Challenges on using standardized ELISA method for the assessment of specific IgG Antibody in vaccine trials

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OBJECTIVE

To present the challenges and approaches on assay validation for quantitative determination of specific IgG Antibody against a vaccine in human serum by standardised ELISA method.

INTRODUCTION

Clinical development of vaccines requires specific set of specialized assays to measure immune response that correlate with protection against disease or immunogenicity of the vaccine. Validation of the assays involves many challenges and validation requirements are not yet fully specified in regulatory guidelines or white papers.

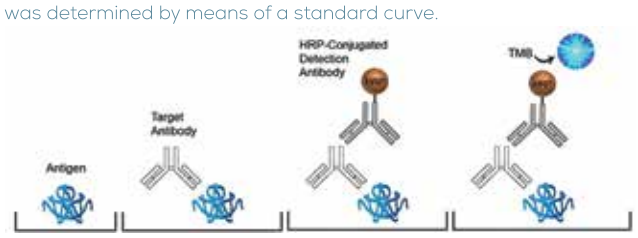
Currently many commercial standardized ELISA kits and automated systems are available to detect Antigen-specific Abs following vaccination. The Enzyme linked immnosorbent assay (ELISA) has been widely used to measure antibody titers for evaluating the immune response of vaccines.

The standardized ELISA methods for the quantitative determination of Diphtheria Toxoid IgG, Tetanus Toxoid IgG, Bordetella Pertussis IgG, Measles Virus IgG, Mumps Virus IgG, Rubella Virus IgG, Varicella Zoster Virus IgG, Anti-S typhi Vi IgG and Haemophilis Influenzae B IgG were validated in human serum at Cliantha Research, Ahmedabad, India.

METHODOLOGY

The specific antibody detection test is based on the principle of the ELISA (Enzyme Linked Immunosorbent Assay).

The wells were coated with antigen. The Specific antibodies of the standards, controls and diluted sample binding to the antigen coated wells were detected by secondary enzyme conjugated antibody specific for human IgG. After the chromogenic substrate reaction, developed blue colour is terminated by the addition of stop solution. Resulting dyes of the samples were measured spectrophotometrically. Concentration of IgG antibodies of samples was determined by means of a standard curve.



CHALLANGES AND APPROCHES

CHALLENGES ENCOUNTERED	
Regulatory guideline / aspect	No guideline for bio analytical method validation of vaccine immunogenicity exists.
Commercial research based kit	Procured research based ELISA kit from reputed vendor.
Preparation of Quality controls	No quality controls available in the kit.
Reference material	There's no commercially availability of reference material (Abs to vaccine). Not easily available of reference material i.e. NIBSC Reference Material (WHO)

APPROACHES / STRATEGIES FOR OVERCOMING THE CHALLENGES	
Regulatory guideline / aspect	FDA and EMA guidelines for bio analytical method validation were adopted and adjusted for necessary method validation experiments.
Commercial research based kit	The commercial kits were used to construct calibration curve.
Preparation of Quality controls	Human serum samples of pathogen exposed or vaccinated individual were screened. Quality controls / validation samples at Two levels (High & Low) were prepared from pooled human serum, mimicing reference sample and were used for precision, accuracy and stability experiments. Also used for vaccine evaluation studies.
Reference material	The National Institute for Biological Standards and Controls (NIBSC) reference materials with assigned antibody titers to relevant evaluation of vaccines procured from registered agency of NIBSC. Control samples prepared from NIBSC reference material and used in validation experiment and vaccine evaluation studies.

VALIDATION EXPERIMENTS SUMMARY

Screening of serum samples Preparation of Validation Samples / Quality Controls	Different human serum samples of pathogen exposed or vaccinated individual were screened for presence of specific antibody to the antigen. Two level (high & low) samples were prepared from pooled human serum considering the screening antibody titer of sample.
Estimation of Validation Samples / Quality Controls	Evaluated the nominal concentration (mean value) of two level validation samples (High and low) from multiple estimation batches.
Accuracy & Precision	Nominal concentration of High and Low validation samples was used to perform intra-day and inter-day Precision and Accuracy experiments.
Stability	Nominal concentration of High and Low validation samples was used to perform various stability experiments i.e. Bench Top Stability (BTS), Freeze & Thaw Stability (FTS) and Long Term Freezer Stability (LTS).
Dilution Integrity	NIBSC reference material respective to vaccine antigen was used to perform Dilution Integrity for concentration above the calibration curve. Additionally Prozone-Hook effect was also performed to check signal interference at high concentration i.e. beyond the highest calibration standard.
Partial Validation	Partial validation experiment was performed on every ELISA kit lot change assaying one Precision & Accuracy experiment.

RESULTS

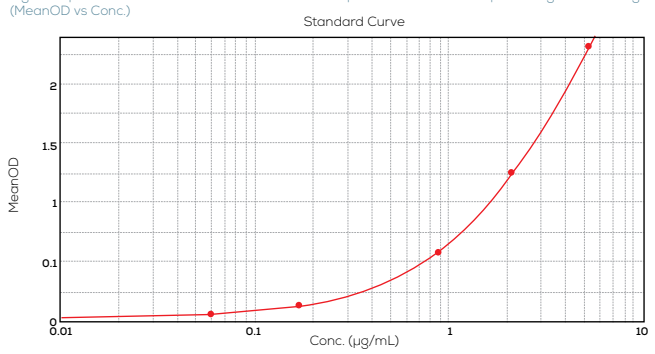
CALIBRATION STANDARDS

The precision and accuracy of standard concentrations were measured from multiple assays of performed over several days.

HAEMOPHILUS INFLUENZAE B (HIB) IgG: PRECISION AND ACCURCY OF STANDARD CONC.

Calibration Std. Conc. (µg/mL)	Mean Result Con. (µg/mL)	%CV	%Bias	Accuracy%	n
5.260	5.250	0.8	-0.2	100	10
2.100	2.112	1.7	-0.6	101	10
0.880	0.876	1.4	-0.5	100	10
0.170	0.170	0.6	0.0	100	10
0.060	0.060	0.0	0.0	100	10

Fig.1- Representative Calibration Standard Curve parameter of Haemophilus Ingluenzae B IgG (MeanOD vs Conc.)



Std (Standard (µg/mL)@HiB IgG_MVP18006.00:MeanOD vs Conc) Weighting: 1/(MeanOD)^2

Curve Fit: 4-Parameter Logistic $y = D + \frac{A - D}{1+\left(\frac{x}{C}\right)^B}$ Curve Fit Result ▲

	Parameter	Estimated Value	Std. Error	Confidence Interval
Std. R2 = 1.000 EC50 = 6.106	A	0.023	0.004	[-0.022, 0.068]
	B	1.062	0.034	[0.633, 1.491]
	C	6.106	1.078	[-7.591, 19.80]
	D	5.007	0.549	[-1.968, 11.98]

CALIBRATION STD. CONCENTRATION OF OTHER ANALYTE OF VACCINE

Anaylte	Unit	Calibration Std. Concentration				
Tetanus Toxoid IgG	IU/mL	5.000	2.500	1.000	0.100	-
Diphtheria Toxoid IgG	IU/mL	1.000	0.500	0.100	0.010	-
Bordetella pertussis IgG	U/mL	150.000	45.000	20.000	1.000	-
Measels Virus IgG	mIU/mL	5000.0	1000.0	250.000	50.000	-
Mumps Virus IgG	U/mL	150.000	40.000	10.000	1.000	-
Rubella Virus IgG	IU/mL	500.000	200.000	50.000	10.000	-
Varicella Zoster Virus IgG	mIU/mL	810.000	270.000	90.000	30.000	10.0
Anti S-tyhphi Vi IgG	U/mL	600.000	200.000	66.700	22.200	7.40

Back calculated standard concentrations of all analytes were within ±20% bias and %CV of absorbance was ≤20% for each experiment run. Correlation of coefficient (r2 value) was greater than 0.98.

QUALITY CONTROLS / VALIDATION SAMPLES

Inter-run accuracy and precision were determined by analyzing each sample in replicates in independent runs on separate days.

Anaylte	Unit	Sample	Estimated Conc.	Mean Result	% CV	%Bias	Accuracy%	n
Tetanus Toxoid IgG	IU/mL	VS-High	3.168	3.152	13.8	-0.5	99.5	18
		VS-Low	1.511	1.622	14.7	7.3	107.3	18
Diphtheria Toxoid IgG	IU/mL	VS-High	0.216	0.214	11.2	-0.9	99.1	18
		VS-Low	0.060	0.059	6.8	-1.7	98.3	18
Bordetella pertussis IgG	U/mL	VS-High	112.0	106.006	12.7	-5.4	94.6	17
		VS-Low	62.780	67.595	9.9	7.7	107.7	18
Measels Virus IgG	mIU/mL	VS-High	1690.144	1803.992	12.9	6.7	106.7	18
		VS-Low	385.875	364.965	13.7	-5.4	94.6	18
Mumps Virus IgG	U/mL	VS-High	100.600	101.332	13.4	0.7	100.7	15
		VS-Low	27.880	28.208	10.2	1.2	101.2	14
Rubella Virus IgG	IU/mL	VS-High	233.400	214.234	9.7	-8.2	91.8	15
		VS-Low	73.710	82.103	11.5	11.4	111.4	15
Varicella Zoster Virus IgG	mIU/mL	VS-High	261.500	272.082	4.2	4.0	104.0	18
		VS-Low	73.650	76.777	3.0	4.2	104.2	18
Anti S-tyhphi Vi IgG	U/mL	VS-High	469.822	449.362	9.8	-4.4	95.6	18
		VS-Low	122.750	123.125	8.5	0.3	100.3	18
Haemophilus Influenza B (HiB) IgG	µg/mL	VS-High	2.208	2.182	9.6	-1.2	98.8	36
		VS-Low	1.018	1.028	7.2	1.0	101.0	36

STABILITY OF VALIDATION SAMPLES / QUALITY CONTROLS

Anaylte	BTS (at room temp.)	FTS (-20 ±10ºC temp.)	LTS (-20 ±10ºC temp.)
Tetanus Toxoid IgG	24 Hours	5 cycles	1011 Days
Diphtheria Toxoid IgG	24 Hours	6 cycles	1002 Days
Bordetella pertussis IgG	24 Hours	6 cycles	1009 Days
Measels Virus IgG	24 Hours	4 cycles	248 Days
Mumps Virus IgG	24 Hours	6 cycles	211 Days
Rubella Virus IgG	24 Hours	6 cycles	212 Days
Varicella Zoster Virus IgG	24 Hours	6 cycles	199 Days
Anti S-tyhphi Vi IgG	24 Hours	6 cycles	494 Days
Haemophilus Influenza B (HiB) IgG	24 Hours	6 cycles	in progress

The mean concentration of each stability sample (VSH & VSL) was within ±20% bias of the nominal value and the coefficient of variation (%CV) was ≤ 20%.

DILUTION INTEGRITY AND PROZONE-HOOK EFFECT

Dilution Integrity and Prozone-Hook Effect by assaying NIBSC reference material with assigned antibody titer to relevant evaluation of vaccines

Anaylte	Ref. Material	Unit	Conc. Used	Dilution Factor	Mean Result	%CV	%Bias	n
Tetanus Toxoid IgG	WHO International Standard 1st International Standard for Tentanus Immunoglobulin Human-NIBSC	IU/mL	120.00	30	127.451	-0.5	6.2	3
			60.000	15	67.549	2.3	12.6	3
Diphtheria Toxoid IgG	WHO International Standard 1st International Standard for Diphtheria Antitoxin Human-NIBSC	IU/mL	2.000	4	1.752	5.5	-12.4	3
			2.000	10	2.001	2.6	0.0	3
Haemophilus Influenza B (HiB) IgG	Human anti-Haemophilus influenza B reference serum-NIBSC	µg/mL	69.400	20	64.901	2.9	-6.5	6
			34.700	10	36.128	1.9	4.1	6

Prozone-Hook effect was performed by assaying NIBSC sample concentration above the highest calibration standard. The Response value (mean optical density) of prozone-hook effect sample was above the response value (mean optical density) of highest relevant calibration standard.

CONCLUSION

The ELISA based methods have been optimized for quantitative detection of antibody specific to relevant vaccine antigen in human serum. The major analytical challenges of quality control sample preparation and reference material were overcome and used in method validation experiments and evaluation of vaccine studies for reliable sample analysis.