

Potency Assignment of a Multivalent Reference Standard to Diphtheria, Tetanus, and Pertussis Antigens

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Abstract

Several vaccines that include diphtheria, tetanus and pertussis (DTP) antigens are currently licensed or in development. Pediatric samples are often limiting in volume and a multiplexed DTP assay would conserve critical samples and increase efficiency. A multiplexed DTP assay would also provide value for concomitant testing to assess vaccine interference in the evaluation of other new pediatric, adolescent and adult vaccines. A multivalent international reference serum to pertussis toxin, filamentous hemagglutinin, pertactin, fimbriae 2/3, diphtheria toxin and tetanus toxin does not exist. To support current and future vaccine studies, we have developed a high-titer, large volume, six-valent DTP human reference standard (09 DTP-6, Lot #063-043). The 09 DTP-6 human reference serum is comprised of an ~25 liter pool of serum collected from 25 individuals that were determined to have high antibody titers following immunization with Adacel[®] (Sanofi Pasteur, Swiftwater, PA). The reference standard was calibrated to the World Health Organization and Food and Drug Administration reference standards WHO 06/140, WHO TE-3, WHO 00/496 and FDA Lot 3. The potency of the reference standard is 44 EU/mL, 295 EU/mL, 940 EU/mL, 1710 EU/mL, 3.75 IU/mL, and 16.2 IU/mL for pertussis toxin, filamentous hemagglutinin, pertactin, fimbriae 2/3, tetanus toxin and diphtheria toxin respectively. This reference standard will be useful for pediatric, adolescent and adult vaccine clinical trials, epidemiology studies and inter-laboratory studies.

Objective

To create a large volume, high-titer, multivalent reference standard to the pertussis toxin (PTx), filamentous hemagglutinin (FHA), pertactin (PRN), fimbriae 2/3 (FIM 2/3), diphtheria toxoid (DTd) and tetanus toxoid (TTd) antigens and to calibrate it to the WHO and FDA reference standards WHO 06/140, WHO TE-3, WHO 00/496 and FDA Lot 3.

Materials & Methods

09 DTP-6 Reference Standard

A clinical protocol was performed to immunize 25 adults, collect plasma 30 days post-vaccination, convert the plasma to serum, aliquot the serum in 1 mL volumes and store it at -70°C.

Reference Standards

WHO 06/140 (PTx, PRN, & FHA), WHO 00/496 (DTd), and WHO TE-3 (TTd) were obtained from the National Institute for Biological Standards and Controls and FDA Lot 3 was obtained from the Food and Drug Administration.

Antigens

PTx, FHA, DTd, and TTd were obtained from List Biologicals. FIM 2/3 was obtained from the Health Protection Agency and PRN was from Pfizer Vaccines.

Study Design

All 5 standards were tested side by side in 30 runs performed over 10 days.

Multiplex DTP Luminex Assay

Reference standards were serially diluted in PBS+0.5%BSA+0.05%Tween-20+50%ADHS. All wells had 2500 Ag-microspheres and were incubated for 45 min. with shaking at RT. Plates were washed 3x with 200 µL of PBST+0.5% BSA. A mouse anti-Human IgG₁₋₄ (HP6043) was added to all wells and the plates were incubated for 30 min. with shaking. Plates were washed 3x with 200 µL of PBST+0.5% BSA and 120µL of PBS was added as read buffer. Plates were read on a Luminex 100. **Figure 1** depicts the microsphere regions used and antigen coupled to each microsphere.

Statistical Analysis

A 4-Parameter Logistic fit was applied to all standard curves. Given the similarity in slopes, a parallel line analysis was then performed to estimate potency for each component within 09 DTP-6 via direct comparison to the corresponding component within the international reference standards.

Results

A total of 25 adults were immunized with Adacel[®]. From these donors, plasma aphaeresis was performed 1 month post-vaccination resulting in an ~25 L preparation of serum branded 09 DTP-6. The resulting material was used for the calibration to the International reference standards to determine the antibody levels to the PTx, FHA, PRN, FIM 2/3, DTd and TTd antigens.

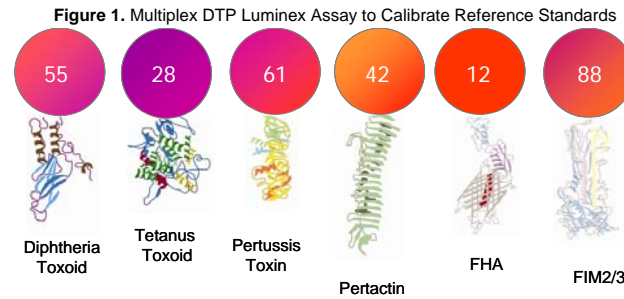
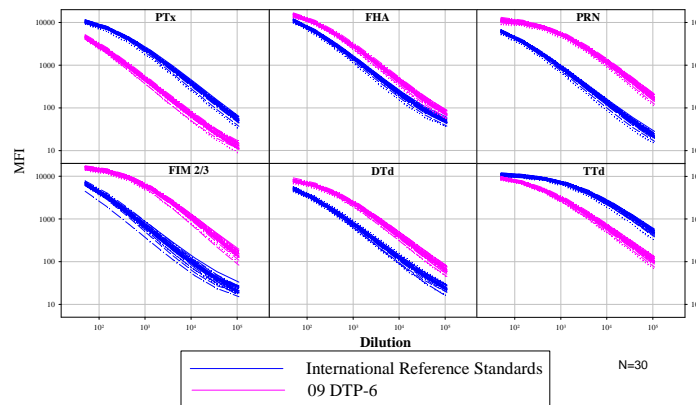


Figure 2. Common Slope, Max and Min Standard Curves Comparing the 09 DTP-6 Reference Standard to the International Reference Standards



Results

The 09 DTP-6 reference standard was calibrated to the 06/140 international reference standard for PTx, FHA and PRN, the anti-FIM 2/3 IgG antibodies were calibrated to the FDA Lot 3 reference standard, the anti-DTd antibodies were calibrated to the 00/496 international reference standard, and the anti-TTd IgG antibodies were calibrated to the TE-3 international reference standard (**Table 1**).

The relative potency assessment is depicted graphically in **Figure 2**. The overall assessment of the adequacy of a common slope model, fit using common minimum and common maximum parameters, was assessed by combining the slope factors across the 30 comparisons to determine the relative potencies for each antigen and is provided in **Table 1**. As shown, the 09 DTP-6 reference standard is more potent than the international reference standards for FHA, PRN, FIM 2/3 and DTd and less potent for PTx and TTd. Antibody concentration assignments for each component of the 09 DTP-6 reference standard are provided in **Table 2**.

Table 1. Determined Relative Potencies and Potency Assessments for 09 DTP-6 to International Reference Standard Calibrators

Antigen	Calibrator RS	Relative Potency	%RSE	International RS Concentration	09 DTP-6 RS Concentration	95% CI
PTx	06/140	0.13	1.0%	335 EU/mL	44 EU/mL	(43, 45)
FHA	06/140	2.27	0.9%	130 EU/mL	295 EU/mL	(289, 300)
PRN	06/140	14.50	0.9%	65 EU/mL	940 EU/mL	(930, 960)
FIM 2/3	FDA Lot 3	17.09	2.7%	100 EU/mL	1710 EU/mL	(1620, 1810)
DTd	00/496	4.69	1.1%	0.8 IU/mL	3.75 IU/mL	(3.67, 3.84)
TTd	TE-3	0.13	1.3%	120 IU/mL	16.2 IU/mL	(15.8, 16.6)

Table 2. Assigned Potency for 09 DTP-6

Target Antigen		International Reference Standards				Exp. Ref. Std.
		06/140	FDA lot 3	00/496	TE-3	09 DTP-6
PTx (EU/mL)	PTx (EU/mL)	335	200			44
	FHA (EU/mL)	130	200			295
	PRN (EU/mL)	65				940
	FIM 2/3 (EU/mL)		100			1710
	DTd (IU/mL)			0.8		3.75
	TTd (IU/mL)				120	16.2

Summary

•A large volume high-titer reference standard for use in epidemiology and vaccine clinical trials to determine antibody levels to PTx, FHA, PRN, FIM 2/3, DTd, and TTd was created

•The novel 09 DTP-6 reference standard was calibrated to FDA and WHO reference standards for the six DTP antigens

•The 09 DTP-6 reference standard can be used for the standardization of assay formats and the comparison of results between labs