Feasibility Study to Develop a Common *In Vitro* D Antigen Assay for Inactivated Poliomyelitis Vaccines

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ABSTRACT

A feasibility study was organised to determine the possibilities for development of a common in vitro assay for determination of D-antigen content in inactivated poliomyelitis vaccines (IPV). 3 different methods were tested on a selection of non-combined IPV vaccines from the European market. The results of this preliminary study suggest that for vaccines with a similar strain composition similar results would be achieved regardless of which of the three methods was used. Nevertheless, for one vaccine with a slightly different strain composition the results obtained depended on which method was applied. This highlights the need to take into account the strain composition in any future development of a common method. The study also highlighted the importance of standardising the statistical approach to analysis of results, since one laboratory obtained different sets of results by applying different statistical analysis to the same raw data. While no immediate need was seen for a large collaborative study to establish a common method, participants encouraged the idea of further study, in particular with respect to the different strain compositions. Adaptation of a common method will also require further analysis of the needs for combined vaccines, including the steps and conditions for de-sorption.

KEYWORDS

Feasibility study, poliomyelitis vaccine (inactivated), IPV, D-antigen assay, Ph. Eur. BRP.

1. AIM

The main objective of this feasibility study is to evaluate the ability of three different methods to determine the D antigen content in a selection of non-combined IPV vaccines from the European market. All three of the methods use polyclonal antibodies for capture while 2 of the 3 use polyclonal antibodies also for the detection and the third method uses monoclonal antibodies for detection. Results obtained will be used to determine the need for development of a common assay.

2. INTRODUCTION

In vitro potency assays are performed regularly in the control of IPV vaccines by both manufacturers and official control authorities throughout Europe and worldwide. The *in vitro* potency is a determination of the D antigen content for poliovirus strains types 1, 2 and 3 in the vaccine using a validated ELISA test and a suitable reference preparation such as the European Pharmacopoeia Biological Reference Preparation batch 2 for IPV D antigen assay [1].

Presently, individual laboratories use validated in-house methods for the determination of D antigen content. These methods differ in the type of antibodies used for capture and detection (polyclonal versus monoclonal), buffers used for the various incubation steps, conjugation or not of the detection antibody plus slight differences in incubation times, blocking and washing steps.

Use of a common reference preparation is an invaluable tool for providing comparability between assays. Nevertheless, there is an interest in developing a common assay for in vitro D antigen determination, in order to allow more direct comparison lab to lab and vaccine to vaccine. This could be especially important in light of the possible introduction of new IPV vaccines as a result of the anticipated cessation of use of OPV vaccines as part of the global polio eradication program [2]. These factors have prompted an investigation into the feasibility of using common reagents and a common protocol for all products.

Comparison of two different assays involving polyclonal antibodies for detection on two vaccines from two different manufacturers suggested that use of polyclonal antibodies for detection could provide a common method suitable for all [S. Morgeaux, personal communication, EDQM unpublished document]. This observation was also supported by the results of the collaborative study to establish European Pharmacopoeia Biological Reference Preparation batch 2 for IPV D antigen assay [1].

It was therefore agreed to perform a small-scale feasibility study, in which 3 official medicines control laboratories would test IPV vaccines available on the European market using 3 different ELISA assays: two involving the use of polyclonal antibodies for capture and detection, the third using polyclonal antibodies for capture and monoclonal antibodies for detection.

The *in vitro* assay for IPV is complicated by the fact that there are 3 different strain types to be assayed separately in each vaccine. In addition, when IPV is found in combined vaccines a de-sorption step is required. Each manufacturer has its own specific de-sorption protocol which may or may not be compatible with the proposed common assay. As a first step it was agreed to investigate the single component IPV vaccines, which do not require an additional de-sorption step.

If a common assay appears to ameliorate the between lab variation and is suitable for all vaccine types the possibility of producing reference reagents (ie antibodies -polyclonal or monoclonal) for use in a common assay would be considered. The ability to use the chosen method on combined vaccines (which will require a de-sorption step) will also have to be addressed before the full benefit of a common method can be evaluated.

3. PARTICIPANTS

Three laboratories from European OMCLs participated in this study. They have been randomly assigned code numbers (1-3) and are referred to as such throughout the report. A list of participants can be found in section 8.

4. STUDY DESIGN, MATERIALS AND METHODS

4.1 Study design

Two ELISA methods using polyclonal antibodies for capture and detection (one direct and one indirect) and one

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ELISA method using polyclonal antibodies for capture and specific monoclonal antibodies for detection were evaluated. 4 different non-combined IPV vaccines were used as samples. They represent the vaccines available on the European market. The Ph. Eur. BRP batch 2 for D antigen assay of IPV vaccines was used as reference in all assays.

Participants were requested to perform three independent assays for D antigen content using each of three different D antigen ELISA methods on all of the samples. Separate assays of an individual method were to be carried out at least one week apart. Six dilutions per sample with at least two dilution series of each were to be performed for each type (1, 2 and 3). A standard plate layout was used. Participants were requested to supply details of any modifications to the method with the results of the experiments.

Participants were encouraged to carry out a preliminary assay to determine appropriate dilutions for each sample. Some indications for starting dilutions were provided in the detailed individual protocols.

4.2 Materials

4.2.1 IPV Ph. Eur. BRP, batch 2

Ph. Eur. BRP for IPV batch 2 produced by Aventis Pasteur, was a concentrated trivalent bulk prepared as routine production batch. An assigned antigen content of 320-67-282 D antigen per ml for poliovirus type 1, 2 and 3 respectively was established in a collaborative study [1]. Strains for type 1, 2 and 3 are Mahoney, MEF 1 and Saukett respectively.

4.2.2 Sample A

This is a routine production trivalent IPV vaccine lot from a European manufacturer. The estimated potency provided by the manufacturer is 34 -6 - 30 D Ag units per ml for types 1, 2 and 3 respectively. Strains for type 1, 2 and 3 are the same as for the Ph. Eur. BRP.

4.2.3 Sample B

This is a routine production trivalent IPV vaccine lot from a European manufacturer. The estimated potency provided by the manufacturer is 69.2 - 14.2 - 63.8 D Ag units per ml for types 1, 2 and 3 respectively. Strains for type 1, 2 and 3 are the same as for the Ph. Eur. BRP.

4.2.4 Sample C

This is a routine production trivalent IPV vaccine lot from a European manufacturer. The estimated potency provided by the manufacturer is 27 - 5 - 10 D Ag units per ml for types 1, 2 and 3 respectively. Strains for type 2 and 3 are the same as for the Ph. Eur. BRP, a different strain, derived from Brunehilde, is used for type 1.

4.2.5 Sample D

This is a routine production trivalent IPV vaccine bulk from a European manufacturer. The estimated potency is 87 - 16.8 - 66 D Ag units per ml for types 1, 2 and 3 respectively. Strains for type 1, 2 and 3 are the same as for the Ph. Eur. BRP.

4.2.6 Method A

Method A is an indirect ELISA using polyclonal antibodies specific for the type and strain in question for plate coating and capture. After incubation with the test vaccines the bound antigen is detected with polycolonal antibodies specific for the type and strain in question. Anti-species-specific peroxidase-conjugated antibody is used for visualisation.

Method A kit provided to participants contained sufficient material to perform the three independent assays requested and an additional preparatory assay.

Each kit contained:

– polyclonal coating antibody type 1, coating antibody type 2, coating antibody type 3;

– polyclonal detection antibody type 1, detection antibody type 2, detection antibody type 3.

Polyclonal antibodies were raised against Mahoney, MEF 1 and Saukett strain for type 1, type 2 and type 3 respectively.

4.2.7 Method B kit

Method B is a direct ELISA using polyclonal antibodies specific for the type and strain in question for plate coating and capture. After incubation with the test vaccines the bound antigen is detected with polyclonal antibodies specific for the type and strain in question conjugated to peroxidase for visualisation.

Method B kit contained sufficient material to perform the three independent assays requested and an additional preparatory assay.

Each kit contained:

– polyclonal coating antibody type 1, coating antibody type 2, coating antibody type 3;

– polyclonal conjugated detection antibody type 1, conjugated detection antibody type 2, conjugated detection antibody type 3.

Polyclonal antibodies were raised against Mahoney, MEF 1 and Saukett strain for type 1, type 2 and type 3 respectively.

4.2.8 Method C kit

Method C is an indirect ELISA using polyclonal antibodies specific for the type and strain in question for plate coating and capture. After incubation with the test vaccines the bound antigen is detected with monoclonal antibodies specific for the type and strain in question. Anti-species-specific peroxidase-conjugated antibody is used for visualisation.

Method C kit contained sufficient material to perform the three independent assays requested and an additional preparatory assay.

Each kit contained:

– polyclonal capture antibody type 1, capture antibody type 2, capture antibody type 3;

– monoclonal detection antibody type 1 (against Mahoney), monoclonal detection antibody type 2 (against MEF 1), monoclonal detection antibody type 3 (against Saukett).

5. STATISTICAL ANALYSIS AND RESULTS

Three (3) laboratories reported results. Throughout this report they are referred to by their code numbers (1 to 3) allocated at random. Each laboratory carried out 3 assays with each of the three methods under investigation (A, B and C) and for each virus type (1, 2 and 3), as requested. Laboratory 2 reported a problem with a plate for virus type 3 for which no results were obtained. This yielded a total of 80 micro titre plates for statistical analysis. Each plate included the Ph. Eur. BRP Batch 2 and 4 non-combined IPV vaccines (samples A, B, C and D) in duplicate serial dilution series.

Table 1 – *Overview of results (calculated at EDQM)*

			Type 1					Type 2					Type 3				
			Assay					Assay					Assay				
			1	2	3	GM	(gcv)	1	2	3	GM	(gcv)	1	2	3	GM	(gcv)
Sample A	Method A	Lab 1	30.6	35.6	33.1	33.1	(7.9)	6.0	6.5	6.4	6.3	(4.1)	28.3	30.2	26.3	28.2	(7.2)
		Lab 2	38.3	34.9	29.1	33.9	(14.9)	7.5	7.2	6.4	7.0	(8.6)	37.9	29.9	27.2	31.3	(18.7)
		Lab 3	39.5	35.1	38.5	37.7	(6.4)	7.4	7.7	7.2	7.4	(3.6)	35.3	32.1	30.9	32.7	(7.0)
		GM				34.8	(7.2)				6.9	(8.6)				30.7	(7.9)
	Method B	Lab 1	27.0	29.8	26.4	27.7	(6.7)	6.2	6.4	6.2	6.3	(2.1)	25.1	26.0	23.3	24.8	(5.6)
		Lab 2	39.2	34.6	35.4	36.4	(6.8)	7.8	7.2	7.5	7.5	(4.2)	28.2	26.3	26.8	27.1	(3.5)
		Lab 3	31.0	30.0	33.6	31.5	(6.1)	7.0	6.9	6.9	6.9	(0.5)	30.8	26.8	28.1	28.5	(7.2)
		GM				31.7	(14.6)				6.9	(9.4)				26.8	(7.4)
	Method C	Lab 1	31.4	34.4	35.0	33.6	(5.9)	5.9	6.3	7.0	6.4	(9.2)	39.5	33.9	37.3	36.8	(8.1)
		Lab 2	36.2	inv.	22.8	28.7	(38.8)	7.1	6.5	4.7	6.0	(24.3)	32.9	inv.	24.0	28.1	(25.0)
		Lab 3	17.7	18.7	16.9	17.7	(5.1)	3.3	3.5	2.4	3.0	(22.0)	28.0	34.0	33.2	31.6	(11.1)
		GM				25.8	(39.4)				4.9	(51.4)				32.0	(14.5)
			Assay					Assay					Assay				
			1	2	3	GM	(gcv)	1	2	3	GM	(gcv)	1	2	3	GM	(gcv)
Sample B	Method A	Lab 1	60.8	50.4	69.2	59.6	(17.3)	14.0	11.8	13.3	13.0	(9.0)	59.0	55.5	56.3	56.9	(3.2)
		Lab 2	69.4	71.3	49.0	62.4	(23.2)	15.4	15.4	12.4	14.3	(13.2)	74.0	74.1	53.7	66.5	(20.3)
		Lab 3	69.4	62.0	63.7	65.0	(6.1)	15.0	15.1	13.9	14.7	(4.8)	74.1	68.7	54.1	65.1	(17.8)
		GM				62.3	(4.4)				14.0	(6.5)				62.7	(8.8)
	Method B	Lab 1	55.0	54.3	49.0	52.7	(6.5)	13.4	14.0	13.6	13.7	(2.2)	52.9	58.7	52.8	54.8	(6.2)
		Lab 2	69.9	66.2	67.8	67.9	(2.7)	15.8	16.4	16.1	16.1	(2.0)	61.5	51.4	56.4	56.3	(9.4)
		Lab 3	59.0	63.8	62.5	61.7	(4.1)	15.2	15.1	15.8	15.4	(2.3)	62.9	55.7	59.2	59.2	(6.3)
		GM				60.5	(13.6)				15.0	(8.8)				56.7	(4.0)
	Method C	Lab 1	68.2	53.6	67.4	62.7	(14.5)	14.2	13.5	12.8	13.5	(5.2)	77.9	55.0	62.4	64.4	(19.3)
		Lab 2	62.2	inv.	74.1	67.9	(13.2)	14.0	21.3	15.3	16.6	(24.7)	57.6	inv.	85.6	70.2	(32.4)
		Lab 3	76.4	89.8	64.3	76.1	(18.2)	18.5	16.1	10.4	14.6	(35.4)	141.4	150.1	175.5	155.0	(11.8)
		GM				68.7	(10.3)				14.8	(10.9)				88.8	(62.3)
			Assay					Assay					Assay				
			1	2	3	GM	(gcv)	1	2	3	GM	(gcv)	1	2	3	GM	(gcv)
Sample C	Method A	Lab 1	13.3	12.7	11.8	12.6	(6.2)	2.9	3.0	2.6	2.8	(7.4)	8.9	9.1	9.1	9.0	(1.5)
		Lab 2	13.0	13.9	12.6	13.2	(5.1)	3.0	3.2	2.6	2.9	(11.9)	8.8	9.2	8.8	8.9	(2.9)
		Lab 3	12.7	10.9	14.8	12.7	(16.4)	3.3	2.8	1.9	2.6	(32.4)	10.4	9.0	11.4	10.2	(12.8)
		GM				12.8	(2.3)				2.8	(4.8)				9.4	(8.0)
	Method B	Lab 1	8.1	8.5	8.3	8.3	(2.9)	2.8	3.2	3.2	3.1	(7.6)	6.0	6.4	6.4	6.2	(3.6)
		Lab 2	7.7	7.5	8.1	7.8	(4.2)	3.1	3.1	3.3	3.2	(2.5)	6.6	6.9	8.3	7.2	(13.0)
		Lab 3	7.3	8.1	7.7	7.7	(5.0)	3.0	2.4	3.2	2.9	(15.5)	3.5	2.9	3.1	3.2	(10.8)
		GM				7.9	(4.3)				3.0	(5.5)				5.2	(55.3)
	Method C	Lab 1	22.6	19.9	19.3	20.5	(8.7)	3.1	3.2	3.3	3.2	(3.7)	23.3	21.1	18.5	20.8	(12.2)
		Lab 2	20.6	inv.	12.6	16.1	(41.3)	3.1	3.1	2.9	3.0	(3.4)	13.5	inv.	17.7	15.5	(20.9)
		Lab 3	20.7	22.8	21.5	21.7	(5.1)	2.9	3.1	2.3	2.8	(18.2)	18.1	17.8	20.3	18.7	(7.5)
		GM				19.3	(17.1)				3.0	(7.6)				18.2	(16.2)
			Assay					Assay					Assay				
			1	2	3	GM	(gcv)	1	2	3	GM	(gcv)	1	2	3	GM	(gcv)
Sample D	Method A	Lab 1	82.8	76.6	72.9	77.3	(6.6)	17.1	14.6	14.9	15.5	(9.0)	59.0	60.7	59.7	59.8	(1.4)
		Lab 2	54.6	85.2	67.2	67.9	(24.9)	12.0	16.3	14.8	14.3	(17.0)	53.1	67.5	66.5	62.0	(14.4)
		Lab 3	64.5	89.3	89.8	80.2	(20.9)	15.0	17.3	15.3	15.9	(8.1)	69.3	59.8	67.7	65.5	(8.2)
		GM				75.0	(9.2)				15.2	(5.7)				62.4	(4.7)
	Method B	Lab 1	82.7	82.1	76.6	80.4	(4.3)	14.8	15.3	15.7	15.3	(2.9)	61.6	65.4	64.1	63.7	(3.1)
		Lab 2	85.7	85.2	100.3	90.1	(9.7)	16.5	16.3	19.5	17.4	(10.5)	65.3	65.5	71.9	67.5	(5.6)
		Lab 3	76.8	75.7	74.4	75.6	(1.6)	17.7	16.4	16.6	16.9	(4.3)	67.3	65.3	62.5	65.0	(3.8)
		GM				81.8	(9.3)				16.5	(7.1)				65.4	(3.0)
	Method C	Lab 1	88.9	84.0	80.5	84.4	(5.2)	16.4	16.5	20.0	17.6	(12.1)	73.0	59.7	73.8	68.5	(12.7)
		Lab 2	101.5	inv.	59.0	77.3	(46.8)	18.3	13.4	12.2	14.4	(23.9)	63.0	inv.	76.1	69.2	(14.3)
		Lab 3	82.5	92.4	89.5	88.1	(6.0)	15.5	18.3	15.2	16.2	(10.7)	153.1	145.4	179.1	158.5	(11.5)
		GM				83.1	(6.8)				16.0	(10.5)				90.9	(61.8)

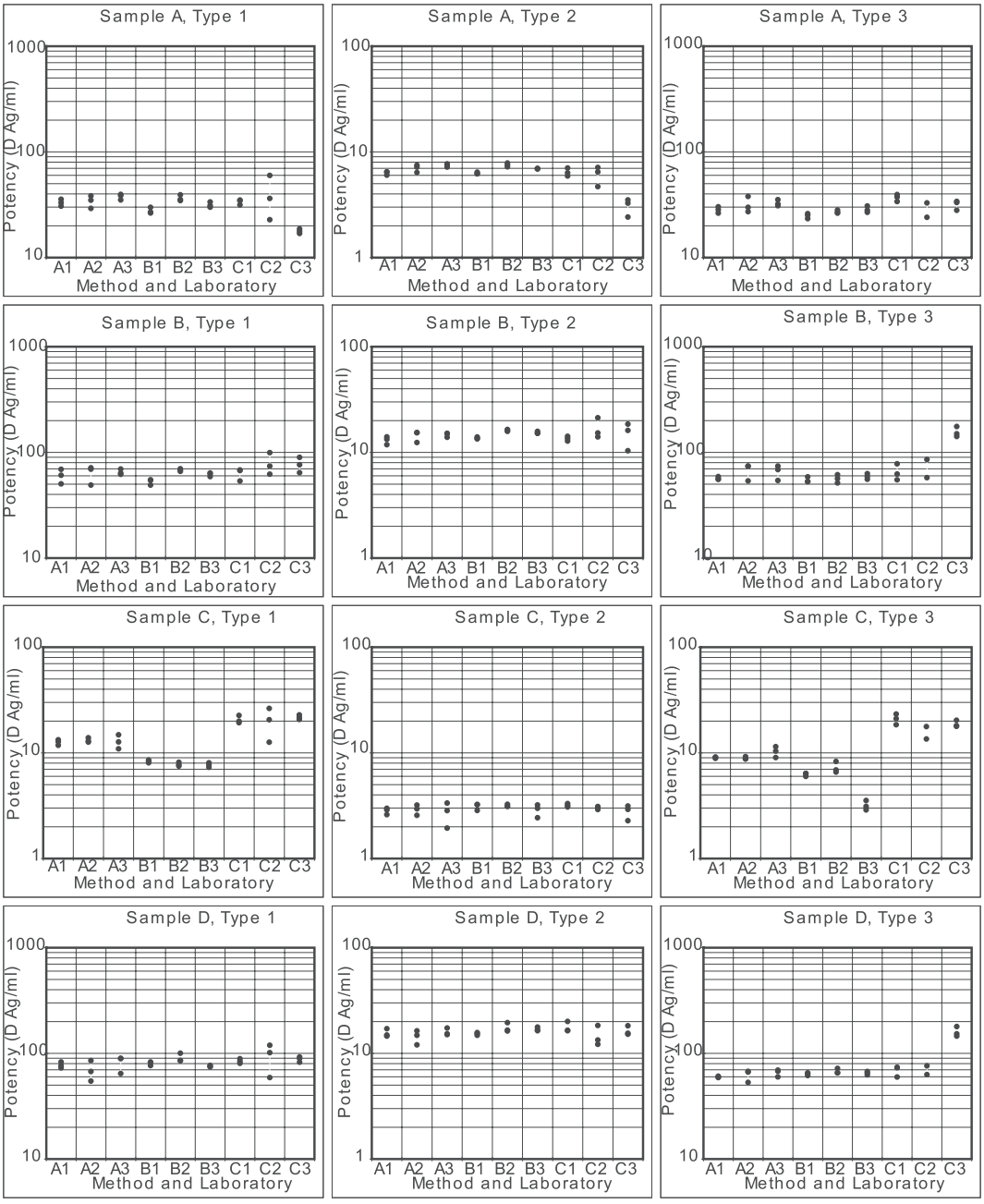
Explanations:
Listed are the estimated potencies of each assay in D Ag/ml.
GM=Geometric mean. gcv=Geometric coefficient of variation.
inv.=invalid assay.

Table 2 – *Overview of results (calculated by participants)*

			Type 1				Type 2				Type 3			
			Assay				Assay				Assay			
			1	2	3	GM (gcv)	1	2	3	GM (gcv)	1	2	3	GM (gcv)
Sample A	Method A	Lab 1	29.8	38.6	34.2	34.0(13.8)	6.2	6.2	6.4	6.3 (1.8)	29.6	30.2	28.4	29.4 (3.2)
		Lab 2	34.9	32.4	27.9	31.6(12.1)	7.3	7.4	6.7	7.1 (5.5)	36.6	32.3	28.0	32.1 (14.3)
		Lab 3	29.2	37.8	37.7	34.7(16.1)	7.0	7.7	7.0	7.2 (5.8)	30.9	36.6	16.8	26.7 (50.4)
		GM				33.4 (5.0)				6.9 (8.1)				29.3 (9.7)
	Method B	Lab 1	31.2	34.8	33.8	33.2 (5.8)	6.4	6.6	6.6	6.5 (1.8)	27.8	28.0	26.0	27.3 (4.2)
		Lab 2	39.3	34.0	38.6	37.2 (8.2)	7.9	7.2	8.3	7.8 (7.5)	28.7	26.4	30.7	28.5 (7.9)
		Lab 3	32.8	31.9	35.9	33.5 (6.4)	6.6	6.9	6.8	6.8 (2.2)	29.4	27.0	30.9	29.1 (7.0)
		GM				34.6 (6.5)				7.0 (9.6)				28.3 (3.4)
	Method C	Lab 1	33.0	34.0	35.8	34.2 (4.2)	6.2	6.0	7.6	6.6 (13.7)	35.6	31.6	34.4	33.8 (6.3)
		Lab 2	33.3	50.9	35.3	39.1(25.9)	7.9	6.2	4.6	6.1 (31.1)	29.6	inv.	24.0	26.7 (16.0)
		Lab 3	18.5	18.1	16.9	17.8 (4.7)	3.1	3.4	2.4	2.9 (20.7)	58.3	119.7	115.6	93.1 (50.0)
		GM				28.8(52.3)				4.9 (56.6)				43.8 (94.3)
			Assay				Assay				Assay			
			1	2	3	GM (gcv)	1	2	3	GM (gcv)	1	2	3	GM (gcv)
Sample B	Method A	Lab 1	59.4	53.4	63.0	58.5 (8.7)	14.2	13.0	14.0	13.7 (4.9)	62.6	59.8	60.8	61.1 (2.3)
		Lab 2	68.4	69.2	50.4	62.0 (19.7	15.8	15.6	12.3	14.5(15.1)	76.0	73.1	44.8	62.9 (34.3)
		Lab 3	47.6	63.4	69.2	59.3 (21.6	14.5	15.2	14.5	14.7 (3.0)	72.2	78.7	52.3	66.7 (24.1)
		GM				59.9 (3.1)				14.3 (3.7)				63.5 (4.6)
	Method B	Lab 1	63.6	65.8	57.8	62.3 (7.0)	13.8	15.2	13.8	14.3 (5.7)	57.4	63.4	57.0	59.2 (6.1)
		Lab 2	69.0	65.9	66.5	67.1 (2.5)	16.0	16.2	15.8	16.0 (1.3)	63.8	52.5	62.4	59.3 (11.3)
		Lab 3	64.9	69.3	68.5	67.5 (3.5)	14.9	14.8	15.6	15.1 (2.7)	60.5	52.8	68.3	60.2 (13.7)
		GM				65.6 (4.6)				15.1 (6.0)				59.6 (0.9)
	Method C	Lab 1	71.2	57.8	66.2	64.8(11.2)	14.8	13.6	12.0	13.4(11.1)	75.8	58.8	65.2	66.2 (13.6)
		Lab 2	56.6	87.6	92.1	77.0(30.7)	14.9	19.0	15.0	16.2(14.8)	60.1	inv.	63.7	61.9 (4.2)
		Lab 3	70.0	110.1	63.9	79.0(33.8)	17.4	15.6	9.9	13.9(35.0)	299.3	354.2	inv.	325.6 (12.6)
		GM				73.3(11.3)				14.5(10.5)				110.1(155.9)
			Assay				Assay				Assay			
			1	2	3	GM (gcv)	1	2	3	GM (gcv)	1	2	3	GM (gcv)
Sample C	Method A	Lab 1	14.2	14.6	12.4	13.7 (9.1)	2.8	3.0	2.8	2.9 (4.1)	8.0	8.4	8.2	8.2 (2.5)
		Lab 2	13.8	14.5	13.2	13.8 (4.8)	3.0	3.5	2.4	2.9 (20.9)	8.7	8.1	7.3	8.0 (9.2)
		Lab 3	10.8	11.4	15.7	12.5(22.4)	3.0	2.9	1.6	2.4 (40.3)	9.6	9.4	11.3	10.0 (10.8)
		GM				13.3 (6.0)				2.7 (11.4)				8.7 (13.3)
	Method B	Lab 1	8.6	inv.	9.0	8.8 (3.3)	2.8	3.2	3.4	3.1 (10.4)	6.4	6.8	6.8	6.7 (3.6)
		Lab 2	7.3	7.6	8.8	7.9 (10.3)	3.1	3.2	3.4	3.2 (4.8)	6.6	6.9	9.0	7.4 (18.3)
		Lab 3	6.4	8.2	8.1	7.5 (14.8)	3.2	2.4	3.1	2.9 (17.2)	3.6	2.6	3.1	3.0 (18.2)
		GM				8.1 (8.3)				3.1 (6.1)				5.3 (62.9)
	Method C	Lab 1	inv.	20.8	20.8	20.8 (0.0)	3.0	3.0	3.2	3.1 (3.8)	14.2	16.0	14.2	14.8 (7.1)
		Lab 2	18.6	22.4	15.0	18.4(22.2)	3.4	3.0	2.9	3.1 (8.7)	12.5	inv.	13.5	13.0 (5.6)
		Lab 3	18.8	23.1	20.7	20.8(10.7)	2.6	3.1	2.2	2.6 (18.6)	30.1	37.2	60.0	40.6 (42.4)
		GM				20.0 (7.3)				2.9 (9.6)				19.8 (86.8)
			Assay				Assay				Assay			
			1	2	3	GM (gcv)	1	2	3	GM (gcv)	1	2	3	GM (gcv)
Sample D	Method A	Lab 1	86.0	80.8	74.6	80.3 (7.4)	16.6	15.0	15.8	15.8 (5.2)	61.4	60.8	60.8	61.0 (0.6)
		Lab 2	56.6	87.9	80.1	73.6 (26.1	12.3	17.2	14.4	14.5 (18.3)	55.0	66.6	66.6	62.5 (11.7)
		Lab 3	84.6	94.9	91.4	90.2 (6.0)	15.2	17.6	14.1	15.6 (11.7)	73.7	74.4	55.4	67.2 (18.3)
		GM				81.1(10.7)				15.3 (4.7)				63.5 (5.2)
	Method B	Lab 1	83.6	84.8	82.8	83.7 (1.2)	15.8	15.6	16.6	16.0 (3.3)	64.0	64.6	64.8	64.5 (0.6)
		Lab 2	85.8	84.3	108.4	92.2(15.1)	16.8	16.4	19.2	17.4 (8.9)	63.8	64.8	73.4	67.2 (8.0)
		Lab 3	85.7	81.2	81.6	82.8 (3.1)	17.1	17.2	16.4	16.9 (2.8)	61.6	65.6	63.6	63.6 (3.2)
		GM				86.1 (6.1)				16.8 (4.4)				65.1 (2.9)
	Method C	Lab 1	86.4	83.4	86.4	85.4 (2.1)	16.8	16.6	21.6	18.2(16.0)	68.2	60.6	70.8	66.4 (8.5)
		Lab 2	101.2	111.7	73.8	94.1(24.2)	19.0	14.3	12.1	14.9(25.6)	64.6	inv.	50.9	57.3 (18.4)
		Lab 3	88.3	87.7	42.5	69.0(52.3)	14.9	17.2	14.7	15.6 (9.3)	277.0	390.5	602.8	402.5 (47.6)
		GM				82.2(17.2)				16.1(11.2)				115.3(196.0)

Explanations:
Listed are the estimated potencies of each assay in D Ag/ml.
GM=Geometric mean. gcv=Geometric coefficient of variation.
inv.=invalid assay.

Figure 1 – *Graphical overview of estimated potencies (D Ag/ml)*



Explanations: each dot represents a potency estimate.
The method and laboratory code are indicated on the x-axes. (e.g. A1 means Method A, Laboratory 1).

For each micro titre plate, a least squares 4-parameter logistic curve model was fitted to the data (optical density (*y*) versus ln dose (*x*)) using the equation $y = d + \frac{a-d}{1+e^{-b(x-c)}}$. In some cases there were insufficient data points available to estimate the upper asymptote. In those cases an exponential model with addition was fitted using the equation $y = d + e^{b(x-c)}$. In these equations *d* is the lower asymptote, *a* the upper asymptote, *b* the common slope and *c* the location parameter for each preparation.

The potencies thus obtained were compared with the calculated potencies by the participants. In some cases a quite large difference between EDQM calculations and participants' calculations was observed, notably for laboratory 3.

Assay 2 with Method C from Laboratory 2 was excluded from analyses due to unacceptably high intra-assay variation. All other assays were judged valid for inclusion in the analysis, although the laboratories themselves rejected some of these. Assays were judged valid if there were no significant deviations from linearity and/or parallelism ($p \geq 0.01$). If significant deviations were observed, the assays were nevertheless judged valid if the correlation coefficient (r) of the fit was at least 0.99 and visual inspection of the plots did not reveal any anomalies. A complete overview of potency estimates is given in Table 1 (EDQM calculations). The geometric mean (GM) and the geometric coefficient of variation (gcv) of the 3 assays are also listed, together with an overall GM and inter-laboratory gcv for each sample, method and virus type. A graphical impression is given in Figure 1 (EDQM calculations). Results from calculations by the participants are presented in Table 2 for information, but further discussion of results will be based on the central calculations at EDQM.

Most striking is that results for Sample C, Types 1 and 3 depend clearly on the method used. For type 1 the methods A, B and C give about 12.8, 7.9 and 19.3 D Ag/ml respectively. For type 3 they give about 9.4, 5.2, and 18.2 D Ag/ml respectively. This demonstrates the need to consistently use the same method for this vaccine. Samples A, B and D seem to depend less on the method used or in any case, such a dependency is less obvious.

In terms of repeatability method B performs best with a median gcv of 4.3%, followed by method A with a median gcv of 8.4% and method C with a median gcv of 12.5%. Because sample C had a somewhat different virus strain composition, the repeatability was also calculated excluding this sample. This had hardly any influence on the result, with median gcv's in this case of 4.3% for method B, 8.6% for method A, and 13.2% for method C. In terms of reproducibility methods A and B perform equally well with a median gcv of 6.8% and 7.2% respectively followed by method C with a median gcv of 15.4%. It should be noted, however, that method C is the method the laboratories are least familiar with.

Table 3 shows the overall GM for each sample and method, together with the estimates provided by the respective manufacturers obtained with their in-house methods. A large discrepancy between the manufacturer's result and the participants can be observed for sample C, Type 1 with methods A and B and to a lesser extent also with method C. For Sample D and B, Type 3 a large difference can be observed with method C. This is however entirely due to the very high results from Laboratory 3. For sample A, Type 1 and 2, a lower value is seen using method C.

Table 3 – *Overview of average results*

	Sample A					
	Type 1		Type 2		Type 3	
Manufacturer	34,4		5,6		30,4	
Method A	34,8	(7,2)	6,9	(8,6)	30,7	(7,9)
Method B	31,7	(14,6)	6,9	(9,4)	26,8	(7,4)
Method C	25,8	(39,4)	4,9	(51,4)	32,0	(14,5)

	Sample B					
	Type 1		Type 2		Type 3	
Manufacturer	69.2		14.2		63.8	
Method A	62,3	(4,4)	14,0	(6,5)	62,7	(8,8)
Method B	60,5	(13,6)	15,0	(8,8)	56,7	(4,0)
Method C	68,7	(10,3)	14,8	(10,9)	88,8	(62,3)

	Sample C					
	Type 1		Type 2		Type 3	
Manufacturer	27,0		5,0		10,0	
Method A	12,8	(2,3)	2,8	(4,8)	9,4	(8,0)
Method B	7,9	(4,3)	3,0	(5,5)	5,2	(55,3)
Method C	19,3	(17,1)	3,0	(7,6)	18,2	(16,2)

	Sample D					
	Type 1		Type 2		Type 3	
Manufacturer	87,0		16,8		66,0	
Method A	75,0	(9,2)	15,2	(5,7)	62,4	(4,7)
Method B	81,8	(9,3)	16,5	(7,1)	65,4	(3,0)
Method C	83,1	(6,8)	16,0	(10,5)	90,9	(61,8)

Explanation:
Listed are for each method the overall GM and gcv between parentheses of the participating laboratories.
Also listed are the manufacturer's assay result from the release protocol using their in-house method.

6. DISCUSSION

The differences observed in values calculated in laboratory 3 as compared to those calculated at EDQM involved primarily type 1 for sample B and D and type 3 for all samples using method C. Investigation by laboratory 3 revealed that most of the observed differences were linked to the use of a statistical program that automatically selects points for optimal linearity and parallelism. Re-calculation of the data using a 4 parameter curve with no automated selection gave results which more closely resembled those from EDQM. This highlights the importance of standardisation of statistical approaches to data evaluation. The results from lab 3 for type 3 using method C, on samples B and D are significantly higher than those for the other 2 laboratories, even using the EDQM calculation. The protocol and dilution strategies were reviewed and no immediate explanation was found for this observation.

With the exception of sample C types 1 and 3 the results using methods A and B are similar. These two methods both use polyclonal antibodies for capture and detection. For method C, which uses monoclonal antibodies for detection, if the inexplicably heterogeneous results for type 3 samples B and D from lab 3 are excluded the remaining results are also largely in agreement with the other methods. It is not clear however if suppression of the data from laboratory three is appropriate. Reports from routine users of method C have also indicated that higher values for type 3 have been observed for the one vaccine tested, as compared to the

manufacturers values which are determined with an assay using polyclonal antibodies. Method C also appears to have the greatest variability of the 3 methods both in and between laboratories. It should however be noted that laboratories were least familiar with this method.

The strain composition of all 4 sample vaccines are the same for types 2 and 3 (MEF 1 and Saukett respectively) however for type 1 sample C differs from samples A, B and D.

The 3 methods give very different results for type 1 of sample C. There is a 2-fold difference between the values obtained with method A and B. The difference may relate to the production of the antibodies. While both method A and B use polyclonal antibodies raised against the Mahoney strain, which may have a weaker affinity for the virus strain used for type 1 of Sample C (Brunehilde), the preparation of the antigen used to produce the antibodies was somewhat different for the two methods. In any case for type 1 all of the methods used give results far below that assigned by the manufacturer which were obtained using an ELISA with polyclonal antibodies raised against a like strain. This suggests that a like versus like situation is preferable and argues against the establishment of a common method for vaccines with different strain compositions.

For sample C type 2 the values obtained with the 3 candidate methods are consistent with each other but are all lower than that predicted by the in-house method of the manufacturer which uses a monoclonal antibody against type 2 MEF 1 as detection antibodies in the ELISA.

For sample C type 3 the differences observed between the methods is more difficult to explain since the same strain is involved as for the other samples, however it has been noted that Saukett strains can vary considerably [3]. The value for method A is approximately the same as that proposed by the manufacturer using their in house method, which uses a monoclonal antibody against Saukett type 3 strain for detection. The value for method B is however almost half that value and method C is almost double the proposed value. The reasons for the higher values observed for type 1 and 3 using method C are unclear. Nevertheless, for evaluation of this vaccine it would appear that use of the same method is important if comparisons are to be made lab to lab.

7. CONCLUSION

The major conclusions of the study are the following.

1) If the results from lab 3 method C type 3 are excluded it appears that for vaccines of the same strain composition the method used does not significantly alter the results obtained. Similar results are observed for 3 of the 4 vaccine types using all 3 methods.

2) The differences observed with sample C highlight the need to pay attention to strain composition and the effect on the method.

3) It is important to use harmonised statistical approaches and tools to ensure comparability of results.

Following on from the results of this study, before a decision is taken to go ahead with a large collaborative study to develop a common method, a number of issues need to be considered. Among the aspects to be clarified are the possibilities of a using common method for vaccines with different strain types. This would entail further investigation of sample C and other vaccines available on the market with different strain composition. In addition, more data on the use of common methods for combination vaccines would be useful. This would include the need to investigate the interchangeability of de-sorption buffers. Participants felt that ideally, a common, manufacturer-independent method is still desirable [EDQM unpublished meeting report], especially in light of the WHO eradication program for poliomyelitis and the probable increased use of inactivated poliomyelitis vaccines as live oral polio vaccines are discontinued [2].

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9. ABBREVIATIONS

BRP: Biological Reference Preparation, EDQM: European Directorate for the Quality of Medicines, ELISA: Enzyme-Linked Immunosorbent Assay, gcv: geometric coefficient of variation, GM: Geometric Mean, IPV: Inactivated Poliomyelitis Vaccine, OMCL: Official Medicines Control Laboratory, OPV: Oral Poliomyelitis Vaccine, Ph. Eur.: Pharmacopée Européenne/European Pharmacopoeia.

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