VALIDATION OF POTENCY TEST OF IPV COMPONENT IN MULTIVALENT VACCINE MANUFACTURED BY SANOFI PASTEUR

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Abstract: According to the World Health Organization (WHO) guidelines, a vaccine's quality control procedure must be validated before it can be used for routine testing. Procedure validation is the process of establishing one or more characteristics, such as accuracy, precision, linearity, linear range, and specificity, to ensure the method's suitability. To quantify the IPV antigen, we fully validated the potency assay procedure for the polio component (IPV) manufactured by Sanofi and used in a combination vaccine. The method used for this quantification was the Enzyme-linked Immunosorbent Assay (ELISA). Within the scope of this paper, we focus on two key validation criteria: specificity and linearity. The results from the validation of the IPV vaccine component showed that the procedure met the requirements for both linearity and specificity. Specifically, the linearity for serotypes 1, 2, and 3 was excellent, with R^2 values of 0.99, 0.99, and 0.98, respectively.

***Keywords**: potency, ELISA, validation. Specificity, linearity.*

1. Introduction

Currently, there are several commercial IPVs (Inactivated Poliovirus Vaccine) from Aventis Pauster, Chiron – Behring, GlaxoSmithKline, National Biological Laboratory, Rijks Institute, Statens Seruminstitu [1]. In 1959, World Health Organization (WHO) published IPV specification for the first time, and updated some complemented versions until June, 2015, the completed version WHO – TRS 910, annex 3 was established to apply in process control, inspection and manufacturer's release standards of IPV vaccines [2]. In this technical specification, D-antigen method is recommended for IPV vaccine potency that based on ELISA technique, which use specific antibody for antigen-antibody reaction. The specific antibody is linked with enzyme that will transform the added substrate to create a signal determined by light intensity (Optical Density-OD). The result is calculated by Excel or specialized software [3]. In IPV potency test of Sanofi, we have used in-house chemicals such as reference standard, antibody [4]. To ensure the reliability of potency test for IPV component, the full validation were performed which included accuracy, precision (repeatability and intermediate precision), linearity, specificity and robustness [5,6]. Therefore, the project "Validation of potency test for

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IPV component in Sanofi combination vaccine by D-antigen method (ELISA)" was performed in Quality control of Viral vaccine Department, however, only specificity and linearity indicators are focus and presented in this article.

2. Objectives and research method

2.1 Research object, duration and location

- Research object: Procedure of potency test for IPV component in Sanofi combination vaccine by ELISA

- Research location: Quality control of Viral vaccine Department, National Institute for Control of Vaccines and Biologicals (NICVB);

- Duration: From 04/2020 - 12/2020

2.2 Research method

Descriptive, retrospective and prospective methods.

2.3 Materials

2.3.1 Materials and chemicals:

Reference standard lot 11.07.07 – Sanofi, Primary antibody serotype 1 code 437517-Sanofi, Primary antibody serotype 2 code 934952-Sanofi, Primary antibody serotype 3 code 405115-Sanofi, Secondary antibody serotype 1 code 123-95 - Sanofi, Secondary antibody serotype 2 code 3495-Sanofi, Secondary antibody serotype 3 code 355-94 - Sanofi. ELISA plate Nunc MaxiSorp (Thermo) and other commercial chemicals.

2.3.2 Sample:

Tetraxim vaccine.

Equipment and consumables: Pipetteman 5000 µl, Pipetteman 1000 µl, Pipetteman 200µl, Pipetteman 100µl, Pipette aid (Eppendorf), Vortex (Maxi mix II), ELISA system (Human), water bath (Shel-lab), Sample storage cabinet (Sanyo), incubator (Sanyo).

2.4. Research design

Linearity is the ability of technical method that given proportional result between OD value and substrate concentration. Performance: Serial dilute reference standard to 1/70, 1/140, 1/280, 1/560, 1/1120 for all three serotypes, follow SOP, measure OD value and calculate the correlation between OD and substrate concentration. Specificity is the ability to evaluate the presence of an analyte in a complex including impurities, degradation products, matrix. Performance: Positive control: Reference standards serotypes I, II, III in 2-fold diluted (use 1/35 dilution), samples: Vaccine in 2-fold dilution (use 1/2 dilution), negative control: PBS, repeat 3 times for each serotype. Peformed in 6 days separately. The result of validation of potency test for IPV component should be achieved the following value: the linearity in the range of $0,95 \leq R^2 \leq 1$, $\%CV \leq 20\%$, Δi is about ±15% as theoretical value in each serotype label, specificity indicator should be specific for each serotype and there is no cross-activity between serotypes.

2.5. Procedure [According to SOP of manufacturer]

Day 1: Dilute primary antibody for plate coating.

- Dilute antibody according to manufacturer's recommendation (1/1000).

- Each serotype coated 9 columns with 50 µl each well. Seal the plate and store at 2-8^{0}C overnight.

- *Day 2:* Wash plate 4 times with 200 µl/well PBS 1X+ Tween (0,025%) + milk 2%

Table 1. Reference vaccine dilution

Dilution	Serotype 1 (DU/ml)	Serotype 2 (DU/ml)	Serotype 3 (DU/ml)
1/35	32,8	7,68	29,0
1/70	16,41	3,84	14,5
1/140	8,21	1,92	7,25
1/280	4,10	0,96	3,63
1/560	2,05	0,48	1,81
1/1120	1,03	0,24	0,91

- Dilute sample in 2-fold serial dilution then transfer 50 µl/well. Seal the plate and incubate at 37±1^{0}C in 1 hour. Dilute detected antibody with PBS milk 2%.

- Dilute secondary antibody to 1/1000.

- Wash 3 times then add 50 µl diluted antibody to each well. Seal the plate and incubate at 37±1^{0}C in 1 hour. Wash 3 times. Dilute conjugated enzyme and then add 50 µl diluted conjugated enzyme to each well. Seal the plate and incubate at 37±1^{0}C in 1 hour. Wash 3 times. Add 50 µl ABTS buffer solution to each well. Incubate 10 minutes for serotype 1, 15 minutes for serotype 2 and 3 at 37±1^{0}C and avoid light. Add 25 µl SDS 1% for each well then read the result at 405nm - 630nm. The final result is calculated by Parallel.

Evaluate the procedure:

The test is valid if:

+ The dilution of reference and sample give linear and parallel OD values by Parallel Line Assay, $R^2 \geq 95\%$

+ In the result data: Parallelism value and Linearity value have no error marks.

+ Deviation from homogeneity of variance (if any) should be no significant difference

+ OD value between wells in the same performance should not different over 20%

+ OD value of blank should be smaller than OD of sample.

+ OD blank = Mean + 3SD: Use diluent as negative control, repeat 3 times

+ Positive: OD sample > OD blank and the solution change to blue color.

Specification: Serotype I: 20-43 DU/dose, Serotype II: 5-9 DU/dose, Serotype III: 17-36 DU/dose

3. Result

Linearity

Table 2. Linear coefficient of Determination R^2 and %CV of Serotype 1

Antibody content (DU/ml)	Serotype 1 (OD)					
	Day 1	Day 2	Day 3	Mean	SD	%CV
16,41	0,950	0,792	0,729	0,823	0,11	13,85
8,21	0,549	0,498	0,450	0,499	0,05	9,88
4,10	0,247	0,235	0,231	0,238	0,01	3,39
2,05	0,164	0,142	0,127	0,145	0,02	12,98
R^2	0,9922	0,9903	0,9872	0,99		

Note: OD: Optical Density, CV: Coefficient of Variation, Mean: Average value, SD: Standard of Deviation.

According to table 2, the average R^2 value of reference standard for serotype 1 was 0,99 that in the range of $0,95 \leq R^2 \leq 1$ and met the requirements.

Table 3. Linear coefficient of Determination R^2 and %CV of Serotype 2

Antibody content (DU/ml	Serotype 2 (OD)					
	Day 1	Day 2	Day 3	Mean	SD	%CV
7,68	1,117	0,806	0,950	0,958	0,16	16,23
3,84	0,798	0,577	0,593	0,656	0,12	18,79
1,92	0,371	0,300	0,386	0,352	0,05	13,00
0,96	0,219	0,197	0,185	0,201	0,02	8,59
R^2	0,9955	0,9763	0,9832	0,99		

Note: OD: Optical Density, CV: Coefficient of Variation, Mean: Average value, SD: Standard of Deviation.

According to table 3, the average R^2 value of reference standard for serotype 1 was 0,99 that in the range of $0,95 \leq R^2 \leq 1$ and met the requirements

Table 4. Linear coefficient of Determination R^2 and %CV of Serotype 3

Antibody content (DU/ml)	Serotype 3 (OD)					
	Day 1	Day 2	Day 3	Mean	SD	%CV
7,26	0,727	0,725	0,633	0,695	0,05	7,70
3,63	0,482	0,447	0,355	0,428	0,07	15,28
1,81	0,267	0,240	0,224	0,244	0,02	8,99
0,91	0,147	0,155	0,137	0,147	0,01	6,15
R^2	0,9795	0,9527	0,9992	0,98		

Note: OD: Optical Density, CV: Coefficient of Variation, Mean: Average value, SD: Standard of Deviation.

According to table 4, the average R^2 value of reference standard for serotype 1 was 0,99 that in the range of $0,95 \leq R^2 \leq 1$ and met the requirements

Table 5. Δi of each point of serotype 1

Serotype 1 (DU/ml)					
Day 1	Day 2	Day 3	Mean	Theoretical value	%Δi
16.04	15.67	15.7591	15.82	16,41	3.59
8.25	9.51	9.3168	9.03	8,21	-10
3.83	3.98	4.2478	4.02	4,10	2.04
2.25	2.04	1.8329	2.04	2,05	0.54

Note: Mean: Average value

According to table 5, %Δi of 4 dilutions value that met the requirements. of serotype 1 in 3 days < ±15% as theoretical

Table 6. Δi of each point of serotype 2

Serotype 2 (DU/ml)					
Day 1	**Day 2**	**Day 3**	**Mean**	**Theoretical value**	**%Δi**
6.74	6.56	7.11	6.8	7,68	4.39
3.36	3.37	3.62	3.45	3,84	3.11
1.68	2.1	1.61	1.8	1,92	-1.1
1.06	0.87	0.9	0.94	0,96	-6.11

Note: Mean: Average value

According to table 6, %Δi of 4 dilutions value that met the requirements. of serotype 2 in 3 days < ±15% as theoretical

Table 7. Δi of each point of serotype 3

Serotype 3 (DU/ml)					
Day 1	**Day 2**	**Day 3**	**Mean**	**Theoretical value**	**%Δi**
7.23	7.81	6.86	7.3	7,26	-0.61
3.64	3.57	4.25	3.82	3,63	-5.26
1.94	1.41	1.95	1.77	1,81	2.53
0.82	0.72	0.87	0.81	0,91	11.02

Note: Mean: Average value

According to table 7, %Δi of 4 dilutions value that met the requirements. of serotype 3 in 3 days < ±15% as theoretical

Specificity

Table 8. Specificity indicator of serotype 1

Duration	**Serotype 1 (OD)**				
	Positive control	**Sample**	**Negative control**		
	(Antibody serotype 1)		**Diluent**	**Antibody serotype 2**	**Antibody serotype 3**
Day 1	1,070 (Positive)	0,964 (Positive)	0,037 (Negative)	0,030 (Negative)	0,031 (Negative)
Day 2	1,022 (Positive)	1,012 (Positive)	0,030 (Negative)	0,036 (Negative)	0,037 (Negative)
Day 3	1,032 (Positive)	1,005 (Positive)	0,032 (Negative)	0,028 (Negative)	0,036 (Negative)
Day 4	1,063 (Positive)	0,919 (Positive)	0,035 (Negative)	0,034 (Negative)	0,035 (Negative)
Day 5	1,132 (Positive)	1,123 (Positive)	0,038 (Negative)	0,035 (Negative)	0,034 (Negative)
Day 6	1,139 (Positive)	1,112 (Positive)	0,037 (Negative)	0,039 (Negative)	0,039 (Negative)

Positive: OD sample > OD blank and the solution changes color.

Negative: No color, OD blank ≤ OD sample.

According to table 8 of serotype 1, positive controls and samples were all positive with antibody serotype 1 in 6 days of performance, negative with antibody serotype 2 and 3. Therefore, the procedure was specific for antibody serotype 1 and no cross-activity with antibody serotype 2 and 3.

Table 9. Specificity indicator of serotype 2

Duration	Serotype 2 (OD)				
	Positive control	Sample	Negative control		
	(Antibody serotype 2)		Diluent	Antibody serotype 1	Antibody serotype 3
Day 1	1,117 (Positive)	1,139 (Positive)	0,029 (Negative)	0,031 (Negative)	0,032 (Negative)
Day 2	0,806 (Positive)	0,805 (Positive)	0,036 (Negative)	0,039 (Negative)	0,035 (Negative)
Day 3	0,950 (Positive)	0,992 (Positive)	0,037 (Negative)	0,030 (Negative)	0,033 (Negative)
Day 4	0,920 (Positive)	1,002 (Positive)	0,029 (Negative)	0,030 (Negative)	0,033 (Negative)
Day 5	0,950 (Positive)	0,970 (Positive)	0,031 (Negative)	0,034 (Negative)	0,034 (Negative)
Day 6	0,819 (Positive)	0,832 (Positive)	0,038 (Negative)	0,033 (Negative)	0,039 (Negative)

Positive: OD sample > OD blank and the solution changes color.

Negative: No color, OD blank ≤ OD sample.

According to table 9 of serotype 2, positive controls and samples were all positive with antibody serotype 2 in 6 days of performance, negative with antibody serotype 1 and 3. Therefore, the procedure was specific for antibody serotype 2 and no cross-activity with antibody serotype 1 and 3.

Table 10. Specificity indicator of serotype 3

Duration	Serotype 3 (OD)				
	Positive control	Sample	Negative control		
	(Antibody serotype 3)		Diluent	Antibody serotype 1	Antibody serotype 2

Day 1	1,208 (Positive)	1,140 (Positive)	0,061 (Negative)	0,064 (Negative)	0,066 (Negative)
Day 2	1,065 (Positive)	1,046 (Positive)	0,060 (Negative)	0,063 (Negative)	0,065 (Negative)
Day 3	1,104 (Positive)	1,092 (Positive)	0,062 (Negative)	0,061 (Negative)	0,070 (Negative)
Day 4	0,994 (Positive)	0,927 (Positive)	0,072 (Negative)	0,066 (Negative)	0,066 (Negative)
Day 5	1,194 (Positive)	1,245 (Positive)	0,067 (Negative)	0,063 (Negative)	0,067 (Negative)
Day 6	1,148 (Positive)	1,174 (Positive)	0,069 (Negative)	0,068 (Negative)	0,067 (Negative)

Positive: OD sample > OD blank and the solution changes color.

Negative: No color, OD blank ≤ OD sample.

According to table 10 of serotype 3, positive controls and samples were all positive with antibody serotype 3 in 6 days of performance, negative with antibody serotype 1 and 2. Therefore, the procedure was specific for antibody serotype 3 and no cross-activity with antibody serotype 1 and 2.

4. Discussion

The quality of IPV vaccine is determined by the number of D unit by ELISA method. The most common in vitro test is indirect ELISA method used by most manufacturers of IPV vaccines in quality control of final products [7]. It is noted that most scientific articles in the world focus on ELISA to evaluate the immunogenicity of IPV vaccines. There are not many published studies on ELISA to evaluate invitro standards. During the quality control of vaccine, WHO or European and American pharmacopoeias only provide guidance on the use of antibodies and standards suitable for ELISA [2,8,9]. Currently, WHO and National Institute for Biological Standards and Control (NIBSC) do not have standard vaccines and specific antibodies for commercial IPV vaccines originating from type 1 (Mahoney), type 2 (Mef-1), and type 3 (Saukett) strains. Addtionally, there are no commercial kits for quantifying IPV antigen yet. Therefore, with each manufacturer's process, we conduct a process validation to ensure the most accurate quality control process for vaccines and biological products.

The evaluation criteria in this process of the article that need to be achieved are specificity and linearity. To establish specificity, each serotype were performced independently on 6 different days. Specificity is also evaluated by cross-activity which means the antibody of this type has no specific to the antigen of other

types and negative controls. Currently in Vietnam, there are combination vaccines from other manufacturers such as Glaxo Smith Kline, however, we did not use these vaccines because ELISA reaction is conducted on primary antibodies and secondary antibodies that may match specifically with each manufacturer's vaccine. Besides, antigen unit of each vaccine is different, therefore, the procedure of a vaccine can not be applied for anothers. We only proved that there was no cross-activity between serotypes because there are no commercial kit . The results showed that the specificity indicator was achieved for serotype 1, 2 and 3 and there was no cross-activity between different types.

For linearity, we conducted separately on 4 dilutions of each serotype in 3 days. The result should mêt the criteria of manufacturer which is $0,95 \leq R^2 \leq 1$ and validation specification of % CV ≤ 20% and và Δi in the range of ±15% as theoretical value [4,6]. The results showed a positive correlation between antigen concentration and OD value. The correlation of serotype 1,2,3 gave the corresponding results R^2 = 0.99, 0.99, and 0.98, which were within the manufacturer's standard range of $0.95 \leq R^2 \leq 1$. On the other hand, the content values of the reference standard have been confirmed in accuracy indicator to make the method more rigorous. In the study of accuracy, 5 dilutions 1/70, 1/140, 1/280, 1/560, 1/1120 were arranged from the highest concentration to the lowest concentration to build a linear curve including the dilutions of samples so that the calculation gave the most accurate results. Accuracy is the closeness between the average value of a series of test results and true value. The accuracy (t) of serotype 1, 2, 3 were respectively confirmed as follow: serotype 1 = 0.45; serotype 2 = 1.5; serotype 3 = 0.21. The t values were all guaranteed to be less than the limit of t_{α} = 4.303. Therefore, the test also gave the required accuracy, which is a factor to prove that our linear arrangement is more rigorous. In addition, in linear research, there is also a bias value of Δi. Δi is the average difference between the measurement results of validation method and the actual value of the reference standards. Each Δi represents the recovery point when diluting the reference standard vaccine of each type. The indicator meets the requirements when Δi < ± 15% and the Δi results of serotype 1, 2, and 3 were all within the accepted limit of Δi < ± 15%.

5. Conclusion

The validation of potency test for IPV vaccine of Sanofi by ELISA pererformed in Laboratory of Quality control of Viral vaccines reached the requirements with high reliability of specificity and linearity indicators.

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