Mertsola [c], Qiushui He [b,d,*]

[a] Department of Biotechnology, University of Turku, Turku, Finland
[b] Department of Medical Microbiology and Immunology, University of Turku, Turku, Finland
[c] Department of Pediatrics and Adolescent Medicine, Turku University Hospital, Turku, Finland
[d] Department of Medical Microbiology, Capital Medical University, Beijing, China

ARTICLE INFO

Article history:
Received 8 December 2017
Received in revised form 24 January 2018
Accepted 25 January 2018
Available online xxxx

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

ABSTRACT

Current serological diagnosis of pertussis is usually done by ELISA. 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. While lateral flow immunoassays (LFIA) are simple to use and ideal for point-of-care diagnostics, they were limited to qualitative assays until recently. In this study, we developed a quantitative LFIA with fluorescent Eu-nanoparticle reporters for the detection of anti-pertussis toxin (PT) IgG. The assay was evaluated by testing 198 serum samples with varying anti-PT IgG levels and the result was compared to those obtained with standardized anti-PT IgG ELISA. At the diagnostic cutoff of 100 IU/mL in ELISA, the LFIA had a concordance of 92% with the ELISA, with a specificity of 96% [95% confidence interval (CI): 89–99%] and a sensitivity of 88% [CI: 77–94%]. The developed LFIA has a turnaround time of one hour and requires only a simple manipulation by the user and an instrument for the quantitative detection of the signal. We conclude that the LFIA is specific and sensitive for serological diagnosis of pertussis and is suitable for a POC test.

1. Introduction

Despite a high childhood vaccination rate, pertussis remains endemic worldwide [33]. Furthermore, the number of pertussis cases in many countries with high vaccination coverage has increased in the last decade [24]. Currently, the diagnosis of pertussis infections in early stage is based on culture and PCR and in late stage on serology through ELISA or immunoblotting. Although serology is not recommended for diagnosing of pertussis in infants, it is particularly useful for children, adolescent and adults and in providing valuable information about the level of pertussis circulation in a population. In fact, the introduction of ELISA assays has significantly increased the detection of pertussis in adults [11]. In ELISA assays, use of purified pertussis toxin (PT) is recommended, since it is solely produced by *Bordetella pertussis* [19]. In addition, mixtures of antigens or whole cell lysates are not recommended in ELISAs due to poor specificity and problems in quantification of the immune response [27,37]. Furthermore, measurement of IgG antibodies against PT is recommended and widely accepted, since it has a higher sensitivity than anti-PT IgA antibodies [23].

The PT antigen is included in all acellular pertussis vaccines. This means that separating vaccine-induced anti-PT IgG response from a recent pertussis infection is especially difficult after three to six months from latest pertussis vaccination, and vaccine-induced immune response may compromise diagnostics even up to 2 years [5,25]. Besides vaccinations, *B. pertussis* is also continuously circulating in adult and adolescent populations [19]. One solution to separate a recent pertussis infection from a past infection or vaccination-induced response is to use quantitative anti-PT immunoassays and population-specific cutoffs with known personal vaccination history and possible exposure information.

Quantitative anti-PT IgG antibody values are reported as international units/mL (IU/mL), standardized against the WHO 06/140 or 06/142 standards [37]. The cutoffs used for positive diagnosis vary between 40 and 125 IU/mL, as the lower cutoffs are useful in outbreak situations [9]. At our laboratory, the Finnish National Reference Laboratory for pertussis, the cutoff is 100 IU/mL. For epidemiological studies, values higher than 100 IU/mL indicate a recent infection within a year, and values between 50 and 100 IU/mL an infection in the past few years. A value below 50 IU/mL is commonly a marker of exposure to pertussis several years ago,

* Corresponding author at: Department of Medical Microbiology and Immunology, University of Turku, Turku, Finland.
E-mail address: qiushui.he@utu.fi (Q. He).
[1] These authors contributed to the work equally.

https://doi.org/10.1016/j.vaccine.2018.01.064
0264-410X/

so a lower cutoff may lead to an overestimation of the incidence rate in epidemiological studies [2].

Clinical symptoms of pertussis in adolescents and adults are often atypical, which leads to missed cases and misdiagnoses [8]. Furthermore, pertussis is highly contagious and tends to occur in epidemics at 2- to 5-year intervals [24]. Together with the difficulty of clinical diagnosis, this suggests a need for epidemiological studies and constant vigilance about pertussis prevalence also in adult populations. 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. Immunoblots are somewhat simpler to use, but cannot quantify antibodies and thus are poor at providing accurate clinical diagnosis of pertussis [15].

Lateral flow immunoassays (LFIA), also known as immunochromatographic assays, provide a simple platform for point-of-care (POC) diagnostics. Until recently these assays were limited to visually read labels, and suffered from the same problem of quantification as the anti-PT immunoblots. However, the LFIA can be made quantitative and their sensitivity improved by using fluorescent particles as labels. A wide variety of different fluorescent labels have been used in LFIAs [13]. Lanthanide chelates are fluorescent labels containing an ion of the lanthanide group of elements, such as europium (Eu), with high sensitivity and easy quantification [10]. In addition, lanthanide chelates produce long-lifetime fluorescence that can be measured with a time-gated instrument, eliminating the autofluorescence of the test materials and sample matrix. When these chelates are doped into approximately 100 nm diameter surface-functionalized polystyrene nanoparticles, they provide a promising label for sensitive and quantitative LFIA.

In this study, we used the LFIA platform with fluorescent lanthanide nanoparticle reporters to develop a serological anti-PT immunoassay, which is quantitative, easy to use and suitable for POC environments outside the central laboratory. The LFIA assay was compared to a standardized ELISA with serum samples.

2. Materials and methods

2.1. Samples and reference assays

The study subjects included 138 patients randomly selected from serologically diagnosed pertussis patients (age range: 2–83 years; median: 15.5) at the Department of Medical Microbiology and Immunology, the University of Turku, Finland, in 2015–2016. The diagnosis was based on IgM and IgA antibody levels measured by ELISA using sonicated *B. pertussis* bacteria as a coating antigen, and was done by laboratory of clinical microbiology and immunology, Turku University Hospital, Turku, Finland [12]. However, the anti-PT IgG antibodies of these selected sera were further measured with the standardized ELISA at the National Reference Laboratory for Pertussis as previously described [3]. Five samples with anti-PT IgG ELISA levels of 0 IU/mL were scored as 1 IU/mL for data analysis.

Second panel of sera consisted of 60 serum samples randomly selected from the sera submitted in the period of 2012–2013 for the diagnosis of celiac disease (age range: 20–39 years; median: 25.5) at the Department of Medical Microbiology and Immunology. However, these samples were tested as negative for celiac disease. Of the 60 sera, 32 were found to have detectable anti-PT IgG antibodies by the standardized ELISA.

A total of 170 samples were used for sensitivity and specificity calculations. These samples consisted of all the 138 serologically diagnosed pertussis patient samples, as well as those 32 samples tested negative for celiac disease but with a measurable amount of anti-PT IgG in the ELISA assay. The line of best fit and the sensitivity and specificity of the LFIA at different cutoffs were calculated in comparison to the ELISA results.

All above-mentioned sera were sent for diagnostic purposes to the diagnostic laboratory of Department of Medical Microbiology and Immunology, and stored at −70 °C. Prior to the inclusion into the study, all patient data, except for age and sex, were anonymized. Furthermore, no clinical data of the patients were handled at all. The study was approved by the Ethical Committee of the Hospital District of Southwest Finland and accepted for publication by the Chief of the Operative Group of Turku University Hospital (Decision 14/17 MBG).

WHO pertussis reference serum 06/142 (Anti-PT IgG, 106 IU/ml) and 06/140 (335 IU/ml) were included in the study as standards [37].

2.2. Reagents and conjugates

The assay buffer consisted of 10 mM phosphate buffer (pH 8.0) containing 135 mM NaCl, 0.5% Tween-20, 1% BSA. Capture reagents on the test strips were purified PT, kindly provided by GlaxoSmithKline, Belgium and rabbit anti-goat IgG (G4018, Sigma-Aldrich, USA).

Monoclonal goat anti-human IgG antibody was purchased from Thermo Fisher Scientific (USA). This was conjugated with fluoroMax carboxyl modified microparticles containing europium chelates (diameter 99 nm, Thermo Fisher Scientific), based on a method by Valanne et al. [32] with following modifications: $1.23 * 10^{12}$ particles in 180 μL of 10 mM PBS (pH 7.0) and 1.2 mM EDC and 9 mM sulfo-NHS (FlukaBiochimica, Switzerland) were used to activate the particle surface [32]. A total of 125 μg of goat anti-human IgG was attached to 360 μL of activated Eu-nanoparticles in 20 mM MES buffer (pH 6.1) containing 0.475% NaCl. 15 mM Tris-HCl buffer (pH 9.0) containing 0.1% Brij 35, 0.1% BSA, was used to block remaining active groups. Particles were stored in 20 mM Tris-HCl buffer (pH 9.0) containing 0.01% Tween-20.

2.3. Lateral flow test strips

Lateral flow test strips were assembled on a plastic support (G&L Precision Die Cutting, USA) with a cellulose absorbent pad (CFSP223000, Millipore, USA) and a glass fiber sample pad (G041, Millipore). Test line was done by dispensing 200 ng/cm of PT in 10 mM Tris-HCl (pH 8.0) buffer on the nitrocellulose membrane (Hi-Flow Plus HF90, Millipore) with Linomat 5 (Camag AG, Switzerland) liquid dispenser. The control line was printed 8 mm from the test line with 0.4 μg/cm of rabbit anti-goat IgG in Tris-HCl. The test line was dispensed with a liquid flow speed of 80 nL/s and the control line with 200 nL/s. The width of the strips was 4.8 mm and total length 65 mm (Fig. 1). The strips were then dried for three hours at +35 °C and stored in room temperature, protected from humidity.

2.4. LFIA procedure

Secondary antibody immunoassay was performed using four assay steps. First, strips were placed in wells (polypropylene 96-well plate (cat. no. 655201, Greiner Bio-One, Germany)) with serum diluted 1:250 in 20 μL of assay buffer, with two replicate strips for each sample (Fig. 1. For the WHO standards, a dilution series was made ranging from 20 to 335 IU/mL. Sera tested negative for anti-PT IgG were added to the WHO standard dilutions so that the overall amount of sera in all samples remained constant. Once the samples were absorbed, the strips were moved to

Please cite this article in press as: Salminen T et al. A rapid lateral flow immunoassay for serological diagnosis of pertussis. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2018.01.064