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Automated capillary Western dot blot method for the identity of a 15-valent pneumococcal conjugate vaccine

Melissa Hamm, Sha Ha, Richard R. Rustandi *

Vaccine Analytical Development, Merck Research Laboratories, West Point, PA 19486, USA

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ABSTRACT

Simple Western is a new technology that allows for the separation, blotting, and detection of proteins similar to a traditional Western except in a capillary format. Traditionally, identity assays for biological products are performed using either an enzyme-linked immunosorbent assay (ELISA) or a manual dot blot Western. Both techniques are usually very tedious, labor-intensive, and complicated for multivalent vaccines, and they can be difficult to transfer to other laboratories. An advantage this capillary Western technique has over the traditional manual dot blot Western method is the speed and the automation of electrophoresis separation, blotting, and detection steps performed in 96 capillaries. This article describes details of the development of an automated identity assay for a 15-valent pneumococcal conjugate vaccine, PCV15–CRM197, using capillary Western technology.

Streptococcus pneumonia bacterial infections are responsible for the majority of mortality and morbidity seen in very young infants (< 2 years), the elderly, or the immune compromised population [1,2]. Currently, there are four licensed vaccines available on the market to prevent this pneumococcal disease: Merck Pneumovax 23, Pfizer Prevnar-7, Pfizer Prevnar-13, and GlaxoSmithKline Synflorix. Prevnar-7 and Prevnar-13 are conjugated with a CRM197 carrier protein to boost T-cell-dependent responses in young infants, and they contain 7 and 13 serotypes of pneumococci antigens, respectively. Recently, a preclinical evaluation of a 15-serotype pneumococcal CRM197-conjugated vaccine has been described [3]. Production and manufacturing of these complex multi-serotype polysaccharide conjugate vaccines are very difficult and challenging. Rigorous analytical characterization and the standard release and stability assays are required [4]. The World Health Organization (WHO)[1] and Pharmacopoeia institutes provide guidance and recommendations on the type of release, stability, and characterization testing that should be performed at a minimum [5,6]. One of those critical tests is an identity assay for each polysaccharide serotype including the carrier protein.

WHO has recommended that an identity test needs to be performed for pneumococcal conjugate vaccines in both the monovalent bulk product and the final container product containing all of the intended polysaccharide serotypes and carrier protein. The identity test should be highly specific and based on unique aspects of the particular polysaccharide and protein structure. Typically, the identity test is qualitative in nature. Nuclear magnetic resonance (NMR) spectroscopy is the gold standard for identity testing of non-conjugated polysaccharides [7]; however, this method does not work for the conjugate polysaccharides due to the addition of a protein carrier. Hence, traditional immuno-based assays, such as an enzyme-linked immunosorbent assay (ELISA) and/or a manual dot blot Western, are employed as identity tests for polysaccharide conjugate vaccine products, but both techniques are usually very time-consuming and labor-intensive [8,9]. Therefore, a faster and more automated method is highly desirable. More recently, an automated capillary electrophoresis (CE)-based Western blot called Simple Western has been described [10]. We recently evaluated this technology for qualitative and quantitative analysis for vaccine protein components and residual bovine serum albumin (BSA) in viral vaccines [11,12]. This CE-based Western provides advantages of being fully automated in operation except for sample preparation and being robust, providing a coefficient of variation (CV) of less than 10% (if quantitation is needed) [11–13]. In this article, we describe the development of a novel automated qualitative pseudo dot blot identity assay using capillary Western technology for a 15-serotype pneumococcal conjugate vaccine.

* Corresponding author. Fax: +1 215 993 2244.
E-mail address: richard_rustandi@merck.com (R.R. Rustandi).

[1] *Abbreviations used:* WHO, World Health Organization; ELISA, enzyme-linked immunosorbent assay; CE, capillary electrophoresis; HRP, horseradish peroxidase; DTT, dithiothreitol; MW, molecular weight; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

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Materials and methods

Chemicals and reagents

Secondary antibody goat anti-rabbit–HRP (horseradish peroxidase) conjugate, goat anti-mouse–HRP conjugate, dithiothreitol (DTT), molecular weight (MW) fluorescent standards, luminol-S, hydrogen peroxide, sample buffer, antibody diluent, running buffer, stacking matrix, separation matrix, wash buffer, matrix removal buffers, and capillaries were purchased from ProteinSimple (Santa Clara, CA, USA). The polyclonal and monoclonal antibodies specific for each serotype were either produced in-house against our own polysaccharide or purchased from Staten Serum Institutes (SSI, Copenhagen). The CRM197 protein, monovalent bulk conjugate (drug substance) for each serotype, and 15-serotype pneumococcal conjugate vaccine final container (drug product) were provided by Merck Research Laboratories [3,14,15].

Sample preparation

Drug substance and drug product samples were diluted to target concentrations of 1 μg/ml for CRM197 protein analysis and 0.1 μg/ml for polysaccharide analysis for all serotypes except 6B and 19F, which were targeted to 4 and 1 μg/ml, respectively. All sample dilutions were made in antibody diluent (part no. 041-426) obtained from ProteinSimple. A monoclonal antibody specific for CRM197 detection was diluted 1000-fold in antibody diluent. Type-specific antibodies for the detection of the polysaccharides were either polyclonals or monoclonals. All of the polyclonal antibodies were diluted 5000-fold in antibody diluent, and the monoclonals were diluted either 500-fold (6B) or 1000-fold (3, 6A, 9V, and 19F) in antibody diluent. The master mix for CRM197 protein detection was prepared by adding 72 μl of MilliQ water, 20 μl of 10× sodium dodecyl sulfate (SDS)-containing sample buffer, and 8 μl of 1 M DTT into a 10× fluorescent standard single-use vial and pipetting up and down to mix. This master mix was combined 1:1 with the diluted CRM197 protein sample, and the sample mixtures were heated at 95 °C for 10 min. After heating, the samples were cooled at room temperature and centrifuged briefly before being loaded onto the assay plate. The master mix for polysaccharide detection was prepared by adding 80 μl of MilliQ water and 20 μl of 10× sample buffer into the 10× fluorescent standard single-use vial and pipetting up and down to mix. This master mix was combined 1:1 with the diluted pneumococcal conjugate sample. Samples were vortexed prior to being loaded onto the assay plate. Samples, primary antibody, secondary antibody, stacking matrix, separation matrix, and luminol/peroxide were added to the plate as per the vendor's instructions.

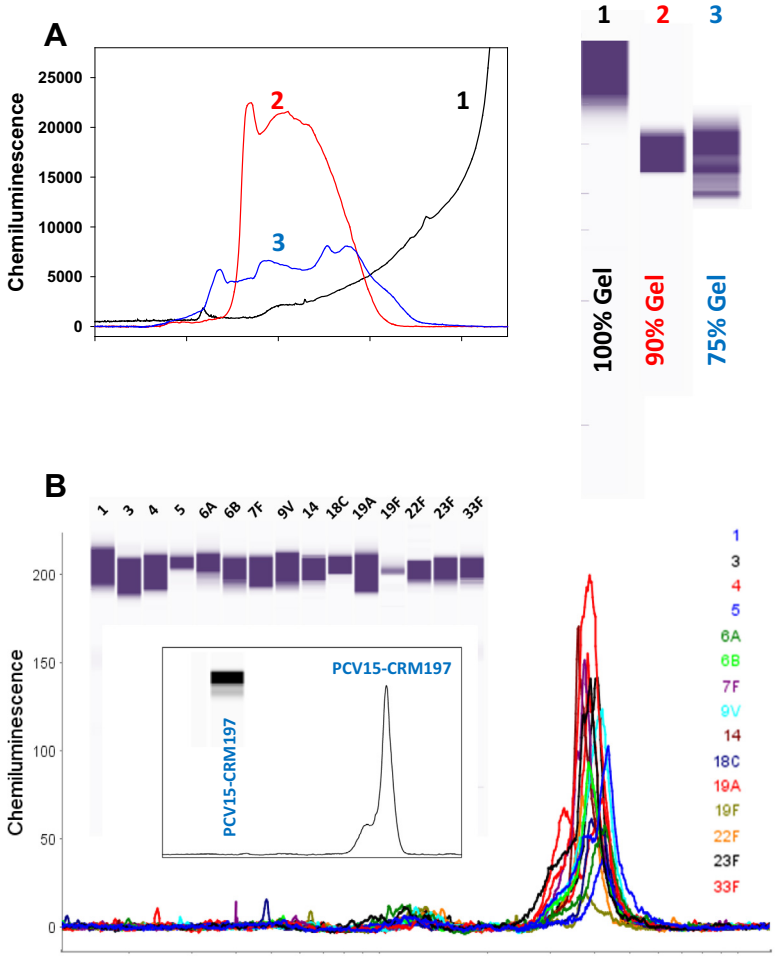

Fig.1. (A) Electropherogram traces comparing 100% (trace 1, black), 90% (trace 2, red), and 75% (trace 3, blue) separation matrix, including the virtual gel image. (B) Electropherograms and gel image of CRM197 identity test in all 15 serotype pneumococcal polysaccharide conjugate vaccines detected using anti-CRM197 specific monoclonal antibody. Inset: Identity CRM197 test in 15-valent pneumococcal conjugate vaccine final container. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)