**Title**

Single Immunogenicity potency Assay for Diphtheria, Tetanus, and acellular Pertussis (DTaP) in combination vaccines by Luminex® serology

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**Background and aim**

The potency of Diphtheria (D), Tetanus (T) and acellular Pertussis (aP) vaccines is currently tested by different compendial potency assays, which imply the use of laboratory animals (according to EU/US/Chinese/Japanese Pharmacopeias, and WHO recommendations). These tests measure either the inhibition of toxin-induced clinical signs or vaccines’ immunogenicity upon antibody quantitation in blood by ELISA.

In compliance with the principles of Reduction, Refinement and Replacements (3Rs) of animal use for laboratory testing that are part of the European regulatory directives, Sanofi Pasteur has developed an alternative D-T-aP potency test suppressing D and T toxin challenges.

While complying with current compendial requirements, the Single Immunogenicity Assay (SIA) in guinea-pig refines and reduces animal use by suppressing toxin challenges and simultaneously quantitating anti-D, anti-T and anti-aP antibodies in the same serum sample by using the multiplex end-point Luminex® technology.

The SIA is designed to improve Quality Control throughput (e.g. by reducing cycle time) and to reinforce compliance to 3Rs by combining refinement and reduction.

**Method**

Step 1\_Immunization of animals

Animals are injected with increasing doses of vaccine containing D, T, and aP (namely pertussis Toxoid, PT, and Filamentous HaemAgglutinin, FHA) antigens.

Step 2\_Luminex® titration of sera

Sera are collected and anti-D, -T, -PT, and –FHA antibodies are simultaneously quantitated by Luminex® technology.

Step 3\_Determination of vaccine potency

Individual serum titers at given vaccines doses are used to plot vaccines’ dose-response profiles that allow measuring of vaccine potency.

**Results**

Luminex®-detected Median Fluorescence Intensities (MFIs) show antigen-specific, dose-dependent linear regression log/log profiles for all applied vaccines doses.

Antigen-specific, vaccine-induced immunogenicity is measured by dose-dependent, linear regression profiles at the given vaccine doses for all anti-antigen responses.

**Conclusions**

By the simultaneous quantitation of anti-D, anti-T, anti-PT and anti-FHA antibodies in guinea pig sera by Luminex® serology, the Single Immunogenicity Assay (SIA) developed by Sanofi Pasteur is an alternative approach to currently applied multiple in vivo potency tests. The application of SIA as a routine test for release of commercial DTaP combination vaccines will therefore allow to: 1) abolish test animal suffering related to the use of microbial toxins, 2) considerably reduce the use of animals for vaccine testing purposes and 3) significantly improve the overall vaccine testing cycle time for Quality Control.

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