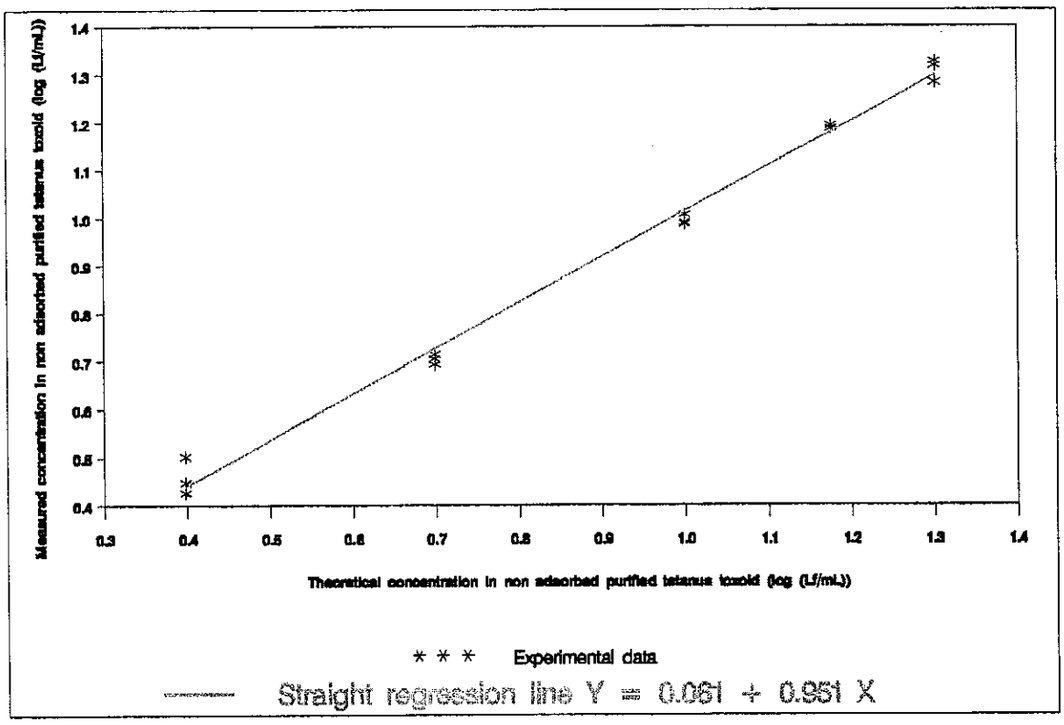
Figure 1: Linearity Graph – Measured Concentration versus the Concentration in Non-Adsorbed Purified Tetanus Toxoid (log(Lf/mL))



2.2.2.1.3 Accuracy

The experimental design was: 3 separated series were performed by 2 operators, on different days.

Each run included the assay of a range of 5 concentrations of purified tetanus toxoid in Hexaxim vaccine (5 spikes of purified tetanus toxoid – Batch no. FA269112 in supernatants of matrix without purified tetanus toxoid – Batch no. BBO09-078).

Batches used for the study are representatives of the production.

2.2.2.1.3.1 Analytical Results

The data subjected to analysis is the concentration in purified tetanus toxoid in Hexaxim vaccine, expressed in Lf/mL.

Table 31 summarizes the results of the study.

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2.2.2.1.3.2 Analysis

The accuracy is tested through the following steps, applied to the data from Table 31:

- Percent recoveries are calculated for each theoretical expected concentration level and for each group;
- Homogeneity of intra-level variances is verified by Cochran's test;
- Non-significance of differences between theoretical expected concentration levels is proved by an analysis of variance if Cochran's test is not significant or by a Welch weighted Anova if Cochran's test is significant;
- When proved the equality of inter-levels means, the average percent recovery is calculated with the 95% confidence limits.

→ Cochran's test is in limit of significance. The slight heteroscedasticity is accepted.

→ The analysis of variance does not allow to conclude to the equality of inter-levels means.

→ The Student-Newman-Keuls' test does allow putting together similar percent recoveries: the theoretical level +1 (2.5 Lf/mL) on one hand and theoretical levels from 2 (5.0 Lf/mL) to +5 (20.0 Lf/mL) on the other hand, whose results are homogeneous together.

→ The average recovery and 95% confidence limits are as follows:

Table 33: Accuracy: Mean Percent Recoveries by Concentration

Calculation of recoveries (%):	
Theoretical concentration level	**Mean**
2.5 Lf/mL	115%
5 Lf/mL	101%
10 Lf/mL	99%
15 Lf/mL	103%
20 Lf/mL	102%

For each theoretical concentration, the mean recovery is included between 80% and 120%.

So, the method is accurate.

2.2.2.1.4 Precision

The experimental design was:

- 3 series were carried out under conditions of intermediate precision: assays were conducted independently using the same method, on a homogeneous primary sample, in the same laboratory, by 3 operators, on different days;

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- Within each serie, 6 assays were carried out under conditions ensuring repeatability: assays were conducted independently using the same method, on a homogeneous primary sample, in the same laboratory, with the same equipment, by the same operator, on 1 day.

The precision is studied with the batch no. IND09014 of Hexaxim Final Bulk Product, representative of the production.

Precision is studied on results expressed in Lf/mL (concentration of non-adsorbed purified tetanus toxoid).

For information, the precision study is also carried out on results expressed in % of adsorption. The results in % are obtained by the following formula:

$$\text{Results \%} = 100 - \frac{\text{Result (Lf/mL)}}{\text{Theoretical concentration of Purified Tetanus Toxoid in vaccine}} \times 100$$

With: concentration TT in vaccine = 10 Lf/dose, that is 20 Lf/mL.

All results are summarized in the following table.

Table 34: Precision: Concentration of Non-Adsorbed Purified Tetanus Toxoid (Lf/mL) and % of Adsoprtion

Serie 1		Serie 2		Serie 3	
Lf/mL	%	Lf/mL	%	Lf/mL	%
13.06	34.7	12.52	37.4	12.41	38.0
12.93	35.4	12.48	37.6	12.07	39.7
13.40	33.0	12.80	36.0	12.09	39.6
13.55	32.3	12.75	36.3	13.27	33.7
12.83	35.9	12.86	35.7	13.24	33.8
14.43	27.9	12.81	36.0	12.85	35.8

2.2.2.1.4.1 Precision on Results Expressed in Lf/mL

The precision of the method is tested through the following steps, applied to the data from Table 34. The homogeneity of intra-groups variances is verified by Cochran's test.

- When acquired the homogeneity, parameters of repeatability and intermediate precision are calculated;
- Overall mean = 1.110 that is **12.90 Lf/mL** in arithmetic form;
- Cochran's test shows that the variances of the 3 series are homogeneous.

Table 35: Precision: Repeatability and Intermediate Precision Characteristics

Characteristics	Relative standard deviation	Standard deviation	95% confidence interval
Repeatability characteristics (1 run)	3.60%	0.016	± 0.033 ; that is ×/ 1.08 Lf/mL in arithmetic form
Intermediate precision characteristics (1 run, 1 measurement)	4.47%	0.019	± 0.041 ; that is ×/ 1.10 Lf/mL in arithmetic form

The 95% confidence intervals of repeatability and intermediate precision are lower or equal than x/÷ 1.4. So, the method is precise.

2.2.2.1.4.2 Precision on Results Expressed in Percent of Adsorption

The precision of the method is tested through the following steps, applied to the data from Table 34.

- The homogeneity of intra-groups variances is verified by Cochran's test;
- When acquired the homogeneity, parameters of repeatability and intermediate precision are calculated;
- Overall mean = 35.5%;
- Cochran's test shows that the variances of the 3 series are homogeneous.

Table 36: Precision: Repeatability and Intermediate Precision Characteristics

Characteristics	Relative standard deviation	Standard deviation	95% confidence interval
Repeatability characteristics (1 run)	6.66%	2.363	± 5.036%
Intermediate precision characteristics (1 run, 1 measurment)	8.27%	2.933	± 6.189%

2.2.2.2 Conclusion

The method is specific.

The method is linear on the range [2.3 - 23.8] Lf/mL.

The accuracy is proved on the same range, since all the percent recoveries are included between 80% and 120%.

The method is precise since:

- The 95% confidence interval of repeatability is x/÷ 1.08;
- The 95% confidence interval of intermediate precision is x/÷ 1.10.

For the analysed data **expressed in % of adsorption**, the relative standard deviation of repeatability and intermediate precision are respectively equal to 6.7% and 8.3%, and the confidence interval of intermediate precision is ± 6% for **1 run with 1 measurement** usually done.

The immunoelectrophoresis method is valid to quantify the non-adsorbed purified tetanus toxoid in the Hexaxim vaccine.

2.2.3 Rat Immunogenicity Assay for IPV

This assay is based on the Ph. Eur. 2.7.20. For compendial assays, test methods described in a monograph of a compendium such as Ph. Eur. or any other recognized pharmacopeia need not be validated for accuracy and reliability, but their suitability under actual conditions of use should be verified. Since this is a compendial method, only intermediate precision was assessed. The results are summarized in Table 37.

Table 37: Rat Immunogenicity Assay for IPV - Validation Summary

Characteristics	Acceptance criteria	Results
Intermediate precision	The values for the individual endpoint titers for each analyst on the same day did not differ by more than ± 2-fold of the rounded mean endpoint titer	Analyst 1: < 2 fold Analyst 2: < 2 fold

2.2.3.1 Intermediate Precision

Intermediate precision refers to within-laboratory variations, which is demonstrated by tests that are performed on different days, by different analysts or using different equipment.

Intermediate precision was assessed from results obtained for the in-house standard serum, tested by 2 analysts on 2 days for a total of 4 assays. The endpoint for in-house standard serum was calculated for each analyst. The rounded endpoint titers for the 2 analysts were averaged to obtain a rounded mean endpoint titer. The results were then compared. The values for the individual endpoint titers for each analyst on the same day did not differ by more than ± 2-fold of the rounded mean endpoint titer (Table 38 and Table 39).

Intermediate precision was also determined using results obtained by 2 analysts on 2 days using different 5 increasing concentrations of serum samples. The $\log_2$ titers for each set of 10 rats for each poliovirus serotype were calculated. The titers for 2 analysts were averaged to obtain a mean titer and then compared. The mean titers for each analyst were found to be within ± 1 logarithm of the mean titer (Table 40 and Table 38).

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