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**Report on the WHO pilot study to investigate the collaborative study to establish the 3rd
International Standard (replacement) for inactivated polio vaccine (IPV)**

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Report

1. Introduction

The potency of IPV is measured *in vitro* using a validated ELISA test with suitable reference preparations and it is expressed in D-Antigen units. The 2nd WHO International Standard (IS) for IPV (NIBSC Code: 91/574) was established in 1994 (1-3). It was shown to be suitable for the determination of the antigenic content and immunogenicity of inactivated poliovirus vaccines by *in vitro* and *in vivo* assays, respectively. In anticipation of the depletion of the stock of 91/574, a collaborative study (2009) was designed to assess the suitability of candidate replacements. However, this study demonstrated excessive variability in potency estimates for the candidate standards between laboratories (4). The reasons for these differences between laboratories were not clear. Following suggestions by one of the participants in the study that they may be due to a lack of homogeneity between vials of the candidate standards, a further “pilot study” (2010) was conducted, selecting a subset of 6 laboratories that had participated in the original study (2009). These laboratories were selected to be representative of the range of potency estimates observed in the original study.

The aims of the pilot study were to see if the variability between laboratories from the original study was confirmed, and to assess the contribution of any vial to vial variability to the results of the study. A protocol was agreed by all participants.

2. Materials

There were 30 samples coded with a number. They correspond to 10 ampoules of each of the two candidates (trivalent concentrated bulks) for the 3rd IPV IS used in the last collaborative study plus a third trivalent concentrated bulk not tested in the collaborative study. These 30 ampoules are coded randomly and each laboratory will receive uniquely coded samples. The samples coded with letter/s correspond to 10 ampoules of a particular commercial vaccine (equivalent to a trivalent final fill lot). All vials were randomised and were labelled with a unique code letter or number.

The study samples were:

- 06/110 Candidate standard coded A & C in the original study.
- 07/140 Candidate standard coded X & Y in the original study.
- IMM A vaccine of equivalent potency to sample F in the original study.
- PU91-01 Equivalent to BRP no. 1 (assigned values 430 DU Type1; 95 DU Type 2; 285 DU Type 3).
- The IPV European Pharmacopoeia Biological Reference Preparation batch 2 (BRP No.2) (assigned values 320 DU for Type1; 67 DU for Type 2; 282 DU for Type 3) was used as a reference in the study.

The two candidate samples were produced by the same manufacturer, PU91-01 was produced by a second manufacturer and IMM and BRP no. 2 correspond to a third manufacturer.

3. Study Design

Participants were requested to:

- Determine the D-Antigen content of a panel of 40 coded samples of different non-adsorbed IPV vaccines using their routine in house immunochemical method.
- The D-Antigen content of the 3 poliovirus types should be determined at least once for each study sample against BRP no. 2 and the in-house reference preparation (in case it is part of the routine test).

As mentioned above, six laboratories were sent 10 vials each of 4 samples, along with the reference BRP no. 2.

Full details of the preparation of the candidate standard samples 06/110 and 07/140 are included in the report of the original collaborative study (4).

3.1 Assay methods

Details of ELISA methods and critical reagents used by each participant are shown in the Table below.

| Details of ELISA method used to determine D-Antigen content of IPV samples by collaborating laboratories | | | | | |
|--|---|--------------------------------------|---------------------------------|------------------------------|-----------|
| Lab. | Coating antibody | Detection antibody | Conjugated antibody | Number of replicas/dilutions | Substrate |
| 1 | Bovine polyclonal serum | Rabbit polyclonal serum | Anti-rabbit IgG-HRPO | 2/6 | ABTS |
| 2 | Rabbit polyclonal serum IgG fraction | Rabbit polyclonal serum-HRPO | NA | 2/5 | TMB |
| 3 | Rabbit polyclonal serum | Rabbit polyclonal serum-HRPO | NA | 1/8 | TMB |
| 4 | Sheep (types 1 and 2) or rabbit (type 3) polyclonal serum | Mouse monoclonal antibody | Anti-mouse IgG-HPRO | 2/4 | OPD |
| 5 | Rabbit polyclonal serum | Rabbit biotinilated polyclonal serum | ExtrAvidin peroxidase conjugate | 2/7-8 | TMB |
| 6 | Bovine polyclonal serum | Mouse monoclonal antibody | Anti-mouse IgG-HRPO | 3/5 | TMB |

3.1 Statistical methods

Analysis was based on the potency estimates supplied by the participants, and raw assay data was not re-analysed, although it was provided by the participants. The potency estimates relative to the BRP no. 2 were also used to calculate potency relative to PU91-01 for samples 06/110, 07/140 and IMM, by taking ratios of the potency of 06/110 to BRP no 2 and the potency of PU91-01 relative to BRP no 2, for example. Potencies of IMM (and sample F) and PU91-01 were also calculated relative to the two candidate samples 06/110 and 07/140.

The potency estimates for the replicate vials were combined using un-weighted geometric means to give a single laboratory mean for each sample. Within laboratory variability was assessed using the within laboratory Geometric Coefficient of Variation (%GCV). The individual assay results were also plotted as both histograms and scatter plots to investigate the variability between vials. For each sample, the laboratory means were combined using un-weighted geometric means with variability between laboratories measured by the between laboratory Geometric Coefficient of Variation (%GCV) calculated from the individual laboratory means.

The within laboratory %GCVs were pooled across samples to give a single “average” value per serotype for each laboratory. They were also pooled across laboratories to give a single value per sample. Finally, they were pooled across all three serotypes to give a combined estimate of the between vial variability. The pooled %GCVs were calculated by averaging the variances of the relevant log potency estimates. The pooled (across serotype) values for samples 06/110, 07/140 and IMM were compared to those for sample PU91-01 using a paired Wilcoxon non-parametric test across laboratories.

3.3 Participants

Six laboratories participated in this study. Participating laboratories are listed in section 9 below.

4. Results and data analysis

The six participating laboratories provided potency estimates for each of the 4 samples. Potency estimates were calculated relative to BRP no.2 for polio types 1, 2 and 3. Each laboratory returned potency estimates for each of the 10 individual vials for each sample, with the following exceptions:

Laboratory 2 only provided 8 estimates for 07/140 and IMM for type 1, and stated that the assay results for the other 2 vials were invalid. For type 2 they provided 10 estimates. For type 3, they provided 9 estimates, noting that they had “insufficient volume”.

Laboratory 5 repeated tests on some vials and provided more than 10 estimates. They provided the following numbers of estimates for types 1, 2 and 3 respectively; 06/110 – 13,10,11; 07/140 – 14,10,12; IMM – 20,12,11; PU91-01 – 13,13,10. All estimates were included in subsequent analysis.

4.1 Laboratory Potency Estimates for Pilot Study

Tables 1a to 1c show laboratory mean potency estimates for all samples relative to BRP no.2, for polio types 1 – 3 respectively. The within laboratory %GCVs are also shown.

For sample 06/110, laboratory means ranged from 219 to 365 for type 1, 48 to 68 for type 2 and 195 to 281 for type 3. Within laboratory variability, measured by the within laboratory %GCVs, ranged from 3.0% to 21.2%, as shown in table 1. The overall means were 293, 58 and 233 for types 1,2 and 3, with between laboratory %GCVs of 22, 15 and 17 respectively.

For sample 07/140, laboratory means ranged from 189 to 352 for type 1, 40 to 70 for type 2 and 171 to 284 for type 3. Variability within a laboratory ranged from 2.6% to 27.5% as shown in table 1. The overall means were 279, 56 and 230 for types 1, 2 and 3, with between laboratory %GCVs of 26, 22 and 22 respectively. The overall means across laboratories for 06/110 and 07/140 are very similar.

For sample IMM, laboratory means ranged from 33 to 42 for type 1, 9.2 to 11 for type 2 and 31 to 39 for type 3. Within laboratory variability ranged from 4.2% to 20.4%. The overall means were 40, 10 and 35 for types 1, 2 and 3, with between laboratory %GCVs of 14, 6 and 9 respectively.

Laboratory means for sample PU91-01 ranged from 429 to 564 for type 1, 89 to 109 for type 2 and 290 to 333 for type 3. Within laboratory variability ranged from 2.3% to 12.3%. The overall means were 459, 98 and 307 for types 1, 2 and 3, with between laboratory %GCVs of 11, 9 and 6 respectively. The overall means for PU91-01 were close to the assigned values of 430 DU (type 1), 95 DU (type 2), and 285 DU (type 3).

4.2 Variability between and within laboratories

The individual potency estimates are shown in histogram form, in figures 1a to 3d. They are also shown as scatter-plots by individual laboratory in figures 4a to 6d, for sample and polio type separately. It is clear from the histograms and figures that the variability between laboratories is greater than the within laboratory, between vial variability. An analysis of variance indicated that the additional between laboratory variability was statistically significant ($p < 0.001$) for all samples and types. From figures 1-6 it is also clear that there are differences in the between vial variability between different laboratories. For example, laboratory 6 has very close agreement in potency estimates between replicate vials, whereas laboratories 1 and 4 have poorer agreement. The pooled within laboratory %GCVs, along with the pooled values for individual laboratories or samples are shown in table 2, pooled across serotypes. The values and pattern across laboratories and samples are similar for all three serotypes (data not shown), and so the pooling of estimates across serotypes is justified.

The %GCVs pooled across laboratories for individual samples are slightly higher for the candidate samples 06/110 and 07/140 than for IMM and PU91-01. However, these differences

were not statistically significant ($p>0.05$) relative to the variability across laboratories. The %GCVs pooled across samples for individual laboratories indicate a big difference in variability between laboratories. Laboratories 2, 3 and 6 have good repeatability between vials, with pooled %GCVs ranging between 5.6% and 6.5%. Laboratories 1 and 4 have poorer repeatability, with %GCVs around 14%. Looking at the %GCVs for individual samples within laboratories, it appears that laboratory 1 had high variability for 07/140 compared to the other samples, while laboratory 4 had high variability with 06/110. The reasons for this are not known. Full details of assay layout (i.e. whether the individual vials were tested in the same assay or independent assays) were not available for all laboratories.

4.3 Comparison with the 2009 collaborative study

Tables 3a to 3c show the laboratory means, overall means and between laboratory %GCV's for samples 06/110, 07/140 and IMM or F for both the pilot study and the original 2009 collaborative study. In the current pilot study, potencies of samples were calculated relative to BRP no. 2. The potencies for samples 06/110, 07/140 and IMM were also calculated relative to PU91-01 for the pilot study, and these are also shown in tables 3. The results for the 2009 study are based on the mean of duplicate samples of 06/110 and 07/140, and are relative to BRP no. 1, the reference used in that study. PU91-01 is equivalent to BRP no. 1, and so the results relative to PU91 from the pilot study should be directly comparable to the results from the original 2009 study.

The variability between laboratories in the recent pilot study was higher for samples 06/110 and 07/140 than samples IMM and PU91-01 (table 1). Expressing results relative to PU91, the variability between laboratories for samples 06/110 and 07/140 was also higher than for sample IMM (table 2). However comparing across the two studies, the between laboratory %GCVs for the recent study are much lower than the between laboratory %GCVs obtained in the original 2009 study where they ranged from 31% (06/110 Type 3) to 77% (07/140 Type 1).

Comparisons across studies of the laboratory mean potency estimates for samples 06/110, 07/140 and IMM or F are shown in graphical form in figures 7a-7c respectively. The vertical scale represents the laboratory mean potency estimates (plotted on a log scale). The results for the 2009 study and the 2010 study (relative to both BRP no. 2 and PU91-01) are separated on the horizontal scale, and the results from an individual laboratory are connected. Results for the three polio serotypes are shown as adjacent blocks on the same graph.

Figures 7a and 7b clearly illustrate the excessive variability between laboratory mean estimates of potency for samples 06/110 and 07/140 in the 2009 study, compared to the results for the 2010 study. As noted above, this is a result of particularly low mean estimates in the 2009 study from laboratories 3 and 6, and to a lesser extent laboratory 1 for 07/140. The results from the 2010 pilot study using either BRP no. 2 or PU91-01 as a reference were similar. Figure 7c shows that the results for sample IMM or F (different vaccine samples but of equivalent potency) are consistent across studies and references.

Tables 4a to 4c show the potencies of sample IMM or F relative to the candidate standard samples (06/110 and 07/140) for both studies. There was greater variability between laboratories in the 2009 collaborative study (%GCV's ranging from 27% to 73%) than in the 2010 pilot study (%GCV's ranging from 9% to 19%).

5. Conclusions

The main objective of this study was to investigate the reason for the high variability in D-Ag potency results found between laboratories for the two candidate standards in the original 2009 study to replace the 2nd IS for IPV. The results meant that it was not possible to establish any of the candidate materials as the 3rd IS for IPV.

Samples included in this pilot study were: the Ph Eur BRP No. 2; PU91-01, a working standard equivalent to Ph Eur BRP No. 1; the two candidate vaccine standards (06/110 and 07/140) used in the original study and a monitor vaccine (IMM). 10 vials of each of the 4 samples were tested by six laboratories.

The results showed that the within laboratory (between vial) variability for individual samples were slightly higher for the candidate samples 06/110 and 07/140 than for IMM and PU91-01. However, these differences were not statistically significant ($p > 0.05$) relative to the variability across laboratories. The between laboratory variability was greater than the within laboratory variability. This suggests that any between vial variability cannot be the only factor contributing to the observed differences between different laboratories. For all three types, the between laboratory variability for samples 06/110 and 07/140 was higher than for IMM and PU91. The reasons for this are not clear but it cannot be discarded that the observed differences are due to the quality of ampoules filled at NIBSC (i.e. more fragile to variations in temperature during shipment, handling and/or storage).

There were differences in between vial variability between the laboratories. The two laboratories with the best overall repeatability (labs 2 and 6) had similar, or better, repeatability for the two candidates 06/110 and 07/140 compared to the other two samples. This suggests that a lack of homogeneity between vials for the candidate samples cannot be the sole reason for the variability experienced in other laboratories.

The observed variability in potency estimates within a laboratory is potentially a combination of between vial and between assay components. This may explain some differences in observed variability between laboratories. However, it will not explain the differences in variability between samples experienced by some laboratories, as presumably the assay design and layout would be the same for all samples. The good repeatability between vials in some laboratories suggests that the excessive variability between laboratories observed in the original 2009 study is therefore unlikely to be caused only by a lack of homogeneity between vials. The contrast in the extent of the between laboratory differences in the original 2009 study and the current study also suggest that factors other than vial to vial variability were involved.

As stated above, the variability between laboratories in the recent pilot study was higher for samples 06/110 and 07/140 than samples IMM and PU91-01. When expressing the results relative to PU91, the variability between laboratories for samples 06/110 and 07/140 was also higher than for sample IMM. However comparing across the two studies, the between laboratory variability for the recent study was much lower than the between laboratory variability obtained in the original 2009 study.

This was mainly due to results from laboratories 3 and 6 which had much lower potency estimates in the 2009 study than the other participants. In the 2010 pilot study however, potency estimates for these 2 laboratories were in closer agreement with the results from the other participants. The variability between laboratories for potency estimates for sample IMM or F was similar for both studies.

The similarity of the results from the 2010 pilot study using either BRP no. 2 or PU91-01 as a reference demonstrate that the variability in the 2009 study is not related to the use of BRP no. 1 compared to BRP no. 2.

When the potencies of sample IMM or F relative to the candidate standard samples (06/110 and 07/140) were obtained using data from both studies, there was greater variability between laboratories in the 2009 collaborative study than in the 2010 pilot study. Again this was mainly due to laboratories 3 and 6 which had higher potency estimates of sample F relative to the candidate standards than other participants in the 2009 collaborative study. This is a direct result of these two laboratories having much lower estimates for the candidate standards than other participants. In the recent pilot study, they were in good agreement with the other participants. Again this demonstrates that the difference in between laboratory variability between the 2009 and the 2010 study is not due to using different references, BRP nos. 1 and 2.

Overall, the conclusion is that the differences found between laboratories in the potency of any of the samples tested in the pilot study are likely to be caused by a combination of factors, including differences in the test performance in participating laboratories. The results for the two candidate samples in the pilot study were much more consistent between laboratories than those obtained in the original study and showed mean potency values very close to those assigned by the manufacturer that produced the candidate materials. However, the between laboratory variability for the two candidate samples was higher than that for any of the other samples in the study. Furthermore, there was not a clear explanation for the differences in the potencies of the candidate samples found between studies in laboratories 3 and 6.

6. Comments from participants

Some comments were received from participants mainly concerning suggestions for editorial changes and clarification of some points. The original manuscript was modified to incorporate most of these changes. One participant considered that the study design did not allow a detailed analysis of vial to vial variability.

7. Proposals

Taken into consideration all of the above, it is proposed to design a new study including samples from various IPV manufacturers and to be filled outside NIBSC to exclude both possible product-specific reactions in the ELISA tests performed at different laboratories and possible problems derived from the filling process at NIBSC. A new study will include the detailed characterization of any candidate replacement reference materials alongside existing references.

8. References

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9. List of participants

- AFSSAPS, France
- FDA, USA
- GSK, Belgium
- NIBSC, UK
- RIVM, The Netherlands
- Sanofi Pasteur, Canada

Table 1a: Type 1 - Mean potency estimates of samples relative to BRP no.2

| Lab | Sample | | | | | | | |
|------|---------|------|---------|------|---------|------|---------|------|
| | 06/110 | | 07/140 | | IMM | | PU91-01 | |
| | Geomean | %GCV | Geomean | %GCV | Geomean | %GCV | Geomean | %GCV |
| 1 | 219 | 13.7 | 189 | 22.0 | 35 | 5.8 | 432 | 7.1 |
| 2 | 310 | 5.9 | 316 | 5.5 | 40 | 4.6 | 433 | 6.1 |
| 3 | 359 | 9.5 | 352 | 9.3 | 48 | 6.8 | 564 | 5.2 |
| 4 | 269 | 19.1 | 272 | 9.6 | 37 | 10.9 | 456 | 10.8 |
| 5 | 262 | 8.9 | 245 | 12.2 | 33 | 12.5 | 429 | 5.2 |
| 6 | 365 | 6.5 | 338 | 4.9 | 42 | 6.0 | 455 | 5.9 |
| GM | 292.7 | | 279.2 | | 38.9 | | 459.3 | |
| %GCV | 21.5 | | 26.4 | | 14.3 | | 11.0 | |

Table 1b: Type 2 - Mean potency estimates of samples relative to BRP no.2

| Lab | Sample | | | | | | | |
|------|---------|------|---------|------|---------|------|---------|------|
| | 06/110 | | 07/140 | | IMM | | PU91-01 | |
| | Geomean | %GCV | Geomean | %GCV | Geomean | %GCV | Geomean | %GCV |
| 1 | 48 | 7.9 | 40 | 21.4 | 9.2 | 8.4 | 89 | 9.0 |
| 2 | 59 | 4.3 | 61 | 2.6 | 10.3 | 4.5 | 97 | 2.3 |
| 3 | 67 | 6.1 | 64 | 4.8 | 11.0 | 5.8 | 106 | 4.4 |
| 4 | 53 | 16.5 | 54 | 10.5 | 10.0 | 8.3 | 90 | 8.7 |
| 5 | 55 | 6.2 | 55 | 8.0 | 10.0 | 6.2 | 102 | 8.9 |
| 6 | 68 | 4.2 | 70 | 3.8 | 10.5 | 6.8 | 109 | 5.9 |
| GM | 57.9 | | 56.4 | | 10.2 | | 98.4 | |
| %GCV | 15.0 | | 22.0 | | 6.0 | | 8.9 | |

Table 1c: Type 3 - Mean potency estimates of samples relative to BRP no.2

| Lab | Sample | | | | | | | |
|-----|---------|------|---------|------|---------|------|---------|------|
| | 06/110 | | 07/140 | | IMM | | PU91-01 | |
| | Geomean | %GCV | Geomean | %GCV | Geomean | %GCV | Geomean | %GCV |
| 1 | 197 | 6.0 | 171 | 27.5 | 35 | 11.6 | 309 | 9.9 |
| 2 | 239 | 8.1 | 244 | 4.5 | 31 | 7.0 | 290 | 8.3 |
| 3 | 269 | 8.8 | 271 | 6.5 | 38 | 4.2 | 310 | 3.3 |

| | | | | | | | | |
|-------------|-------|------|-------|------|------|------|-------|------|
| 4 | 231 | 21.2 | 238 | 14.4 | 35 | 20.4 | 333 | 12.3 |
| 5 | 195 | 5.8 | 192 | 7.5 | 33 | 4.9 | 283 | 3.7 |
| 6 | 281 | 3.0 | 284 | 5.6 | 39 | 9.6 | 323 | 8.3 |
| GM | 233.0 | | 229.5 | | 34.9 | | 307.4 | |
| %GCV | 16.6 | | 21.9 | | 8.9 | | 6.4 | |

Table 2: Within laboratory %GCVs pooled across samples, laboratories and serotypes.

| Lab | Sample | | | | Pooled |
|---------------|---------------|---------------|------------|----------------|---------------|
| | 06/110 | 07/140 | IMM | PU91-01 | |
| 1 | 9.7 | 23.7 | 8.9 | 8.7 | 13.9 |
| 2 | 6.3 | 4.4 | 5.5 | 6.1 | 5.6 |
| 3 | 8.3 | 7.1 | 5.7 | 4.4 | 6.5 |
| 4 | 19.0 | 11.7 | 14.0 | 10.7 | 14.1 |
| 5 | 7.1 | 9.4 | 8.5 | 6.3 | 7.9 |
| 6 | 4.8 | 4.8 | 7.6 | 6.8 | 6.1 |
| Pooled | 10.1 | 11.8 | 8.8 | 7.4 | |

Table 3a: Type 1 - Comparison of potency means from IPV 2010 pilot study and IPV 2009 collaborative study (result for collaborative study is a mean of A and C)

| Lab Code | | 06/110 (A/C) | | | 07/140 (x/y) | | | IMM (F) | | |
|----------------|------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 2010 | 2009 | 2010 v BRP2 | 2010 v PU91 | 2009 v BRP1 | 2010 v BRP2 | 2010 v PU91 | 2009 v BRP1 | 2010 v BRP2 | 2010 v PU91 | 2009 v BRP1 |
| 1 | 4 | 219 | 218 | 225/188* | 189 | 188 | 182/144* | 36 | 35 | 41/36* |
| 2 | 2 | 311 | 308 | 306 | 316 | 314 | 302 | 40 | 39 | 41 |
| 3 | 3 | 359 | 274 | 136 | 352 | 268 | 71 | 48 | 37 | 38 |
| 4 | 5 | 269 | 254 | 290 | 272 | 256 | 295 | 37 | 35 | 39 |
| 5 | 11 | 262 | 263 | 303 | 245 | 246 | 286 | 33 | 33 | 39 |
| 6 | 6 | 365 | 344 | 138 | 338 | 319 | 108 | 42 | 40 | 39 |
| Geomean | | 57.9 | 292.7 | 274.1 | 215.0 | 279.2 | 261.4 | 174.5 | 38.9 | 36.4 |
| %GCV | | 15.0 | 21.5 | 17.2 | 42.7 | 26.4 | 21.3 | 76.5 | 14.3 | 7.2 |

*2 different methods used

Table 3b: Type 2 - Comparison of potency means from IPV 2010 pilot study and IPV 2009 collaborative study (result for collaborative study is a mean of A and C)

| Lab Code | | 06/110 (A/C) | | | 07/140 (x/y) | | | IMM (F) | | |
|----------------|------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 2010 | 2009 | 2010 v BRP2 | 2010 v PU91 | 2009 v BRP1 | 2010 v BRP2 | 2010 v PU91 | 2009 v BRP1 | 2010 v BRP2 | 2010 v PU91 | 2009 v BRP1 |
| 1 | 4 | 48 | 51 | 46/44* | 40 | 43 | 40/30* | 9.2 | 9.9 | 8.9/9.0* |
| 2 | 2 | 59 | 58 | 60 | 61 | 60 | 61 | 10.3 | 10.0 | 9.0 |
| 3 | 3 | 67 | 60 | 24 | 64 | 57 | 22 | 11.0 | 9.9 | 7.9 |
| 4 | 5 | 53 | 56 | 58 | 54 | 57 | 60 | 10.0 | 10.6 | 9.1 |
| 5 | 11 | 55 | 52 | 61 | 55 | 51 | 61 | 10.0 | 9.3 | 8.5 |
| 6 | 6 | 68 | 60 | 48 | 70 | 61 | 36 | 10.5 | 9.2 | 9.0 |
| Geomean | | 57.9 | 55.9 | 46.8 | 56.4 | 54.4 | 41.5 | 10.2 | 9.8 | 8.8 |
| %GCV | | 15.0 | 7.3 | 38.1 | 22.0 | 14.7 | 49.1 | 6.0 | 5.3 | 5.2 |

*2 different methods used

Table 3c: Type 3 - Comparison of potency means from IPV 2010 pilot study and IPV 2009 collaborative study (result for collaborative study is a mean of A and C)

| Lab Code | | 06/110 (A/C) | | | 07/140 (X/Y) | | | IMM (F) | | |
|----------------|------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 2010 | 2009 | 2010 v BRP2 | 2010 v PU91 | 2009 v BRP1 | 2010 v BRP2 | 2010 v PU91 | 2009 v BRP1 | 2010 v BRP2 | 2010 v PU91 | 2009 v BRP1 |
| 1 | 4 | 197 | 182 | 171/169* | 171 | 158 | 123/94* | 35 | 32 | 36/33* |
| 2 | 2 | 239 | 235 | 212 | 244 | 240 | 236 | 31 | 30 | 31 |
| 3 | 3 | 270 | 248 | 104 | 272 | 249 | 70 | 38 | 34 | 31 |
| 4 | 5 | 231 | 197 | 201 | 238 | 203 | 213 | 35 | 30 | 32 |
| 5 | 11 | 195 | 197 | 213 | 192 | 193 | 222 | 33 | 33 | 34 |
| 6 | 6 | 281 | 248 | 131 | 284 | 251 | 94 | 39 | 35 | 28 |
| Geomean | | 233.0 | 216.0 | 166.6 | 229.6 | 212.8 | 135.7 | 34.9 | 32.3 | 32.0 |
| %GCV | | 16.6 | 14.5 | 30.9 | 21.9 | 20.0 | 64.1 | 8.9 | 6.9 | 8.5 |

*2 different methods used

Table 4a: Type 1 - Mean potency estimates of sample IMM (F in 2009 study) relative to 06/110 (313 D-Ag units) or 07/140 (307 D-Ag units)

| | | Sample | | | |
|-----------------------|------------|--------------|------------|--------------|------------|
| Lab Code 1 | Lab Code 2 | IMM v 06/110 | F v 06/110 | IMM v 07/140 | F v 07/140 |
| 2010 | 2009 | 2010 | 2009 | 2010 | 2009 |
| 1 | 4 | 51 | 57/59* | 58 | 69/76* |
| 2 | 2 | 40 | 42 | 39 | 41 |
| 3 | 3 | 42 | 87 | 42 | 165 |
| 4 | 5 | 43 | 42 | 41 | 41 |
| 5 | 11 | 40 | 40 | 42 | 42 |
| 6 | 6 | 36 | 89 | 38 | 115 |
| Geometric mean | | 42 | 57 | 43 | 68 |
| %GCV | | 12.2 | 40.4 | 16.5 | 73.4 |

*2 different methods used

Table 4b: Type 2- Mean potency estimates of sample IMM (F in 2009 study) relative to 06/110 (60 D-Ag units) or 07/140 (62 D-Ag units)

| | | Sample | | | |
|-----------------------|------------|--------------|-------------|--------------|-------------|
| Lab Code 1 | Lab Code 2 | IMM v 06/110 | F v 06/110 | IMM v 07/140 | F v 07/140 |
| 2010 | 2009 | 2010 | 2009 | 2010 | 2009 |
| 1 | 4 | 11.6 | 11.6/12.2* | 14.4 | 13.7/18.7* |
| 2 | 2 | 10.4 | 9.0 | 10.4 | 9.1 |
| 3 | 3 | 9.9 | 19.8 | 10.7 | 22.0 |
| 4 | 5 | 11.4 | 9.5 | 11.5 | 9.4 |
| 5 | 11 | 10.8 | 8.4 | 11.4 | 8.6 |
| 6 | 6 | 9.2 | 11.3 | 9.3 | 15.7 |
| Geometric mean | | 10.5 | 11.2 | 11.2 | 13.1 |
| %GCV | | 9.2 | 33.0 | 15.7 | 45.7 |

*2 different methods used

Table 4c: Type 3 - Mean potency estimates of sample IMM (F in 2009 study) relative to 06/110 (232 D-Ag units) or 07/140 (232 D-Ag units)

| | | Sample | | | |
|-----------------------|------------|--------------|------------|--------------|------------|
| Lab Code 1 | Lab Code 2 | IMM v 06/110 | F v 06/110 | IMM v 07/140 | F v 07/140 |
| 2010 | 2009 | 2010 | 2009 | 2010 | 2009 |
| 1 | 4 | 41 | 49/45* | 47 | 68/81* |
| 2 | 2 | 30 | 34 | 29 | 31 |
| 3 | 3 | 32 | 69 | 32 | 102 |
| 4 | 5 | 35 | 37 | 34 | 35 |
| 5 | 11 | 40 | 37 | 40 | 36 |
| 6 | 6 | 32 | 49 | 32 | 68 |
| Geometric mean | | 35 | 45 | 35 | 55 |
| %GCV | | 13.0 | 26.9 | 19.0 | 60.7 |

Figure 1c:

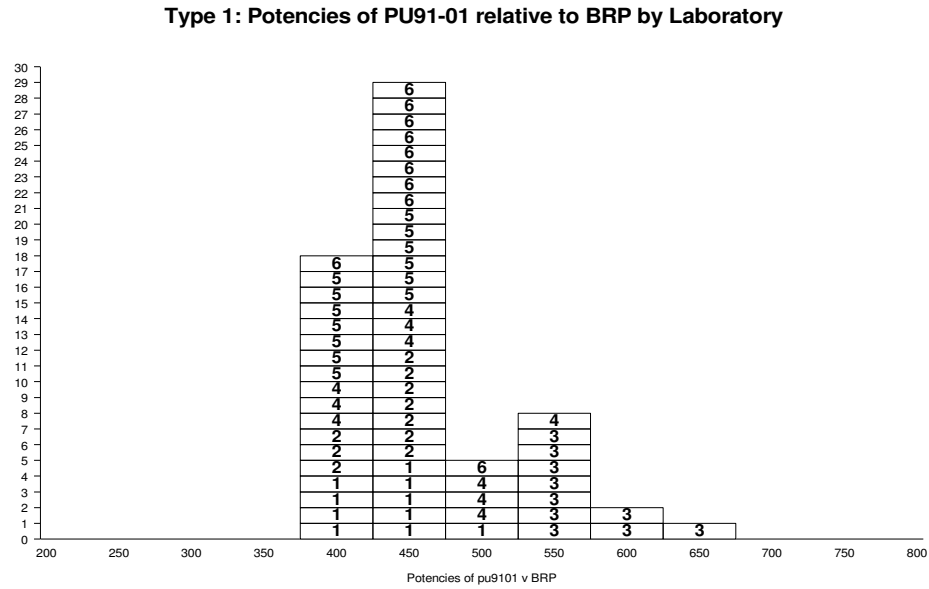


Figure 1d:

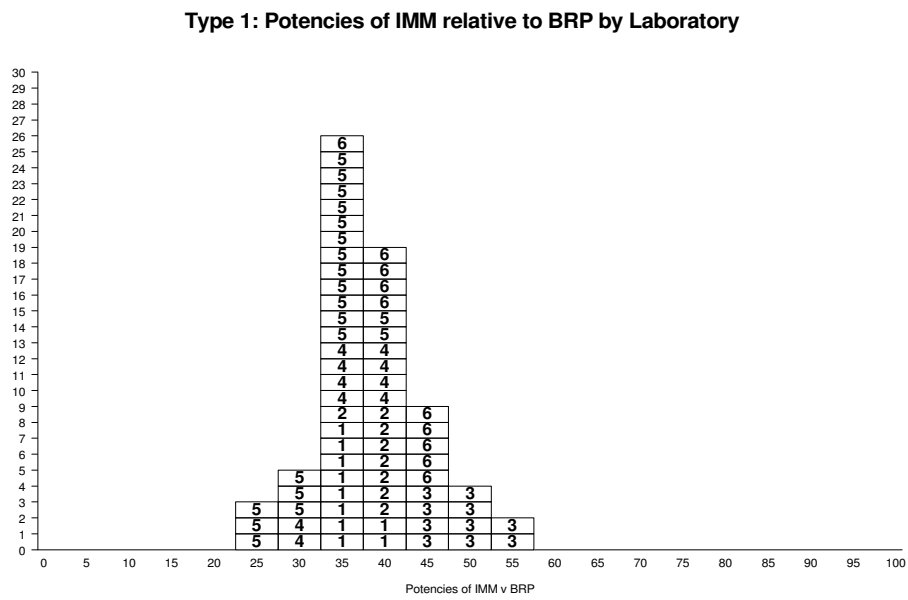


Figure 2a:

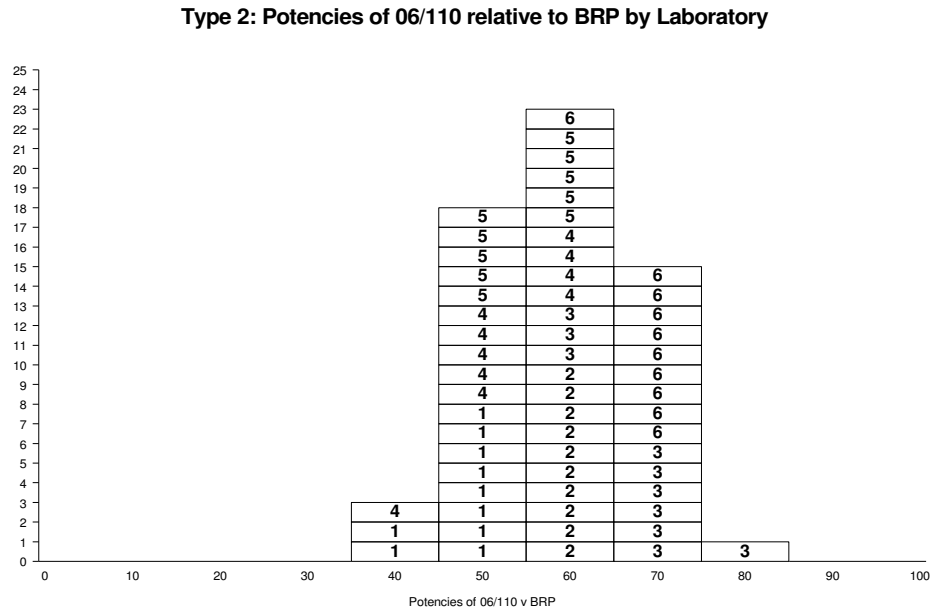


Figure 2b:

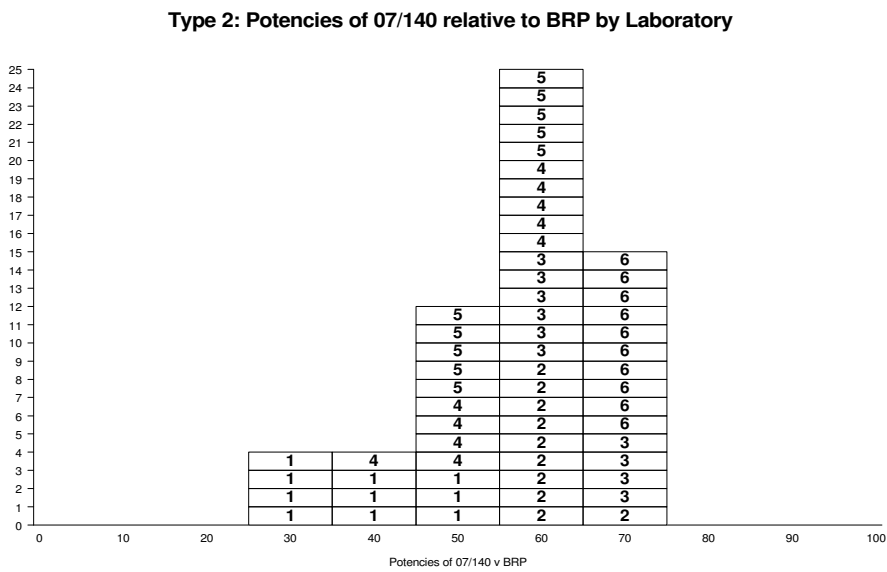


Figure 2c:

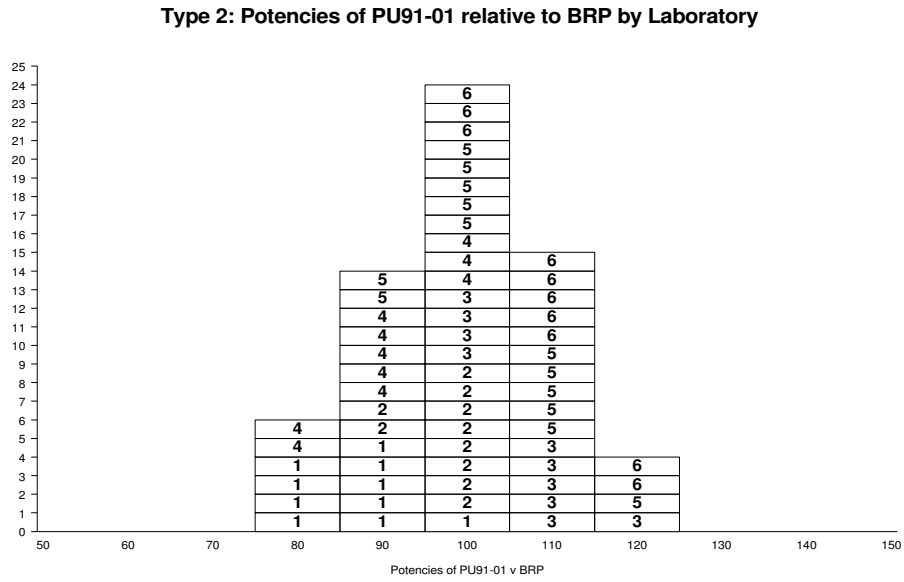


Figure 2d:

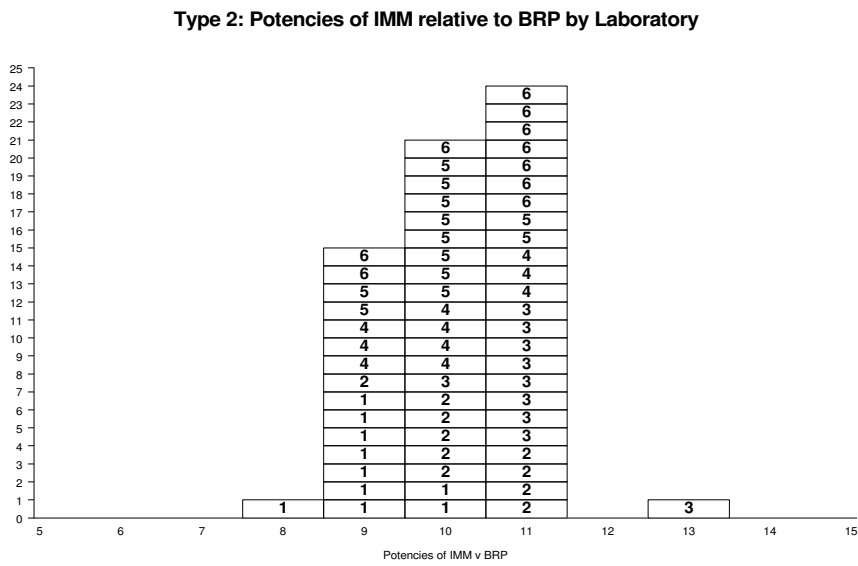


Figure 4a:

Plot of within lab variability for Type 1 sample 06/110

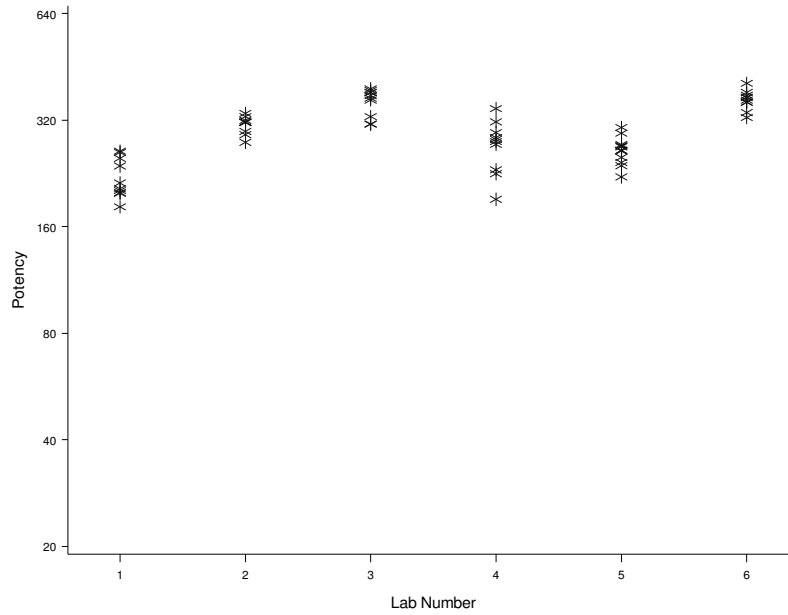


Figure 4b:

Plot of within lab variability for Type 1 sample 07/140

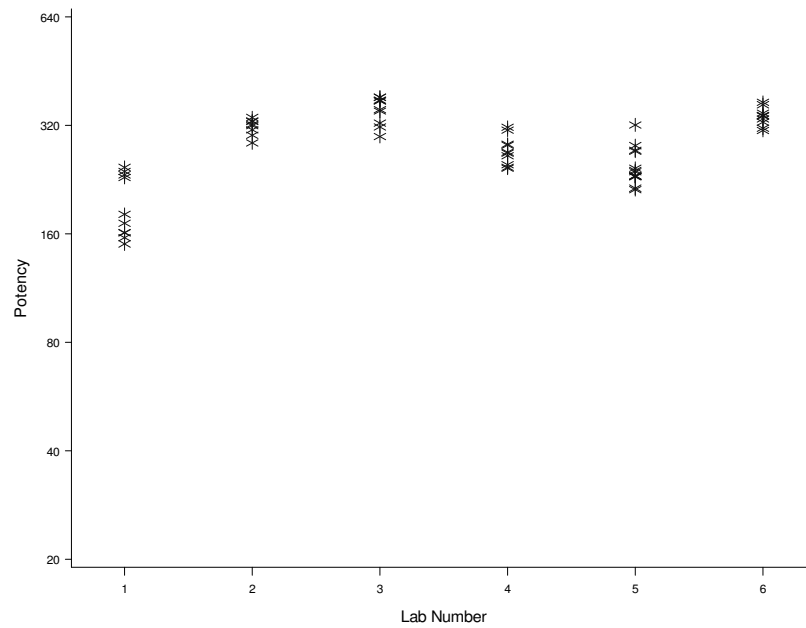


Figure 4c:

Plot of within lab variability for Type 1 sample PU91-01

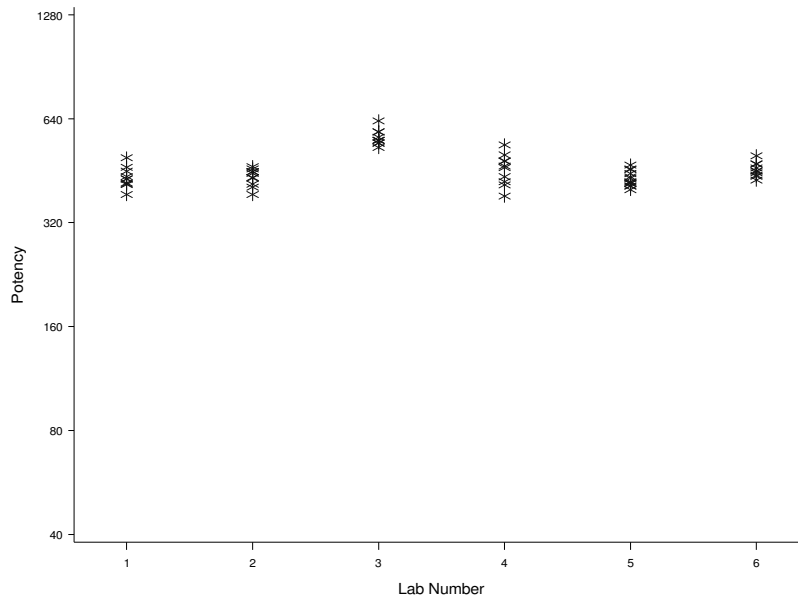


Figure 4d:

Plot of within lab variability for Type 1 sample IMM

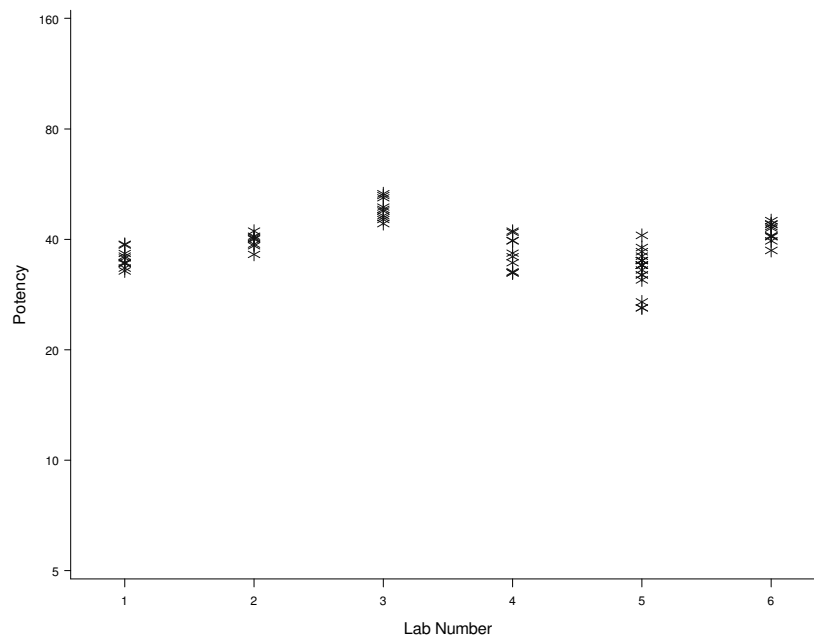


Figure 5a:

Plot of within lab variability for Type 2 sample 06/110

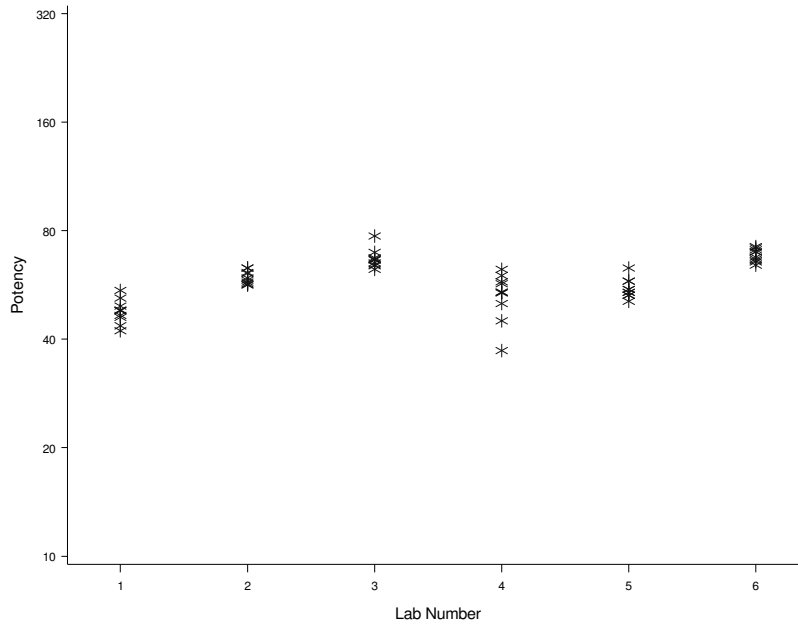


Figure 5b:

Plot of within lab variability for Type 2 sample 07/140

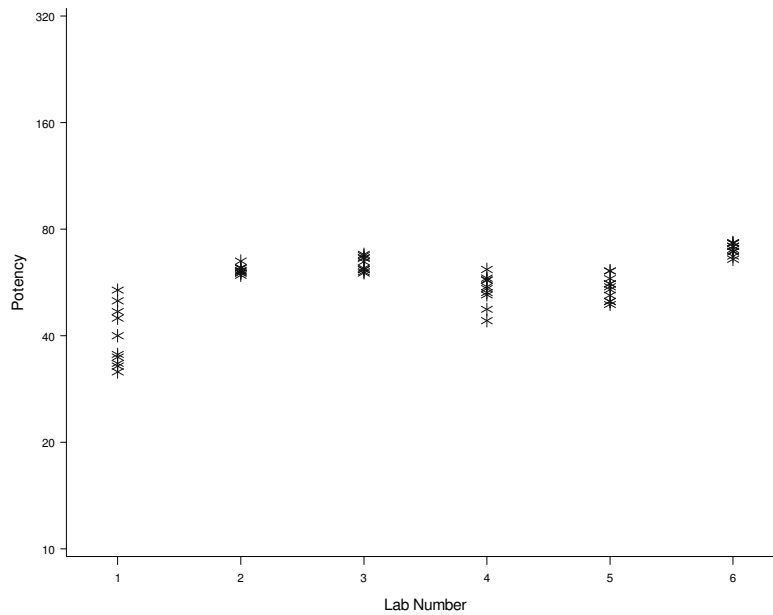


Figure 5c:

Plot of within lab variability for Type 2 sample PU91-01

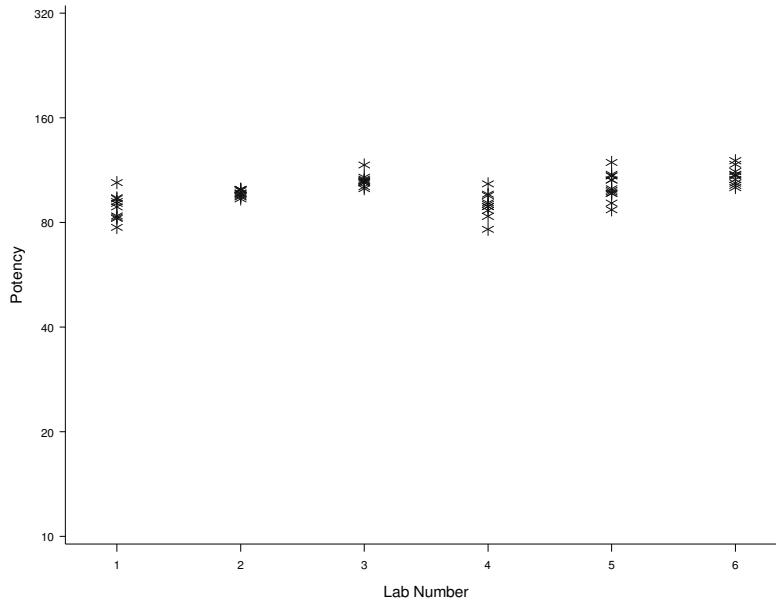


Figure 5d:

Plot of within lab variability for Type 2 sample IMM

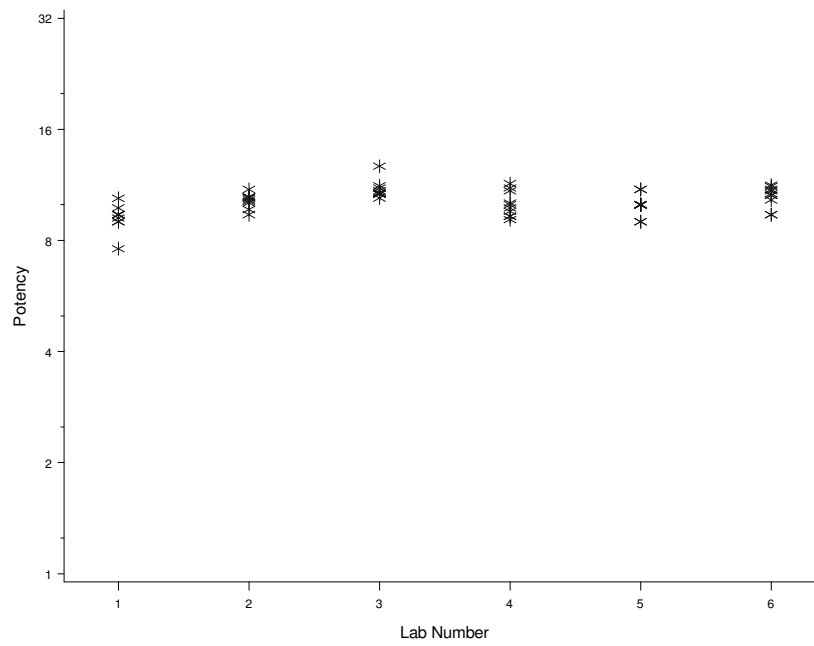


Figure 6a:

Plot of within lab variability for Type 3 sample 06/110

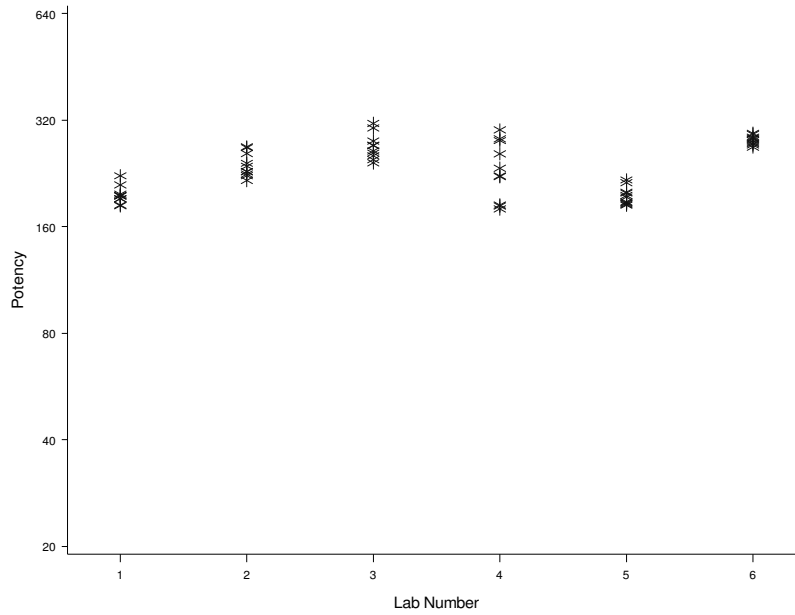


Figure 6b:

Plot of within lab variability for Type 3 sample 07/140

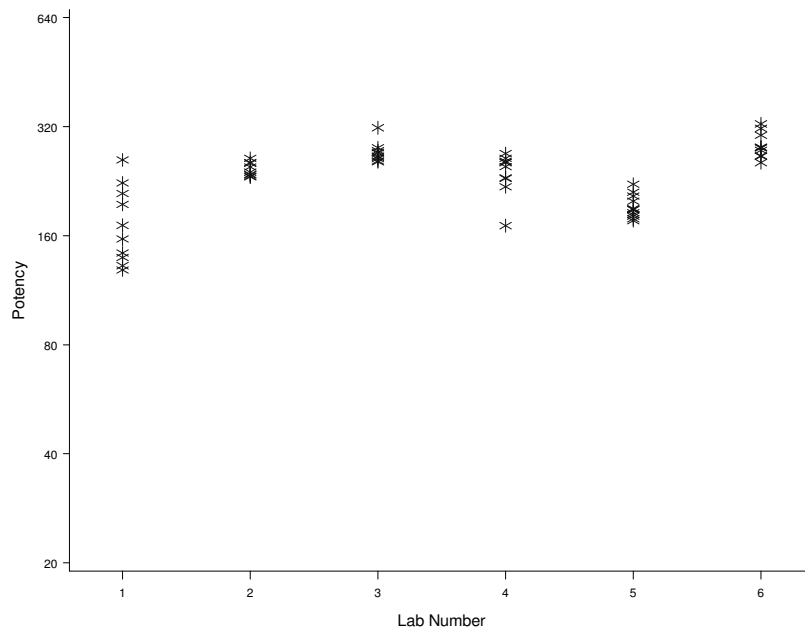


Figure 6c:

Plot of within lab variability for Type 3 sample PU9101

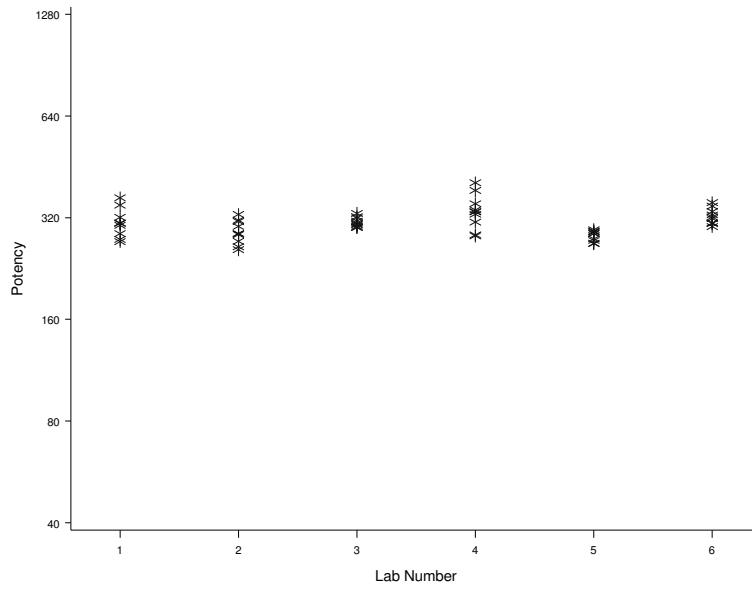


Figure 6d:

Plot of within lab variability for Type 3 sample IMM

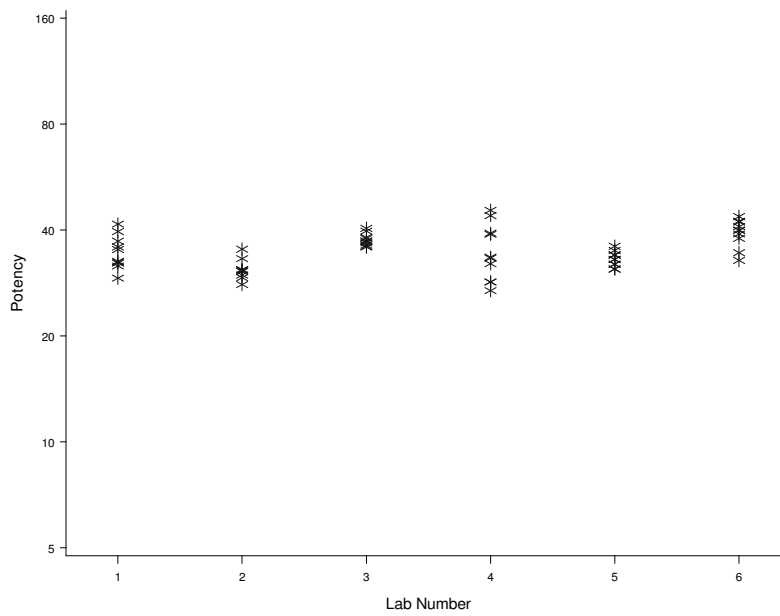


Figure 7a:

Sample 06/110 (2010 study) and A/C (2009 study)

Potencies relative to BRP no.1 (2009), BRP no.2 (2010) and PU91-01 (2010)

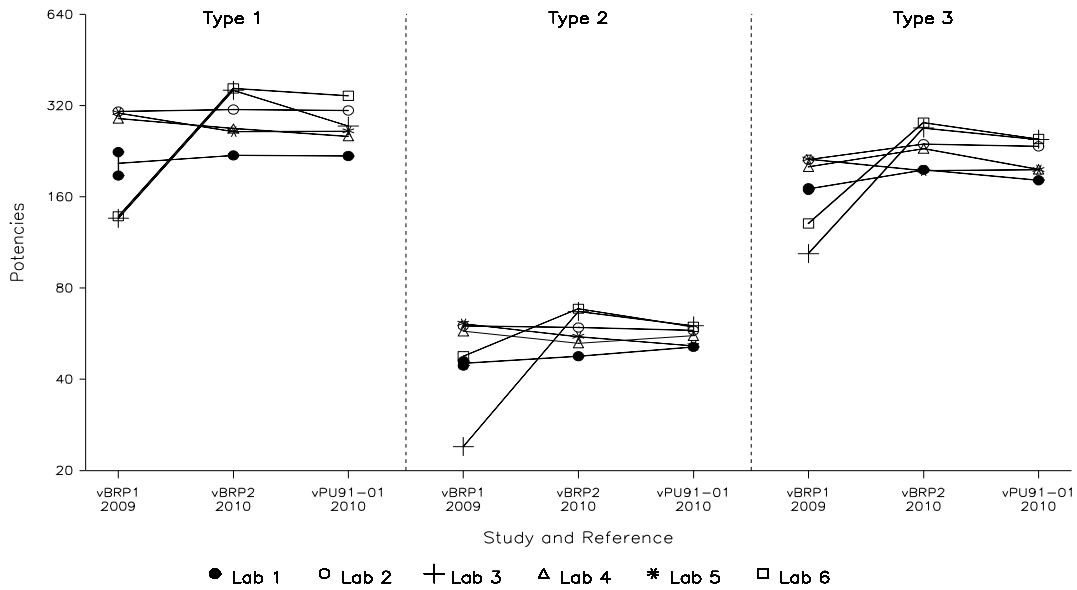


Figure 7b:

Sample 07/140 (2010 study) and X/Y (2009 study)

Potencies relative to BRP no.1 (2009), BRP no.2 (2010) and PU91-01 (2010)

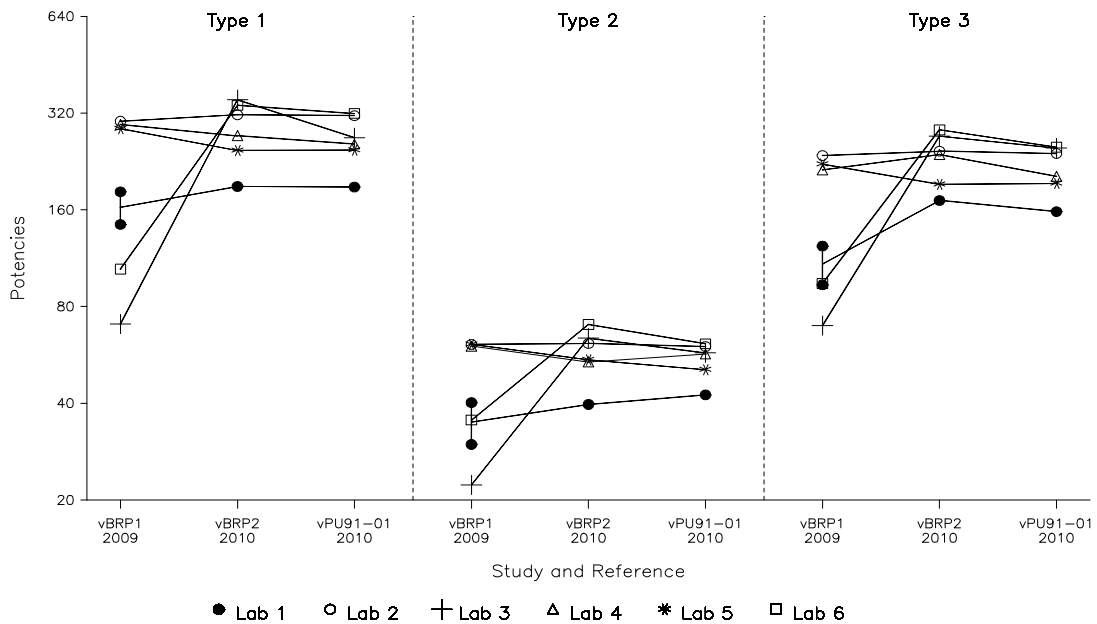


Figure 7c:

Sample IMM (2010 study) and F (2009 study)

Potencies relative to BRP no.1 (2009), BRP no.2 (2010) and PU91-01 (2010)

