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**Report of a collaborative study to assess the suitability of a replacement
International Standard for Oral Polio Vaccine**

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SUMMARY

The potency of a trivalent oral poliovaccine (OPV) preparation was assessed by collaborative study as a candidate replacement for the current International Standard (NIBSC reference 85/659). Thirteen laboratories participated in the study and were drawn from five OPV manufacturers and eight National Control Authorities. Five samples were assessed in the study; the current IS, the candidate replacement (NIBSC reference 02/306), the European Pharmacopoeia Biological Reference Preparation, a commercial vaccine and the same vaccine that had been heat stressed. The candidate preparation was tested as a coded duplicate sample in the study. Laboratories were allowed to use their own anti-sera for neutralization in the test (for which nine elected to do so) but were also asked to include the current NIBSC monoclonals which were freeze dried preparations. The overall levels of within assay variability were 0.18 \log_{10} TCID₅₀ (average root mean square difference in \log_{10} titres between duplicate samples C and D). The within laboratory variation gave an average coefficient of variation of 2.50% (between \log_{10} estimates of the same sample across assays). These figures correspond with previous studies and indicate a high level of consistency within laboratories. For all poliovirus types in all study samples the range of values obtained between laboratories were all within 0.5 \log_{10} TCID₅₀ of the mean – indicating good consistency between laboratories. An overall potency obtained for the candidate 2nd IS was 7.51, 6.51, 6.87 and 7.66 \log_{10} TCID₅₀/ml for type 1, 2, 3 and total virus content respectively. There were no problems encountered in the use of the candidate 2nd IS and all other samples behaved as predicted. No substantial differences were obtained with the NIBSC monoclonal antibodies or the in-house antisera.

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PRODUCT SUMMARY FOR THE PROPOSED 2nd INTERNATIONAL STANDARD FOR TRIVALENT ORAL POLIOVACCINE (02/306)

Presentation	Ampoule
Number of ampoules	4600
Excipients/additives	None
Coefficient of variation of the liquid fill	0.09%

INTRODUCTION

Oral Polio Vaccine (OPV) has a pivotal role in the Global Polio Eradication Program organised by the WHO and will continue to do so until eradication is complete. Even though the eradication program is in its final phase the remaining “hot spots” of endemic poliovirus circulation require significant management which will continue to require large amounts of OPV in the short to medium term. In the medium to long term after eradication there will also be the need to maintain stockpiles of the vaccine to deal with any unexpected re-emergence of the virus.

The manufacture and control of this vaccine must therefore be maintained to the highest level to ensure eradication succeeds; therefore suitable standard preparations must be available to ensure testing meets appropriate regulatory requirements.

The 1st International Standard (IS) for the potency estimation OPV (NIBSC Catalogue number: 85/659) was established in 1995 (A B Heath, D J Wood, P D Minor and D I Magrath, REF 95.1821, TRS No 872, 46th report). This IS has been used for the calibration of regional working references (e.g. the European Pharmacopoeia Biological Reference Preparation - BRP) and the in-house references of a number of manufacturers and National Control Laboratories (NCLs). The stocks of this standard are now very low and it is critical that a replacement is established soon to meet the demands of the final stages of the polio eradication program.

A candidate 2nd IS has been prepared by NIBSC in line with the current IS (85/659), but with slightly higher potency to put it more in line with current vaccines. The composition of the formulation for the candidate 2nd IS was otherwise kept as close as possible to the current IS to allow direct comparison of the two preparations e.g. in stability studies. The aim of this study is therefore to assess the suitability of this candidate as a replacement for 85/659 and, if found to be suitable, to determine the potency to be assigned to the preparation.

STUDY PLAN

Aim of the study

To assess the suitability of a candidate replacement for the current international standard for trivalent OPV (85/569), and to determine a titre to be assigned to the preparation.

Study time-frame

Samples were sent to participants in November 2003 who were asked to complete the study and return their results by the 1st March 2004. Most data were returned before the cut-off date but some laboratories had assay problems. Two participants requested repeat samples due to

technical problems, and two laboratories had a problem with their shipments. Data from thirteen laboratories were eventually returned by May 2004.

Participants

Fourteen laboratories agreed to participate in the collaborative study. Thirteen laboratories (8 NCLs, 5 manufacturers) submitted results. These participants are listed in Appendix 1. In the report, participants are referred to by a code number, which is not necessarily related to the order in the list in Appendix 1.

METHODS AND MATERIALS

Preparation of the Candidate 2nd IS

The candidate 2nd IS was produced at NIBSC from three commercially produced monovalent bulks - one of each poliovirus (Sabin) types 1, 2 and 3. The passage level of the virus in the bulks is: Sabin original (SO) + 3 for type 1, SO+3 for type 2 and a re-derived SO (RSO)+3 for type 3. All three bulks used in the production of this standard were produced on primary monkey cells (PMKC). Each of the monovalent bulks had previously been released by a National Control Authority (OMCL) and can therefore be considered to be in compliance with current licensing, Pharmacopoeial and WHO Requirements.

The candidate standard was prepared by blending the three polio serotypes monovalent bulks in MEM with 1% w/v bovine albumin and sodium bicarbonate buffer. Thermal stabilisers were not added to the preparation. The blended bulk was formulated to give titres of 6.50, 5.50 and 6.00 TCID₅₀/0.1ml.

Filling of the Candidate 2nd IS

The preparation was filled in the standards processing division of NIBSC on the 13th January 2003 in a single working session from a single bulk. The ampoules were ink-jet labelled before filling and the total number of ampoules filled was 4837; 26 were sampled at regular intervals through the filling process to establish the precision of the fill. The ampoules were all of neutral borosilicate glass.

The mean weight of the fill was 0.8031g (0.8022 – 0.8045) with a coefficient of variation of 0.09%. The ampoules were sealed under an atmosphere of normal air and then labelled. A total of 4600 ampoules are available for use as a standard. The ampoules are stored at –70^oC at NIBSC and the custodian is Gillian Cooper department of Virology. The NIBSC reference number of the candidate 2nd IS is 02/306.

Stability Studies

Samples of 02/306 were removed from storage at –70^oC and exposed to 4^oC, 20^oC, 37^oC and 45^oC for varying amounts of time. After exposure the samples were refrozen at –70^oC until they were assayed. One vial was used for each temperature/exposure time combination. Assays were conducted at NIBSC using the NIBSC monoclonal antibodies for neutralisation.

Study Samples

All Study Samples (A-F) were stored at –70^oC prior to the study and shipped on dry ice with the recommendation for storage after receipt at –70^oC

Study sample A - WHO IS for OPV

The WHO IS for OPV was supplied by NIBSC (NIBSC code 85/659). Four ampoules were provided to each participant for the study. The assigned titre of the 1st IS is given below.

Standard	Assigned potencies (TCID ₅₀ /0.1 ml)		
	Type 1	Type 2	Type 3
1 st WHO IS 85/659	5.60	4.60	5.20

Study sample B -Ph. Eur. BRP (batch 3)

The BRP was supplied by the European Directorate for the Quality of Medicines (EDQM). Four vials were provided to each participant for the study. The assigned virus content is given below.

Standard	Assigned potencies (TCID ₅₀ /0.1 ml)			
	Type 1	Type 2	Type 3	Total virus
BPR Batch 3	5.99	5.06	5.83	6.20

Study samples C and D - the candidate 2nd IS

The candidate 2nd IS comprised samples C and D. The duplicate sample was included to allow within assay variation to be assessed.

Study sample E - commercial vaccine

This sample was a commercially produced, magnesium chloride stabilised, vaccine (20 dose presentation) formulated to specifications for UNICEF usage (Type 1 \geq 6.0, Type 2 \geq 5.0, Type 3 \geq 5.8 Log₁₀ CCID₅₀ /0.1ml). Each participant was provided with four vials for the study.

Study sample F - low potency sample

Sample F was produced from study sample E by heat treatment for 80 hrs at 37⁰C. Preliminary studies at NIBSC showed the treated sample to have reduced potency. Each participant was provided with four vials for the study.

Monoclonal anti-poliovirus antibodies

Participants were provided with monoclonal antibodies (MAb) use in the study. The MAb's are freeze dried preparations that had been produced at NIBSC. Two vials were provided of each of the MAb's as listed below:

Poliovirus type 1 monoclonal antibody serum 02/256 (NIBSC batch number 425)

Poliovirus type 2 monoclonal antibody serum 02/258 (NIBSC batch number 267)

Poliovirus type 3 monoclonal antibody serum 02/260 (NIBSC batch number 495)

MAb's were stored at -20⁰C prior to the study and shipped on dry ice. Participants were asked to reconstitute one vial of each polio serotype in 1ml of assay media and distribute the contents in 100ul aliquots. These aliquots were to be stored at -20⁰C until required. The second vial of each MAb was to be kept at -20⁰C as a back-up.

The NIBSC MAb's are routinely used globally by a number of manufacturers and NCL's for the assay of trivalent OPV. A recent EDQM collaborative study (BSP048) for the establishment of the new Ph. Eur BRP (batch 3) used these MAb's to evaluate the candidate standard. A working

dilution of 1:500 (after reconstitution in 1 ml) for each of the MAb's had been previously established and was therefore recommended to the participants for the current study.

In-house antisera

Participants were also given the opportunity to assay the study samples using their own antisera - if different from the NIBSC MAb's. The details of the in-house antisera used by the participants that elected to take this option are shown in Table 1.

Study Methodology

Participants were asked to assay the coded samples A-F concurrently on three separate occasions, ideally one week apart. Those participants intending to use their in-house antisera in addition to the monoclonal antibodies were asked to perform the additional assay in parallel, using a common titration.

Participants were requested to make one titration per sample and to determine the virus titre of each of the three polio serotypes and the total virus content for each of the coded samples. Total virus content of each sample was the titre in the absence of antibody neutralisation. Laboratories used their in-house potency methods to evaluate the samples. Type specific monoclonal antibodies were provided by NIBSC for the neutralisation of two of the three polio serotypes present in the samples.

Participants were also requested to vary the order the samples (A-F) were tested in each assay - this was to ensure that there was no bias present in the testing schedule. Sufficient samples were provided to each participant for a repeat assay if the dilution series used in the in-house method did not give 0% and 100% infection for each test sample.

Documentation and submission of results

Participants were asked to return all raw data and calculated potencies to NIBSC on the forms provided. It was requested that the data be submitted electronically and that a hard copy of the results also be sent.

Statistical Methods

Participants provided calculated potencies using the methods shown in Table 1. Potencies were reported as TCID₅₀/0.1ml. All subsequent analysis was based on the potencies calculated by the participants.

Variation between duplicate samples C and D was assessed by calculating root mean square (r.m.s.) difference in log₁₀ titres. This is calculated by taking the difference in titre between C and D within each assay, taking the square, taking the mean of the square across assays, and taking the square root, to give an average difference in units of log₁₀ titre. The r.m.s. values were calculated for each laboratory and polio type separately.

Variation between assays was assessed by calculating the percentage coefficient of variation (cv) of the log₁₀ titre estimates across the three assays for each of the laboratories, polio types and study samples. These cv's were then pooled across the six samples to give a single figure for each laboratory and polio type. Mean values for individual laboratories, polio types and an overall mean were also calculated.

Variation between laboratories was assessed graphically using histograms of laboratory mean titres. Between laboratory ranges (highest - lowest) and standard deviations were also calculated.

RESULTS

Stability

The candidate standard was prepared in the same manner as the current IS 85/659 inasmuch as it was produced from monovalent polio bulks without the addition of thermal stabilisers. The expectation would therefore be that under long-term storage it would behave in a very similar manner to the current IS.

Available data on the current IS are summarised in Table 2A. These data show that there has been no detectable loss of activity over the course of 18 years storage at NIBSC at -70°C . The real-time data currently available for the candidate 2nd IS is summarised in Table 2B along with the accelerated thermostability test data at 4°C , 20°C , 37°C and 45°C . There are no data for the current IS with which to compare the accelerated thermostability data.

The real time data for the candidate 2nd IS shows that it has good stability at -70°C and is in line with the data available on the current IS. As the two samples have been formulated in a similar way we would expect the candidate IS to show at least the same stability as the current IS i.e. no loss of activity over 19 years storage at -70°C .

We will continue to annually monitor the potency of the candidate 2nd IS at NIBSC.

Study Data returned

The raw data reported was well presented did not indicate any problems with the assays performed and of achieving the estimates from the dilution series used. No variations from the study protocol were noted.

Thirteen laboratories (coded 1 to 13) returned results from three assays performed using the NIBSC MAb's. In addition, nine laboratories (1, 2, 5, 6, 8, 9, 10, 11, and 12) performed the study using their own in-house antisera. Laboratory 12 currently uses the NIBSC monoclonal antibodies as their in-house antisera but is using tissue culture preparations rather than freeze-dried material. Details of the in-house antisera used by the above laboratories can be found in Table 1.

Within assay variation

The average root mean square (r.m.s) difference between the \log_{10} titres of the coded duplicate samples C and D was used as a measure of within assay variation. The r.m.s difference was $0.18 \log_{10} \text{TCID}_{50}$ when averaged across all polio types and total virus content and across all laboratories (Table 3). The range of results for individual laboratories was 0.07 to $0.35 \log_{10} \text{TCID}_{50}$ (data not shown).

Within laboratory variation

The coefficient of variation (cv) of the titre difference in the three assays performed averaged over the 6 samples and across all polio types and total virus was 2.50% (Table 4).

Seven out of the thirteen laboratories had cv's below 2.50% for the three polio types and total virus across all samples.

Comparison of Antisera

Mean values, standard deviations and coefficient of variation were calculated for each of the samples, polio types and total virus for both in-house and the NIBSC monoclonal antibodies (Tables 5-10). The results show there is good agreement between the titres obtained when either the NIBSC MAb's or the in-house antisera are used.

Titre estimates for samples

Laboratory mean titre estimates for each of the samples are given in Tables 5-10. The distribution by laboratory of these mean estimates are shown in Figures 1-4. These data show that overall the estimates of titre obtained between laboratories were very consistent. In fact, there were only two results which were noticeably lower than for other laboratories: sample A type 3 (laboratory 7), and sample F type 3 (laboratory 8) – both using the NIBSC monoclonal antibodies.

Mean estimates and standard deviations for each sample by virus type are given in Tables 5-10. Overall the coefficients of variation across samples were very similar although Sample E type 3 and sample F type 2 and 3 had slightly higher cv's than the other samples. The between laboratory variation in titres is shown in Table 11. These again show good consistency across virus type and samples with the mean range observed for assays with NIBSC antibodies being $0.58 \log_{10} \text{TCID}_{50}$, and for assays with in-house antibodies being $0.46 \log_{10} \text{TCID}_{50}$.

Assay sensitivity

The current WHO IS (sample A) and the Ph.Eur BRP (sample B) were included in this study to help evaluate assay sensitivity across the study. All laboratories obtain titres within $\pm 0.5 \log_{10} \text{TCID}_{50}$ of the assigned potencies for both these references demonstrating that the sensitivity of assay methods, cells and antisera used by the participants were comparable and that methods were, overall, well standardised.

Individual Study samples

Sample A - Current IS

The overall mean titres obtained in the study (5.79, 4.67, 5.22 $\text{TCID}_{50}/0.1\text{ml}$ for Types 1, 2 and 3 respectively) are very close to the assigned values for this reference i.e. 5.6, 4.6 and 5.2 for $\text{TCID}_{50}/0.1\text{ml}$ for Types 1, 2 and 3 respectively.

Sample B – Ph. Eur. BRP

The overall mean titres obtained for this sample (6.24, 5.18, 5.86 and 6.43 $\text{TCID}_{50}/0.1\text{ml}$ for Types 1, 2 and 3 and Total Virus respectively) as for the current IS, were very close to the assigned values for this reference (5.99, 5.06, 5.83 and 6.20 $\text{TCID}_{50}/0.1\text{ml}$ for Types 1, 2 and 3 and Total Virus respectively)

Samples C and D – Candidate 2nd IS

As the candidate 2nd IS was included in the study as samples C and D the overall mean titres from all assays were calculated. This gave titres of 6.51, 5.51, 5.87 and 6.66 $\log_{10} \text{TCID}_{50}/0.1\text{ml}$ for type 1, 2, 3 and Total Virus respectively.

Sample E – Commercial Vaccine

Sample E was a batch of vaccine formulated to UNICEF specifications i.e.

Type 1 ≥ 6.0 , Type 2 ≥ 5.0 , Type 3 ≥ 5.8 Log TCID₅₀/0.1 ml. This batch had previously been QC tested and released by the manufacturer. While some laboratories obtained titre estimates outside of the release criteria for this vaccine, most of these (5) were for type 3 (Laboratories 5, 8, 10, 12, 13) and only two (Laboratories 3, 13) and one (Laboratory 3) for Type 2 and 1 respectively. Four of the five type 3 results were from assays performed with in-house antisera.

Sample F – Low potency sample

Sample F was a batch of heat stressed vaccine and was included as a low potency sample. All laboratories returned estimates below the original specifications (see above) and the overall mean was 5.57, 4.48, 5.48 and 5.90 log₁₀ TCID₅₀/0.1ml for type 1, 2, 3 and Total Virus respectively.

DISCUSSION

The first aim of this study was to assess the suitability of a candidate reference preparation to replace the current International standard (85/659) for the potency assay of trivalent oral polio vaccine (OPV). Real-time stability tests indicate that the sample has stability comparable with the existing IS. In the collaborative study the candidate preparation was tested as a coded duplicate sample, with 9 of the participating laboratories testing the samples with their in-house antisera as well as the NIBSC monoclonal antibodies provided. The results obtained gave very comparable mean titres, low standard deviations and cv's, for both antibody sources used. The stability of the candidate along with its consistent behaviour across the range of assays employed in the collaborative study (Table 1) indicates that the candidate is a suitable replacement for the current IS 85/659.

The overall levels of within assay were 0.18 log₁₀ TCID₅₀ (average r.m.s difference in log₁₀ titres between duplicate samples C and D). The within laboratory variation gave an average coefficient of variation of 2.50% (between log₁₀ titre estimates of the sample across assays).

These figures correspond with previous studies undertaken to assess reference materials for the assay of OPV. The between laboratory variation in titres was 0.58 log₁₀ TCID₅₀ (mean of minimum to maximum range across laboratories) showing reasonable consistency between laboratories. However, for some individual samples and poliovirus types the figures are higher (see Table 11). Such variation in sensitivity between laboratories may lead to vaccines close to the release criterion producing different pass/fail decisions in different laboratories – as is witnessed for Sample E. The fact that different laboratories may reach different decisions for borderline samples does not however affect the fact that overall the level of consistency was good over the whole study.

Given the suitability of the sample and the consistency of the study, the next aim was to assign potency to the candidate replacement for the current IS. Taking all of the titres obtained for the candidate in the study (Samples C and D with both NIBSC and in-house sera) titres of 6.51, 5.51, 5.87 and 6.66 log₁₀ TCID₅₀/0.1 ml were obtained for type 1, 2, 3 and Total Virus respectively.

As it convention to assign the titres of the IS in TCID₅₀/ ml the above should be expressed as:

7.51, 6.51, 6.87 and 7.66 log₁₀ TCID₅₀/ml for type 1, 2, 3 and Total Virus content respectively.

USAGE OF THE CURRENT IS

The current IS, 85/659 was filled in October 1985 with a total of 2145 ampoules available for distribution. The preparation has been used in several collaborative studies (including the current study) and in a number of proficiency panels. Requests for distribution over the last few years have amounted to around 30 ampoules per year. Based on the size of the fill for the candidate we would predict that it will be available for up to 30 years.

RECOMMENDATIONS

The candidate should be established as the 2nd IS for the potency testing of trivalent OPV. The recommended potency for this preparation should be set at:

7.51, 6.51, 6.87 and 7.66 log₁₀ TCID₅₀/ml for type 1, 2, 3 and Total Virus content respectively.

Additional real-time stability data will be continue to be generated to confirm the expected stability at the storage temperature and, unless indications of instability are found, will be reported to the committee in a further 5 years time.

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REFERENCES

1. Magrath DI and Seagroatt V (1985). The standardisation of infectivity titrations of poliovaccines – a WHO collaborative study. *J Biologicals Standardisation*, 13, 159-166.
2. Heath A B, Wood DJ, Minor PD and Magrath DI (1990). WHO Collaborative Study on the proficiency of laboratory estimates of potency of oral poliovaccine. Report on the 2nd round to WHO Expert Committee on Biological Standardisation BS/90.1651.
3. Wood D, Collaborative study for the establishment of a biological preparation for oral poliovirus vaccine. *Pharmeuropa-Bio* 97-2, 19-30 (1997).
4. World Health Organisation (1990). Requirements for poliomyelitis vaccine (oral) (revised 2002). Technical Report Series, 904, 31-93.

Table 1

Details of assay methods used in each laboratory participating in the study

Laboratory	In-house Antiserum	Neutralisation Step (^o C/hrs)	Dilution Series (log ₁₀)	Inoculation Volume (µl)	No well inoculated per dilution	End point Of assay	Cell line	Calculation Method
1	Goat polyclonal	36.5/1.00	0.3	100	8x5	Any cpe	Hep 2C	Reed-Munch
2	Equine polyclonal	37/1.00	1.0	50	10	100%	Hep 2C	Arcsinus
3	N/A	37/1.00	0.6	50	10	100%	Hep 2C	Spearman Karber
4	N/A	35/3.00	0.5	50	8	100%	Hep 2C	Probit
5	Rabbit polyclonal	37/3.00	0.5	50	8	100%	Hep 2C	Spearman Karber
6	Equine polyclonal	37/1.10	1.0	50	10x3	100%	Hep 2C	Combistats
7	N/A	37/1.00	0.6	100	12	100%	Hep 2C	Combistats
8	Rabbit polyclonal	36/3.00	0.5	100	8	100%	Hep 2C	Spearman Karber
9	Type 1+3 Horse Type 2 Monkey	35/3.00	0.5	50	8	100%	Hep 2C	Spearman Karber
10	Polyclonal	35/2.00	1.0	50	8	Cpe	Hep 2C	Spearman Karber
11	Type 1+3 Horse Type 2 Monkey	35-36/3.00	0.5	50	8	100%	Hep 2C	Spearman Karber
12	NIBSC monoclonals	36/3.00	0.5	50	8	100%	Hep 2C	Spearman Karber
13	N/A	37/1.00	0.6	50	10	100%	Hep 2C	Reed-Munch

Table 2

Stability Studies on 85/659 and the Candidate 2nd IS.

A) Long-term stability of 85/659 stored at -70^oC.

B) Accelerated degradation stability of candidate.

A)

Study	Mean Titre (log TCID ₅₀ /ml)			
	Type 1	Type 2	Type 3	Total Virus
Heath et al., 1990 ⁽¹⁾	6.56	5.53	6.15	6.83
Wood and Heath, 1995 ⁽²⁾	6.68	5.69	6.22	6.89
NIBSC 2003	6.6	5.6	6.1	6.7
This Study; 2004	6.79	5.67	6.22	6.96

⁽¹⁾ Assays conducted in

⁽²⁾ Assays conducted in

B)

Temperature	Exposure Time	Titre (log TCID ₅₀ /0.1 ml)			
		Type 1	Type 2	Type 3	Total Virus
-70^oC ⁽¹⁾	0 days	6.8	5.8	6.2	6.8
	367 days	6.8	5.6	5.9	6.7
4^oC	28 days	6.3	5.5	5.8	6.6
	60 days	6.6	5.3	5.9	6.2
	92 days	6.4	5.2	5.7	6.3
	121days	6.2	5.2	5.6	6.3
	183 days	6.1	5.3	5.5	6.0
	274 days	5.6	5.0	5.5	6.0
	367 days	5.4	4.9	5.2	5.6
20^oC	21 days	5.9	5.1	5.7	6.8
	42 days	5.4	4.2	5.0	5.2
37^oC	21 days	<2.50	<2.50	<2.50	<2.50
	28 days	<2.50	<2.50	<2.50	<2.50
	42 days	<2.50	<2.50	<2.50	<2.50
45^oC	28 days	<2.50	<2.50	<2.50	<2.50

⁽¹⁾ Mean of 3 determinations

Table 3

Within assay variation assessed as the root mean squared (r.m.s.) difference between coded duplicates C and D

Laboratory	Average difference r.m.s. between C and D (\log_{10} TCID ₅₀)				
	Type 1	Type 2	Type 3	Total Virus	Overall
All Laboratories	0.18	0.17	0.19	0.17	0.18

Table 4

Within laboratory variation as measured using the coefficient of variation between assay

Laboratory	cv ⁽¹⁾				
	Type 1	Type 2	Type 3	Total Virus	Overall
All Laboratories	2.33	2.63	2.71	2.37	2.50

⁽¹⁾ Percentage coefficient of variation (cv) of the titres between assays averaged over all 6 samples.

Table 5

Laboratory mean estimates, across laboratory means for each antibody source and overall mean, between laboratories standard deviation and % coefficient of variation for Sample A

Laboratory	Titre (log ₁₀ TCID ₅₀ /0.1ml)							
	NIBSC F/D monoclonal antibodies				In-house antisera			
	Polio Type				Polio Type			
	1	2	3	TV	1	2	3	TV
1	5.97	4.77	5.19	N/A	5.88	4.90	5.30	6.05
2	6.03	4.99	5.62	6.13	6.01	4.92	5.50	6.24
3	5.43	4.58	5.28	5.58	N/A	N/A	N/A	N/A
4	5.60	4.67	5.23	6.00	N/A	N/A	N/A	N/A
5	5.89	4.44	5.20	5.87	5.84	4.55	5.11	5.88
6	5.83	4.73	5.23	6.10	5.70	4.77	5.23	5.93
7	6.00	4.62	4.69	5.97	N/A	N/A	N/A	N/A
8	5.70	4.60	5.03	5.91	5.57	4.49	5.03	5.82
9	5.63	4.63	5.32	5.78	5.70	4.78	5.30	5.78
10	6.01	4.51	5.30	6.26	5.86	4.55	5.30	6.24
11	5.66	4.66	5.18	5.82	5.61	4.61	5.18	5.80
12	5.69	4.66	5.19	6.01	5.90	4.78	5.08	6.04
13	5.77	4.60	5.27	5.93	N/A	N/A	N/A	N/A
Mean	5.79	4.65	5.21	5.95	5.79	4.71	5.23	5.98
Overall Mean⁽¹⁾	5.79	4.67	5.22	5.96				
SD⁽¹⁾	0.17	0.14	0.18	0.17				
% cv⁽¹⁾	2.91	3.07	3.41	2.93				

⁽¹⁾ Overall mean, standard deviation (SD) and percentage coefficient of variation (cv) are based on pooled values from both antisera. For type 1,2 and 3 n=22, and for tv n= 21

Table 6

Laboratory mean estimates, across laboratory means for each antibody source and overall mean, between laboratories standard deviation and % coefficient of variation for Sample B

Laboratory	Titre (\log_{10} TCID ₅₀ /0.1ml)							
	NIBSC F/D monoclonal antibodies				In-house antisera			
	Polio Type				Polio Type			
	1	2	3	TV	1	2	3	TV
1	6.43	5.36	5.97	N/A	6.41	5.51	6.10	6.66
2	6.28	5.44	5.93	6.54	6.22	5.44	6.10	6.55
3	5.98	4.98	5.85	6.25	N/A	N/A	N/A	N/A
4	6.40	5.13	6.17	6.53	N/A	N/A	N/A	N/A
5	6.32	5.22	5.66	6.26	6.09	5.07	5.72	6.32
6	6.23	5.20	5.80	6.30	6.20	5.23	5.97	6.60
7	6.39	5.23	5.95	6.50	N/A	N/A	N/A	N/A
8	6.20	5.14	5.80	6.34	6.16	5.05	5.66	6.36
9	6.18	4.95	5.84	6.38	6.18	4.95	5.86	6.38
10	6.42	5.01	5.84	6.46	6.18	5.24	5.62	6.49
11	6.07	5.09	5.86	6.34	6.07	5.09	5.91	6.30
12	6.36	5.18	5.72	6.38	6.29	5.27	5.68	6.58
13	6.30	5.13	5.83	6.47	N/A	N/A	N/A	N/A
Mean	6.27	5.16	5.86	6.40	6.20	5.21	5.85	6.47
Overall Mean⁽¹⁾	6.24	5.18	5.86	6.43				
SD⁽¹⁾	0.13	0.16	0.15	0.12				
% cv⁽¹⁾	2.1	3.1	2.6	1.9				

⁽¹⁾ Overall mean, standard deviation (SD) and percentage coefficient of variation (cv) are based on pooled values from both antisera. For type 1,2 and 3 n=22, and for tv n= 21

Table 7

Laboratory mean estimates, across laboratory means for each antibody source and overall mean, between laboratories standard deviation and % coefficient of variation for Sample C

Laboratory	Titre (\log_{10} TCID ₅₀ /0.1ml)							
	NIBSC F/D monoclonal antibodies				In-house antisera			
	Polio Type				Polio Type			
	1	2	3	TV	1	2	3	TV
1	6.89	5.77	6.09	N/A	6.76	5.91	6.17	6.96
2	6.71	5.83	6.10	6.79	6.66	5.79	6.16	6.79
3	6.23	5.30	5.73	6.33	N/A	N/A	N/A	N/A
4	6.53	5.60	6.30	6.83	N/A	N/A	N/A	N/A
5	6.51	5.39	5.68	6.41	6.13	5.24	5.55	6.41
6	6.47	5.63	5.90	6.63	6.47	5.53	5.77	6.67
7	6.59	5.74	5.80	6.70	N/A	N/A	N/A	N/A
8	6.45	5.40	5.78	6.53	6.22	5.22	5.55	6.49
9	6.45	5.30	5.91	6.68	6.49	5.32	5.95	6.68
10	6.59	5.51	6.26	7.05	6.74	5.42	5.68	6.62
11	6.34	5.28	6.07	6.63	6.30	5.30	6.03	6.61
12	6.68	5.58	5.74	6.76	6.65	5.65	5.83	6.85
13	6.43	5.40	5.73	6.57	N/A	N/A	N/A	N/A
Mean	6.53	5.52	5.93	6.66	6.49	5.49	5.85	6.68
Overall Mean⁽¹⁾	6.51	5.50	5.90	6.67				
SD⁽¹⁾	0.19	0.21	0.22	0.19				
% cv⁽¹⁾	3.0	3.8	3.8	2.7				

⁽¹⁾ Overall mean, standard deviation (SD) and percentage coefficient of variation (cv) are based on pooled values from both antisera. For type 1,2 and 3 n=22, and for tv n= 21

Table 8

Laboratory mean estimates, across laboratory means for each antibody source and overall mean, between laboratories standard deviation and % coefficient of variation for Sample D.

Laboratory	Titre (log ₁₀ TCID ₅₀ /0.1ml)							
	NIBSC F/D monoclonal antibodies				In-house antisera			
	Polio Type				Polio Type			
	1	2	3	TV	1	2	3	TV
1	6.85	5.78	6.06	N/A	6.76	5.49	6.09	6.96
2	6.60	5.76	6.04	6.75	6.63	5.68	5.97	6.66
3	6.28	5.43	5.75	6.55	N/A	N/A	N/A	N/A
4	6.67	5.70	5.97	6.73	N/A	N/A	N/A	N/A
5	6.38	5.37	5.88	6.57	6.26	5.24	5.59	6.57
6	6.50	5.63	5.83	6.51	6.53	5.50	5.83	6.67
7	6.45	5.47	5.66	6.46	N/A	N/A	N/A	N/A
8	6.39	5.26	5.68	6.51	6.28	5.36	5.61	6.57
9	6.49	5.41	6.01	6.70	6.49	5.41	5.93	6.70
10	6.67	5.67	6.18	6.55	6.55	5.55	5.74	6.74
11	6.34	5.34	6.11	6.63	6.26	5.30	6.05	6.59
12	6.63	5.43	5.47	6.72	6.58	5.74	5.49	6.90
13	6.43	5.37	5.77	6.67	N/A	N/A	N/A	N/A
Mean	6.51	5.51	5.88	6.61	6.48	5.52	5.81	6.71
Overall Mean⁽¹⁾	6.50	5.51	5.85	6.66				
SD⁽¹⁾	0.17	0.19	0.21	0.12				
% cv⁽¹⁾	2.6	3.5	3.6	1.8				

⁽¹⁾ Overall mean, standard deviation (SD) and percentage coefficient of variation (cv) are based on pooled values from both antisera. For type 1,2 and 3 n=22, and for tv n= 21

Table 9

Laboratory mean estimates, across laboratory means for each antibody source and overall mean, between laboratories standard deviation and % coefficient of variation for Sample E

Laboratory	Titre (log ₁₀ TCID ₅₀ /0.1ml)							
	NIBSC F/D monoclonal antibodies				In-house antisera			
	Polio Type				Polio Type			
	1	2	3	TV	1	2	3	TV
1	6.42	5.22	6.06	N/A	6.32	5.36	6.16	6.65
2	6.28	5.20	6.31	6.43	6.20	5.36	6.31	6.51
3	5.88	4.83	6.18	6.15	N/A	N/A	N/A	N/A
4	6.37	5.20	6.27	6.67	N/A	N/A	N/A	N/A
5	6.36	5.34	5.84	6.43	6.20	5.26	5.57	6.40
6	6.30	5.10	6.10	6.37	6.13	5.17	5.93	6.30
7	6.11	4.98	6.00	6.41	N/A	N/A	N/A	N/A
8	6.05	5.07	5.82	6.41	5.95	5.05	5.66	6.38
9	6.18	5.05	5.97	6.38	6.18	5.05	5.95	6.38
10	6.22	5.18	6.30	6.55	6.12	5.05	5.67	6.43
11	6.09	5.09	5.88	6.36	6.09	5.07	5.88	6.32
12	6.34	5.16	5.75	6.52	6.19	5.08	5.68	6.50
13	6.20	4.93	5.60	6.37	N/A	N/A	N/A	N/A
Mean	6.22	5.10	6.01	6.42	6.15	5.16	5.87	6.43
Overall Mean⁽¹⁾	6.19	5.13	5.95	6.42				
SD⁽¹⁾	0.14	0.13	0.24	0.12				
% cv⁽¹⁾	2.2	2.6	4.0	1.8				

⁽¹⁾ Overall mean, standard deviation (SD) and percentage coefficient of variation (cv) are based on pooled values from both antisera. For type 1,2 and 3 n=22, and for tv n= 21

Table 10

Laboratory mean estimates, across laboratory means for each antibody source and overall mean, between laboratories standard deviation and % coefficient of variation for Sample F

Laboratory	Titre (\log_{10} TCID ₅₀ /0.1ml)							
	NIBSC F/D monoclonal antibodies				In-house antisera			
	Polio Type				Polio Type			
	1	2	3	TV	1	2	3	TV
1	5.84	4.62	5.65	N/A	5.74	4.87	5.71	6.02
2	5.72	4.68	5.67	5.99	5.47	4.83	5.60	6.06
3	5.25	4.55	5.63	5.65	N/A	N/A	N/A	N/A
4	5.70	4.37	5.67	5.97	N/A	N/A	N/A	N/A
5	5.49	4.36	5.24	5.86	5.49	4.24	5.22	5.95
6	5.60	4.47	5.60	5.83	5.50	4.40	5.57	5.97
7	5.48	4.33	5.53	5.72	N/A	N/A	N/A	N/A
8	5.61	4.51	4.89	5.93	5.43	4.51	5.22	5.87
9	5.61	4.36	5.53	5.86	5.66	4.32	5.55	5.86
10	5.72	4.09	5.76	5.88	5.68	4.18	5.36	6.05
11	5.45	4.80	5.59	5.86	5.36	4.80	5.61	5.93
12	5.57	4.43	5.26	5.80	5.65	4.46	5.30	5.96
13	5.60	4.40	5.40	5.80	N/A	N/A	N/A	N/A
Mean	5.59	4.46	5.49	5.85	5.55	4.51	5.46	5.96
Overall Mean⁽¹⁾	5.57	4.48	5.48	5.90				
SD⁽¹⁾	0.14	0.21	0.22	0.10				
% cv⁽¹⁾	2.5	4.8	3.9	1.75				

⁽¹⁾ Overall mean, standard deviation (SD) and percentage coefficient of variation (cv) are based on pooled values from both antisera. For type 1,2 and 3 n=22, and for tv n= 21

Table 11

Between laboratory variation showing mean, minimum and maximum titres and the range across titres.

		Titre (\log_{10} TCID ₅₀ /0.1ml)							
		NIBSC F/D monoclonal antibodies				In-house antisera			
Sample	Virus type	Mean	Min	Max	Range	Mean	Min	Max	Range
A	1	5.79	5.43	6.03	0.60	5.79	5.57	6.01	0.44
	2	4.65	4.44	4.99	0.55	4.71	4.49	4.92	0.37
	3	5.21	4.69	5.62	0.93	5.23	5.03	5.50	0.47
	TV	5.95	5.58	6.26	0.68	5.98	5.78	6.24	0.46
B	1	6.27	5.89	6.43	0.54	6.2	6.07	6.41	0.34
	2	5.16	4.95	5.44	0.49	5.21	4.95	5.51	0.56
	3	5.86	5.66	6.17	0.51	5.85	5.62	6.10	0.48
	TV	6.40	6.25	6.54	0.29	6.47	6.30	6.66	0.36
C	1	6.53	6.34	6.89	0.55	6.49	6.13	6.67	0.63
	2	5.52	5.28	5.83	0.55	5.49	5.22	5.91	0.69
	3	5.93	5.68	6.30	0.62	5.85	5.55	6.17	0.62
	TV	6.66	6.33	7.05	0.72	6.68	6.41	6.96	0.55
D	1	6.51	6.28	6.85	0.57	6.48	6.28	6.76	0.48
	2	5.51	5.26	5.78	0.52	5.52	5.24	5.74	0.5
	3	5.88	5.47	6.18	0.71	5.81	5.49	6.09	0.6
	TV	6.61	6.46	7.05	0.59	6.71	6.57	6.96	0.39
E	1	6.22	5.88	6.42	0.54	6.15	5.95	6.32	0.37
	2	5.10	4.83	5.34	0.51	5.16	5.05	5.36	0.31
	3	6.01	5.60	6.30	0.7	5.87	5.57	6.31	0.74
	TV	6.42	6.15	6.55	0.4	6.43	6.32	6.65	0.18
F	1	5.59	5.25	5.84	0.59	5.55	5.36	5.74	0.38
	2	4.46	4.09	4.68	0.59	4.51	4.18	4.87	0.69
	3	5.49	4.89	5.67	0.78	5.46	5.22	5.71	0.49
	TV	5.85	5.65	5.99	0.34	5.96	5.86	6.06	0.20
Overall Mean				0.58				0.46	

Figure Legends

Distribution of laboratory mean titres estimates for each laboratory and virus type.
Laboratory code numbers are given in the boxes.

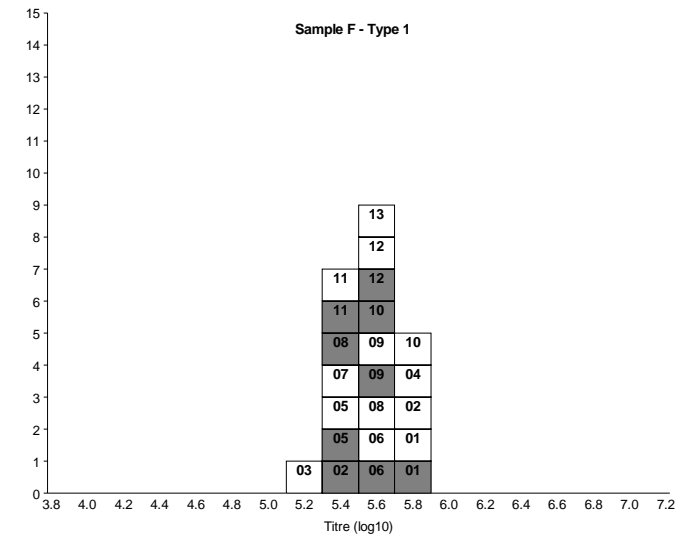
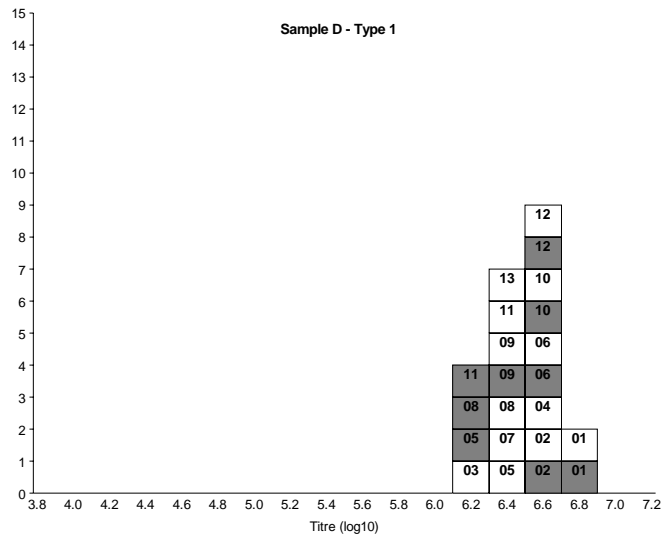
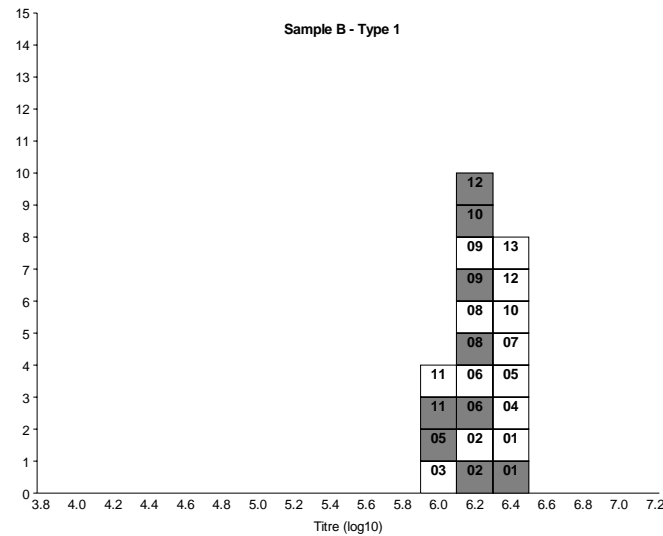
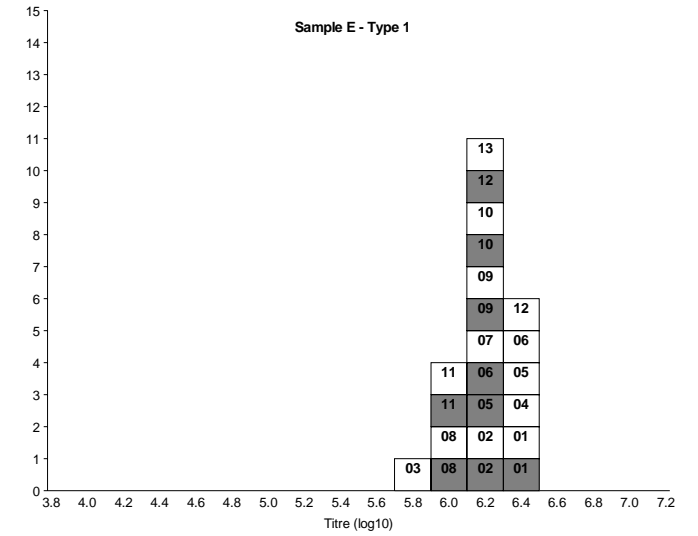
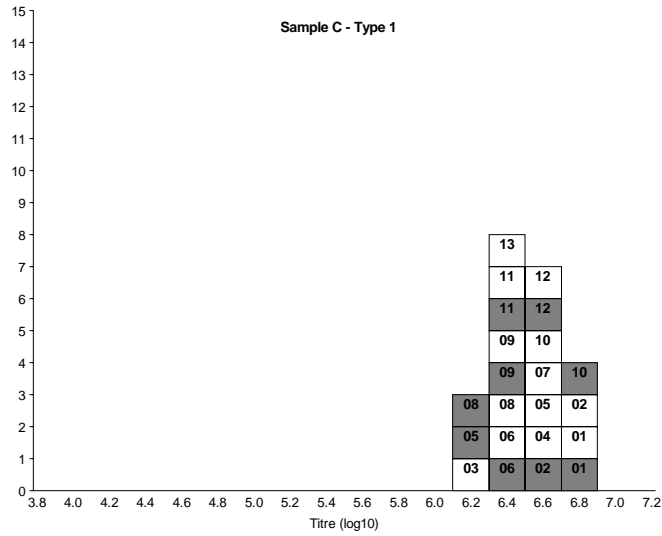
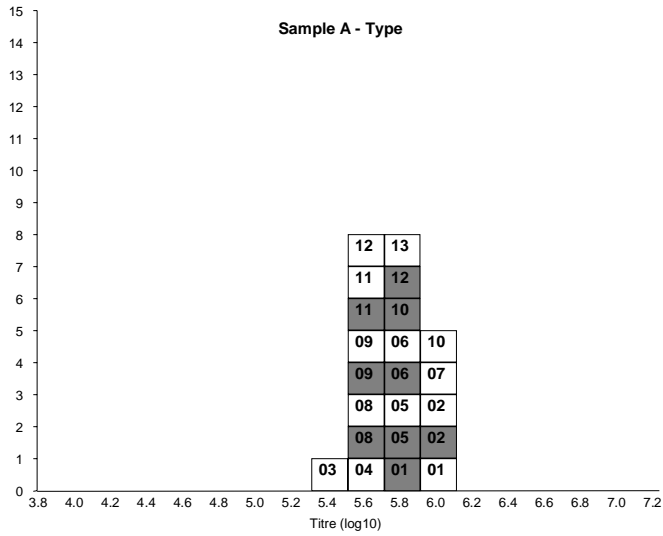
Assays with In-house antisera



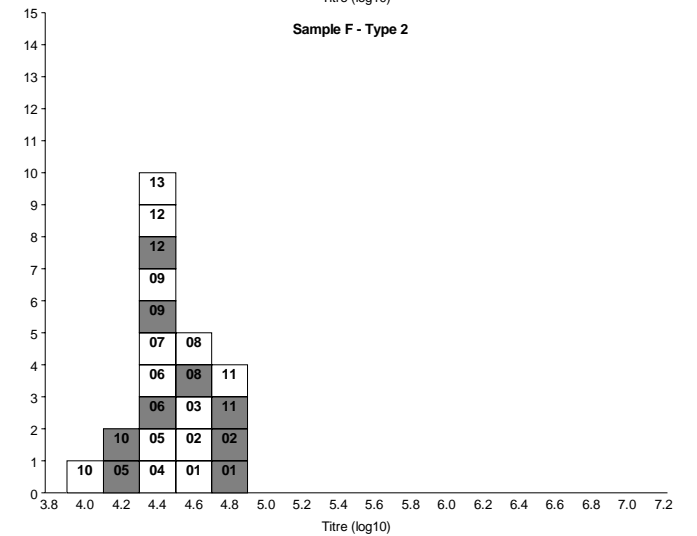
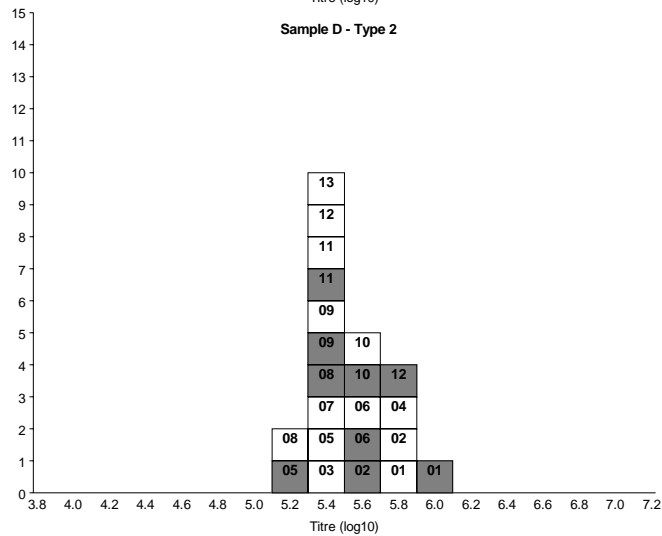
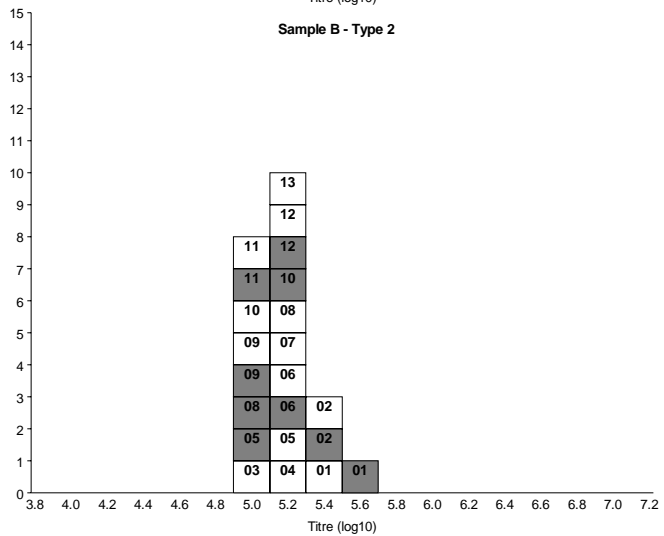
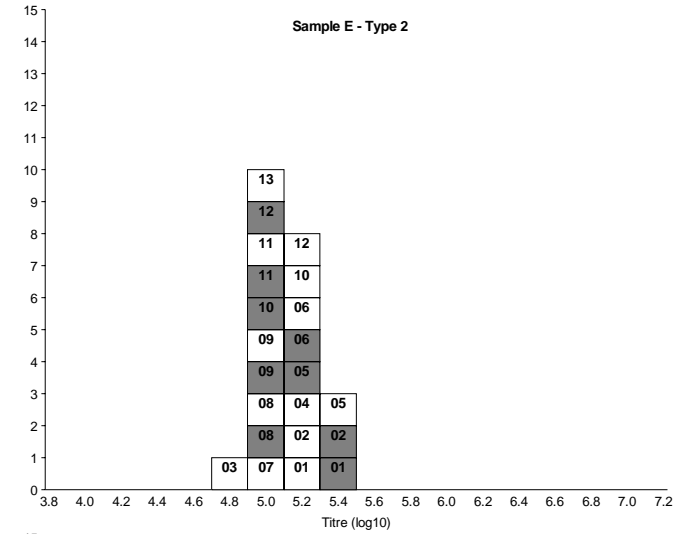
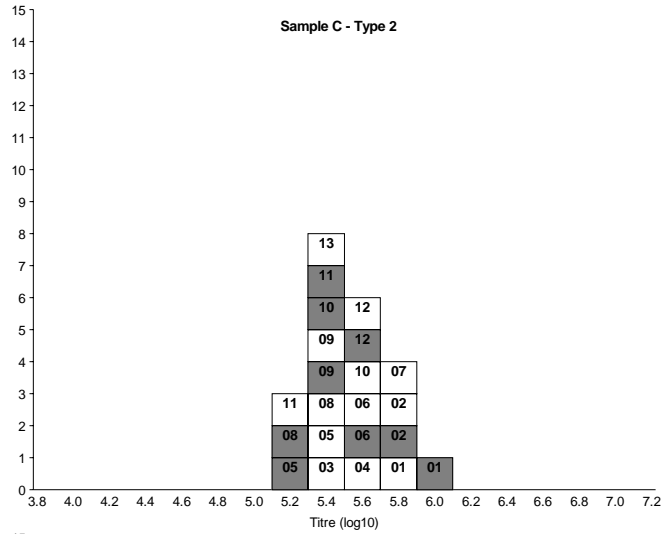
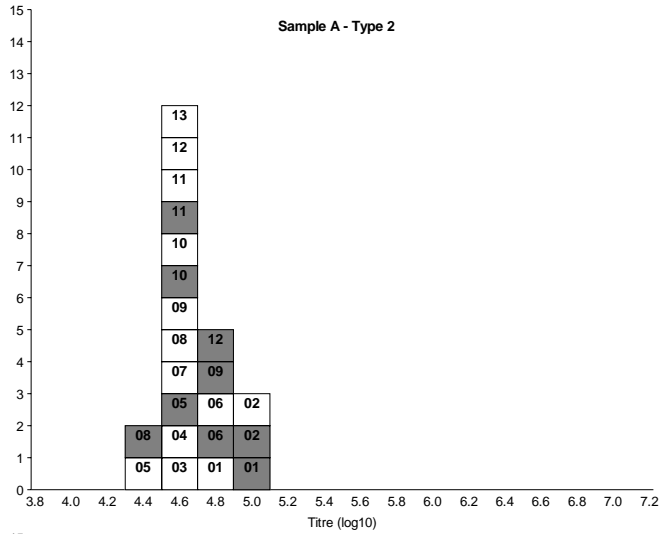
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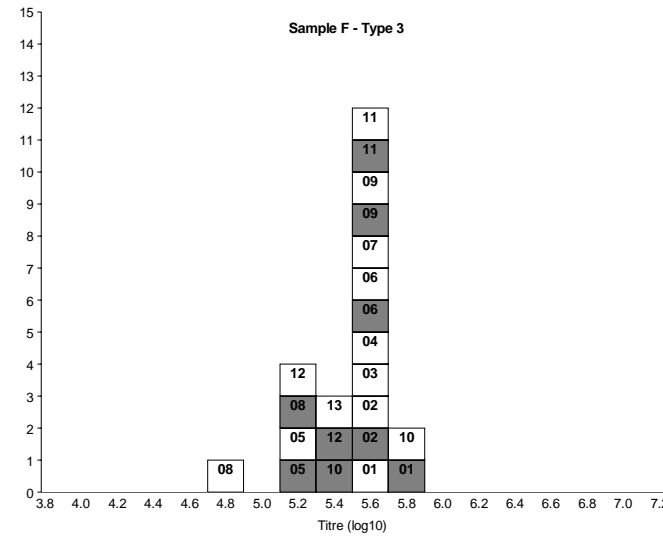
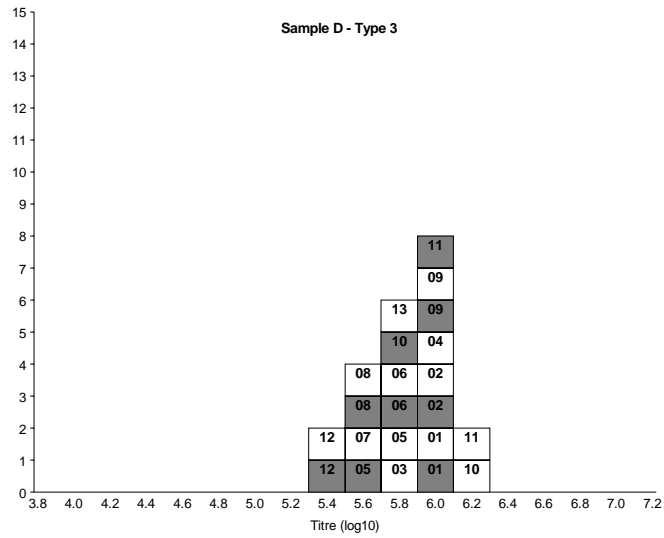
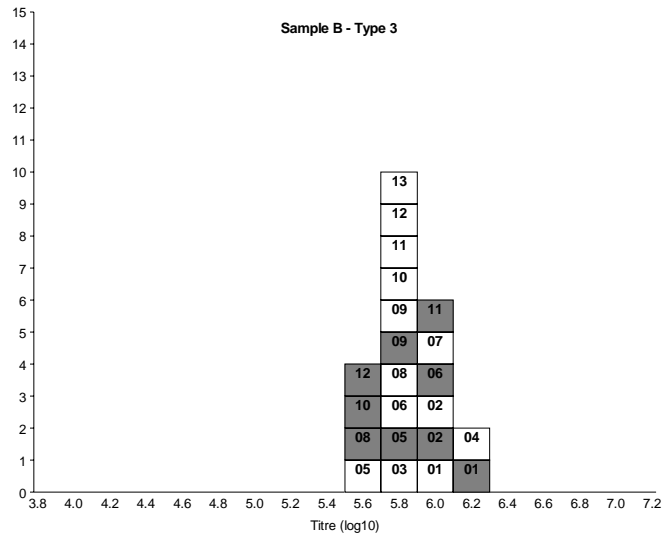
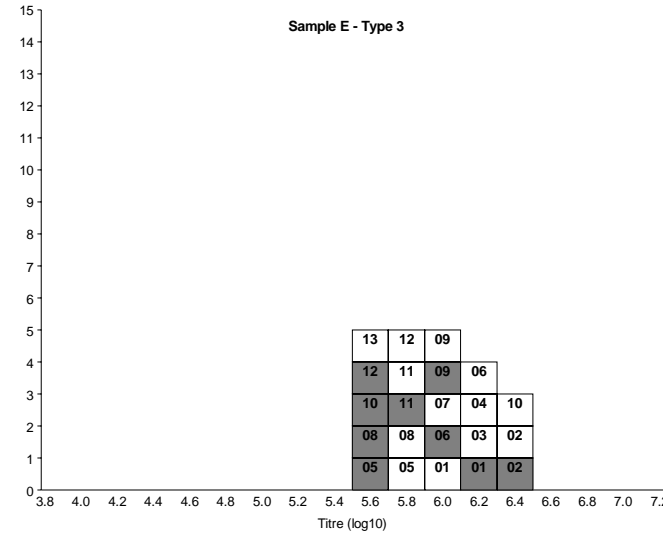
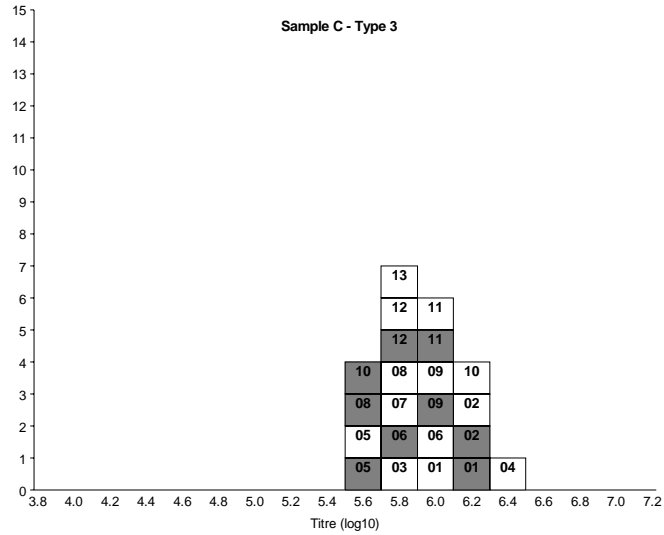
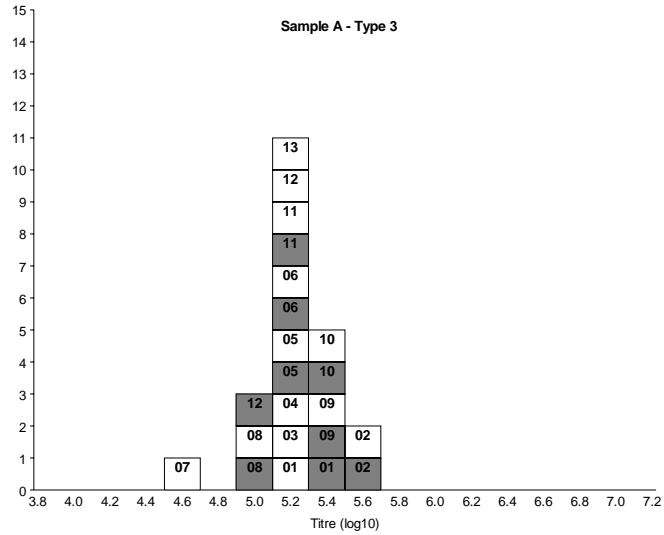
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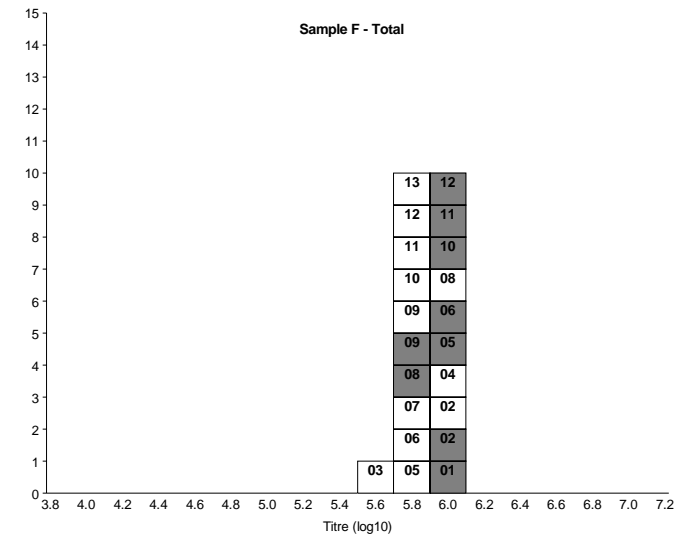
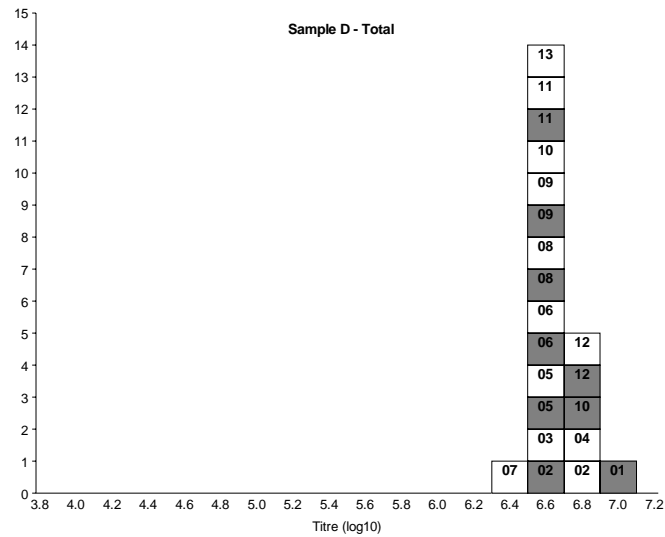
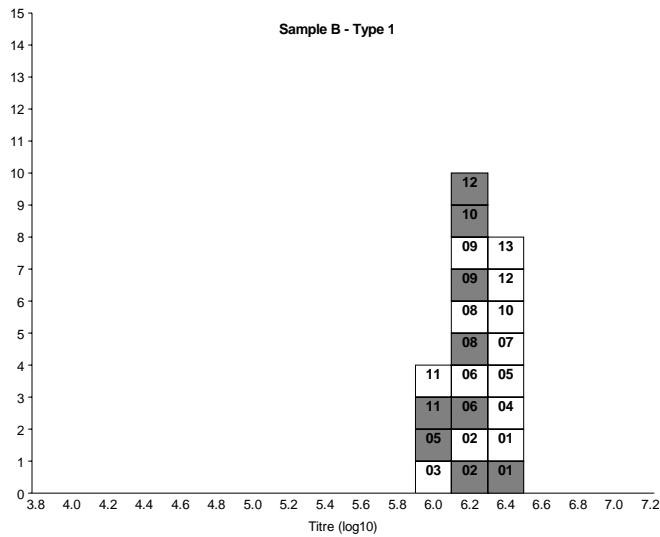
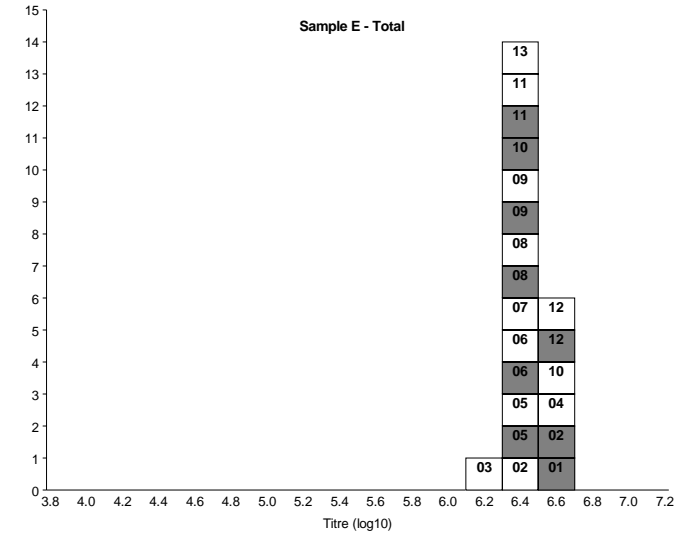
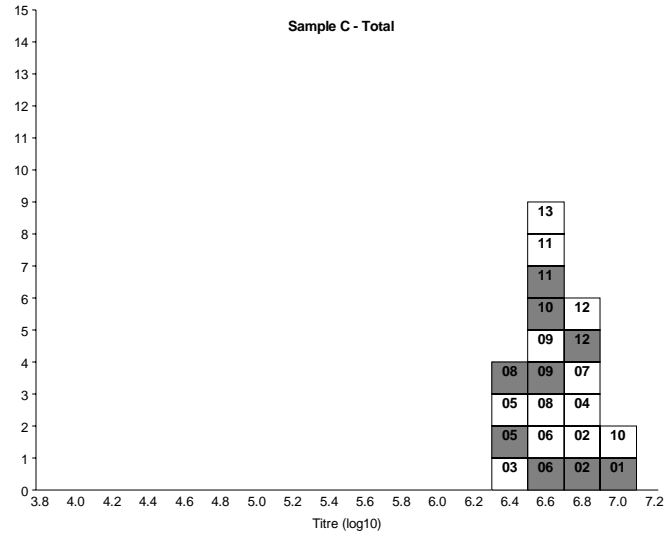
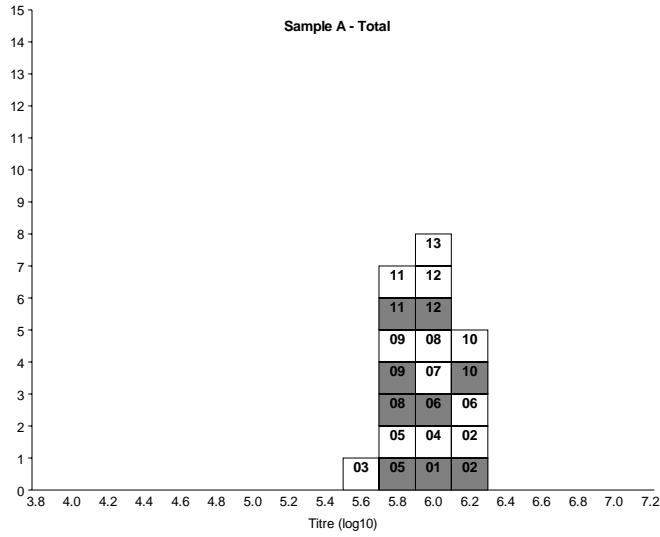
Type 2 - Laboratory Means TCID₅₀/0.1ml



Type 3 – Laboratory Means TCID₅₀/0.1ml



Total Virus Content Laboratory means TCID₅₀/0.1ml



Appendix 1

Study Participants

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<p>Dr Noemi Caro QC Virology Dept Chiron Vaccines Via Fiorentina 1 53100 Siena Italy Tel (+) 39 0577 243124 Fax (+) 39 0577 234495</p>	