**WHO/BS/2018.2335**
# WHO 7ᵗʰ International Standard for Rabies Vaccine

Dianna E. Wilkinson, Jason Hockley, Peter Rigsby and the Collaborative Study Group

# EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

## Geneva, 29 October to 2 November 2018

**International collaborative study to assess the suitability of the candidate 7th WHO International Standard for rabies vaccine**

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# Introduction

- Rabies vaccines for human use are produced in many countries and the **minimum potency requirements are expressed in IU**.

- The WHO International Standard for rabies vaccine (inactivated) is used by manufacturers of human and veterinary vaccines and control testing laboratories for the standardisation of:

  - ➢ **NIH mouse potency tests**

  - ➢ **ELISA and Single Radial Immunodiffusion (SRD) assays for glycoprotein content**

- **The current IS (6th IS NIBSC code 07/162 is essentially depleted**

- A collaborative study was undertaken to assess the suitability of a candidate **7th IS (NIBSC code 16/204),** which was **prepared from a purified and inactivated bulk of Vero cell-derived, Pitman Moore strain, rabies vaccine**, and to calibrate it in International Units (IU).

# Collaborative study samples

| Sample ID | Sample Description | Sample volume filled per tube (mL) | Presentation | Assigned or target potency |
|---|---|---|---|---|
| 07/162 | 6th IS for Rabies Vaccine | 0.5 | Freeze-dried | 8 IU/ml in 1 mL (NIH) <br><br> 6.6 IU/ml in 0.5 mL (ELISA and SRD) |
| RAV | 5th IS for Rabies Vaccine | 1.0 | Freeze-dried | 16 IU/mL in 1 mL (NIH test) |
| Sample A | Candidate 7th IS (NIBSC 16/204) | 0.5 | Freeze-dried | Target formulation potency based on vaccine bulk antigen content. 7 IU/mL by SRD |
| Sample B | Candidate 7th IS (NIBSC 16/204) | 0.5 | Freeze-dried | |
| Sample C | Bulk preparation remaining from 6th IS production | 1.0 | Liquid | Not determined (in vitro assays) |

# Candidate 7th Rabies Vaccine IS production

The bulk vaccine provided to NIBSC for formulating the candidate 7th IS was produced by the same manufacturing process used to prepare the bulk that went into the formulation of the 6th IS.

- Pitman Moore strain

- Vero cell-derived

- Purified inactivated bulk

| | |
|---|---|
| NIBSC code | **16/204** |
| Product name | **7th IS Rabies Vaccine** |
| Collaborative study code | **CS605** |
| Filling machine | **Bausch &Strobel AFV5090** |
| Date of filling | **22 September 2016** |
| No. of ampoules filled | **11,098** |
| Mean fill weight (g) | **0.5238** |
| CV of fill weight (%) | **0.3255 n=372** |
| Freeze dryer | **CS100** |
| Date of completion of lyophilization | **27 September 2016** |
| Mean dry weight (g) | **0.0551** |
| CV of dry weight (%) | **0.3478 n=6** |
| Mean residual moisture (%) | **0.4** |
| CV of residual moisture (%) | **20.5 n=12** |
| Mean oxygen content (%) | **0.13** |
| CV of oxygen content (%) | **81.37 n=12** |
| No. of ampoules available to WHO as of March 2018 | **10,298** |

16/204 was validated in NIH, ELISA (Ref lab 1) and SRD (Ref lab 2) before use in the collaborative study

# Collaborative study design

**Participants were requested to:**

- perform 3 independent assays

- use a freshly opened vial or reconstituted ampoule for each assay.

- reconstitute the freeze-dried samples as directed in the instructions for use.

  - NIH assay: 1mL

  - ELISA and SRD: 0.5mL

- prepare and test a series of dilutions for the 6th IS and each study sample and **test all samples concurrently**

- Report results using the Excel worksheet supplied by NIBSC

# Collaborative study design

## Statistical Analysis:

- Data were analysed using the EDQM software CombiStats

  - **NIH mouse potency** test: **probit bioassay** analysis comparing transformed assay responses to log dose using the **6th IS** (**8 IU/mL** when reconstituted as directed in 1 mL water)

  - **ELISA: parallel line or sigmoid curve model** with untransformed or log transformed responses. Potencies were expressed relative to the **6th IS** (**6.6 IU/ml** when reconstituted as directed in 0.5 ml water i.e. 3.3 IU/ampoule)

  - **SRD assays: slope-ratio assays**. Potencies were expressed relative to the **6th IS** (**6.6 IU/ml** when reconstituted as directed in 0.5 ml water i.e. 3.3 IU/ampoule)

# Collaborative study results

- 16 laboratories from 12 countries returned data sets for 26 assays.
  Argentina (2), Canada (1), France (2), Germany (2), India (2), Mexico (1) Russian Federation (1), Serbia (1), South Africa (1), Thailand (1) UK (1) and USA (1)

- Labs were randomly assigned codes

| Laboratory code | Method |
|---|---|
| 1 | SRD |
| 2 | NIH |
| 3a | NIH |
| 3b | ELISA |
| 3c | SRD |
| 4 | ELISA |
| 5a | NIH |
| 5b | ABT |
| 6a | NIH |
| 6b1 | ELISA technician 1 |
| 6b2 | ELISA technician 2 |
| 7a | NIH |
| 7b | ELISA |
| 7c | SRD |

| Laboratory code | Method |
|---|---|
| 8 | SRD |
| 9a | ELISA |
| 9b | NIH |
| 10 | NIH |
| 11 | NIH |
| 12a | ELISA |
| 12b | NIH |
| 13a | SRD |
| 13b | SRD |
| 14 | NIH |
| 15 | ELISA |
| 16 | ELISA |

Data sets

10 NIH test

9 ELISA

6 SRD

1 ABT laboratory calculated and provided results for their in vitro ABT method.

ABT: Antibody binding test. This method tests the antigen content of a vaccine after neutralization with a standard antibody in competition with rabies strain CVS-11

# NIH Test Results – Sample A (16/204)

| Lab Code | Assay | LCL | Potency (IU/mL) | UCL | Intra-laboratory %GCV | LCL | Laboratory GM Potency (IU/mL) | UCL |
|---|---|---|---|---|---|---|---|---|
| 2 | 1 | ~ | NP | ~ | | | | |
| 2 | 2 | 7.665 | 19.865 | 55.043 | n/a | 3.976 | 7.595 | 14.508 |
| 2 | 3 | 1.530 | 3.665 | 8.514 | | | | |
| 3a | 1 | 2.539 | 6.307 | 15.454 | | | | |
| 3a | 2 | 0.750 | 2.261 | 6.026 | 102% | 3.424 | 5.696 | 9.475 |
| 3a | 3 | 4.146 | 8.704 | 19.113 | | | | |
| 5a | 1 | 3.146 | 8.982 | 25.980 | | | | |
| 5a | 2 | 1.998 | 5.262 | 13.412 | 32% | 3.703 | 6.416 | 11.117 |
| 5a | 3 | 2.500 | 6.022 | 14.359 | | | | |
| 6a | 1 | 7.416 | 26.306 | 114.504 | | | | |
| 6a | 2 | 6.757 | 20.125 | 67.315 | 46% | 8.965 | 17.448 | 33.958 |
| 6a | 3 | 4.556 | 12.426 | 34.924 | | | | |
| 7a | 1 | 3.835 | 7.359 | 13.956 | | | | |
| 7a | 2 | 4.546 | 7.974 | 13.932 | 8% | 5.624 | 7.978 | 11.317 |
| 7a | 3 | 4.644 | 8.600 | 16.076 | | | | |
| 9b | 1 | 1.688 | 7.523 | 33.242 | | | | |
| 9b | 2 | 2.400 | 9.582 | 40.120 | 18% | 3.518 | 7.919 | 17.827 |
| 9b | 3 | 1.819 | 6.958 | 26.087 | | | | |
| 10 | 1 | ~ | NP | ~ | | | | |
| 10 | 2 | 3.536 | 13.167 | 46.724 | n/a | 3.972 | 11.358 | 32.482 |
| 10 | 3 | 1.241 | 8.494 | 46.310 | | | | |
| 11 | 1 | 3.089 | 9.306 | 28.454 | | | | |
| 11 | 2 | 3.558 | 8.373 | 19.759 | 29% | 4.357 | 7.532 | 13.020 |
| 11 | 3 | 2.153 | 5.746 | 13.723 | | | | |
| 12b | 1 | 2.850 | 7.123 | 17.452 | | | | |
| 12b | 2 | 2.047 | 5.401 | 13.271 | 15% | 3.580 | 6.149 | 10.562 |
| 12b | 3 | 2.189 | 5.976 | 15.344 | | | | |
| 14 | 1 | 7.302 | 16.330 | 37.638 | | | | |
| 14 | 2 | 7.799 | 17.448 | 40.095 | 5% | 11.005 | 17.353 | 27.361 |
| 14 | 3 | 8.727 | 18.144 | 38.086 | | | | |

| Sample A | |
|---|---|
| Laboratory GM Potency | |
| Min | 5.696 |
| Max | 17.448 |
| Intra-laboratory %GCV | |
| Min | 5% |
| Max | 102% |

| Sample A | |
|---|---|
| Overall GM (IU/mL) | 8.803 |
| 95% CL | 6.587-11.764 |
| Overall %GCV | 50% |

# NIH Test Results – Sample B (16/204)

| Lab Code | Assay | LCL | Potency (IU/mL) | UCL | Intra-laboratory %GCV | LCL | Laboratory GM Potency (IU/mL) | UCL |
|---|---|---|---|---|---|---|---|---|
| 2 | 1 | 7.848 | 15.479 | 30.681 | | | | |
| 2 | 2 | 6.434 | 16.923 | 47.894 | 11% | 10.678 | 16.905 | 26.762 |
| 2 | 3 | 8.754 | 19.028 | 42.678 | | | | |
| 3a | 1 | 2.911 | 7.844 | 21.101 | | | | |
| 3a | 2 | 1.944 | 5.243 | 13.663 | 46% | 4.952 | 8.265 | 13.796 |
| 3a | 3 | 5.271 | 11.224 | 24.021 | | | | |
| 5a | 1 | 3.112 | 7.866 | 19.858 | | | | |
| 5a | 2 | 2.629 | 6.592 | 16.311 | 35% | 3.593 | 6.163 | 10.571 |
| 5a | 3 | 1.626 | 4.380 | 11.248 | | | | |
| 6a | 1 | 3.847 | 12.952 | 46.895 | | | | |
| 6a | 2 | 4.817 | 16.429 | 64.671 | 25% | 6.412 | 12.658 | 24.990 |
| 6a | 3 | 3.777 | 10.549 | 30.091 | | | | |
| 7a | 1 | ~ | NL | ~ | | | | |
| 7a | 2 | 4.079 | 7.042 | 11.991 | n/a | 4.511 | 6.759 | 10.128 |
| 7a | 3 | 3.439 | 6.412 | 11.680 | | | | |
| 9b | 1 | 2.994 | 14.20 | 83.24 | | | | |
| 9b | 2 | 0.783 | 3.82 | 15.11 | 103% | 2.510 | 5.833 | 13.554 |
| 9b | 3 | 1.203 | 4.69 | 16.31 | | | | |
| 10 | 1 | 1.257 | 5.323 | 19.064 | | | | |
| 10 | 2 | 0.906 | 4.093 | 14.708 | 27% | 2.389 | 5.264 | 11.596 |
| 10 | 3 | 1.585 | 6.597 | 23.670 | | | | |
| 11 | 1 | 6.885 | 18.284 | 52.102 | | | | |
| 11 | 2 | 2.530 | 6.838 | 18.238 | 67% | 5.758 | 10.272 | 18.324 |
| 11 | 3 | 3.240 | 8.854 | 24.352 | | | | |
| 12b | 1 | 3.514 | 8.068 | 18.547 | | | | |
| 12b | 2 | 2.752 | 6.422 | 14.512 | 35% | 3.891 | 6.681 | 11.471 |
| 12b | 3 | 0.988 | 4.448 | 15.436 | | | | |
| 14 | 1 | 8.122 | 17.158 | 36.904 | | | | |
| 14 | 2 | 7.592 | 17.053 | 39.584 | 3% | 11.199 | 17.474 | 27.267 |
| 14 | 3 | 8.709 | 18.130 | 38.069 | | | | |

## Blinded Duplicate

| Sample B | |
|---|---|
| Laboratory GM Potency | |
| Min | 5.264 |
| Max | 17.474 |
| Intra-laboratory %GCV | |
| Min | 3% |
| Max | 103% |

| Sample B | |
|---|---|
| Overall GM (IU/mL) | 8.777 |
| 95% CL | 6.395 - 12.045 |
| Overall %GCV | 56% |

# NIH Test Results – Samples A & B combined

Combined Overall GM Potencies for blinded duplicates of the Candidate 7th IS Rabies Vaccine (16/204) [Samples A and B] in NIH.

| | | LCL | | UCL |
|---|---|---|---|---|
| Samples A and B N=20 | Overall GM Potency (IU/mL) | 7.208 | 8.790 | 10.722 |
| | Overall %GCV | | 51% | |
| | Max | | 17.474 | |
| | Min | | 5.264 | |

Rabies vaccine potencies for 5th IS Rabies Vaccine (RAV) expressed relative to the 6th IS (8 IU/mL in 1 mL) in the NIH Test.

| Lab Code * | Assay | LCL | Potency (IU/mL) | UCL | Intra-laboratory %GCV | LCL | Laboratory GM Potency (IU/mL) | UCL |
|---|---|---|---|---|---|---|---|---|
| 2 | 1 | 4.155 | 8.822 | 18.886 | | | | |
| 2 | 2 | 14.723 | 36.492 | 101.174 | 105% | 9.474 | 16.187 | 27.656 |
| 2 | 3 | 6.705 | 21.350 | 78.096 | | | | |
| 3a | 1 | 9.200 | 21.822 | 54.569 | | | | |
| 3a | 2 | 2.608 | 7.726 | 22.689 | 87% | 11.126 | 18.264 | 29.982 |
| 3a | 3 | 11.663 | 23.720 | 48.776 | | | | |
| 6a | 1 | 8.147 | 32.449 | 152.439 | | | | |
| 6a | 2 | 7.860 | 24.004 | 85.065 | 38% | 10.811 | 24.201 | 54.171 |
| 6a | 3 | 3.687 | 16.965 | 99.305 | | | | |
| 7a | 1 | 4.243 | 7.989 | 14.964 | | | | |
| 7a | 2 | 4.762 | 9.026 | 17.484 | 12% | 6.299 | 9.002 | 12.866 |
| 7a | 3 | 5.632 | 9.945 | 18.009 | | | | |

Note*: Not all laboratories received RAV for testing.

5th IS Rabies Vaccine (RAV) **assigned value 16 IU/mL**

Very close to the assigned potency

| | | LCL | | UCL |
|---|---|---|---|---|
| RAV N=4 | Overall GM Potency (IU/mL) | 8.216 | **15.931** | 30.890 |
| | Overall %GCV | | **52%** | |

# ELISA Results – Sample A (16/204)

| Lab Code | Assay | LCL | Potency (IU/mL) | UCL | Intra-laboratory %GCV | LCL | Laboratory GM Potency (IU/mL) | UCL |
|---|---|---|---|---|---|---|---|---|
| 3b | 1 | 5.076 | 5.190 | 5.305 | | | | |
| 3b | 2 | 5.064 | 5.226 | 5.393 | 4.0% | 5.079 | 5.166 | 5.256 |
| 3b | 3 | 4.614 | 4.868 | 5.137 | | | | |
| 4 | 1 | 5.323 | 5.535 | 5.754 | | | | |
| 4 | 2 | 5.853 | 6.194 | 6.554 | 7.8% | 5.343 | 5.647 | 5.969 |
| 4 | 3 | 5.255 | 5.369 | 5.486 | | | | |
| 6b1 | 1 | 4.754 | 4.989 | 5.234 | | | | |
| 6b1 | 2 | 4.881 | 5.056 | 5.238 | 12.0% | 4.924 | 5.340 | 5.790 |
| 6b1 | 3 | 5.750 | 6.112 | 6.497 | | | | |
| 6b2 | 1 | 5.077 | 5.188 | 5.302 | | | | |
| 6b2 | 2 | 4.831 | 4.963 | 5.098 | 3.2% | 5.032 | 5.156 | 5.284 |
| 6b2 | 3 | 5.202 | 5.276 | 5.352 | | | | |
| **6b1 and 6b2 results on the same reconstituted material combined*** | | | | | 8.1% | 5.056 | 5.209 | 5.366 |
| 7b | 1 | 4.158 | 4.350 | 4.555 | | | | |
| 7b | 2 | 3.897 | 4.349 | 4.861 | 0.6% | 4.210 | 4.333 | 4.459 |
| 7b | 3 | 4.073 | 4.303 | 4.552 | | | | |
| 9a** | 1 | ~ | invalid | ~ | | | | |
| 9a** | 2 | ~ | invalid | ~ | n/d | n/d | n/d | n/d |
| 9a** | 3 | ~ | invalid | ~ | | | | |
| 12a | 1 | 4.389 | 4.563 | 4.743 | | | | |
| 12a | 2 | 4.238 | 4.396 | 4.560 | 4.3% | 4.320 | 4.426 | 4.535 |
| 12a | 3 | 3.955 | 4.197 | 4.451 | | | | |
| 15 | 1 | 5.368 | 5.644 | 5.932 | | | | |
| 15 | 2 | 5.218 | 5.362 | 5.509 | 7.9% | 4.978 | 5.257 | 5.552 |
| 15 | 3 | 4.734 | 4.864 | 4.996 | | | | |
| 16 | 1 | 4.256 | 4.516 | 4.792 | | | | |
| 16 | 2 | 3.808 | 3.962 | 4.122 | 25.2% | 4.103 | 4.781 | 5.570 |
| 16 | 3 | 5.795 | 6.134 | 6.493 | | | | |

| Sample A | |
|---|---|
| **Laboratory GM Potency** | |
| Min | 4.333 |
| Max | 5.647 |
| **Intra-laboratory %GCV** | |
| Min | 0.6% |
| Max | 25.2% |

| Sample A | |
|---|---|
| Overall GM (IU/mL) | 4.954 |
| 95% CL | 4.528-5.421 |
| Overall %GCV | 10.2% |

*For laboratory 6b, the combined operator results were used to determine overall GM and %GCV.

** Assays for laboratory 9a invalid due to confidence intervals for individual assay falling outside the 80%-120% range.

# ELISA Results – Sample B (16/204)

| Lab Code | Assay | LCL | Potency (IU/mL) | UCL | Intra-laboratory %GCV | LCL | Laboratory GM Potency (IU/mL) | UCL |
|---|---|---|---|---|---|---|---|---|
| 3b | 1 | 4.820 | 5.039 | 5.268 | | | | |
| 3b | 2 | 5.308 | 5.420 | 5.535 | 4.9% | 4.973 | 5.170 | 5.374 |
| 3b | 3 | 4.729 | 4.948 | 5.177 | | | | |
| 4 | 1 | 5.236 | 5.463 | 5.700 | | | | |
| 4 | 2 | 5.656 | 5.972 | 6.305 | 4.6% | 5.545 | 5.635 | 5.727 |
| 4 | 3 | 5.528 | 5.631 | 5.735 | | | | |
| 6b1 | 1 | 5.049 | 5.165 | 5.283 | | | | |
| 6b1 | 2 | 5.291 | 5.544 | 5.810 | 5.0% | 5.041 | 5.256 | 5.481 |
| 6b1 | 3 | 4.708 | 5.045 | 5.405 | | | | |
| 6b2 | 1 | 5.132 | 5.216 | 5.302 | | | | |
| 6b2 | 2 | 4.978 | 5.074 | 5.172 | 2.9% | 5.109 | 5.222 | 5.336 |
| 6b2 | 3 | 5.279 | 5.373 | 5.468 | | | | |
| 6b1 and 6b2 results on the same reconstituted material combined* | | | | | 3.7% | 5.144 | 5.229 | 5.316 |
| 7b | 1 | 4.067 | 5.268 | 6.781 | | | | |
| 7b | 2 | 4.426 | 4.755 | 5.105 | 10.1% | 4.283 | 4.400 | 4.520 |
| 7b | 3 | 4.239 | 4.348 | 4.459 | | | | |
| 9a** | 1 | ~ | invalid | ~ | | | | |
| 9a** | 2 | ~ | invalid | ~ | n/d | n/d | n/d | n/d |
| 9a** | 3 | ~ | invalid | ~ | | | | |
| 12a | 1 | 4.542 | 4.731 | 4.926 | | | | |
| 12a | 2 | 4.624 | 4.792 | 4.966 | 7.1% | 4.355 | 4.586 | 4.830 |
| 12a | 3 | 4.039 | 4.228 | 4.424 | | | | |
| 15 | 1 | 5.111 | 5.370 | 5.639 | | | | |
| 15 | 2 | 5.472 | 5.572 | 5.673 | 5.1% | 5.136 | 5.338 | 5.547 |
| 15 | 3 | 4.891 | 5.049 | 5.211 | | | | |
| 16 | 1 | 4.406 | 4.622 | 4.848 | | | | |
| 16 | 2 | 3.732 | 3.914 | 4.104 | 8.8% | 3.965 | 4.222 | 4.496 |
| 16 | 3 | 3.956 | 4.160 | 4.375 | | | | |

| Sample B | |
|---|---|
| Laboratory GM Potency | |
| Min | 4.222 |
| Max | 5.635 |
| Intra-laboratory %GCV | |
| Min | 3.7% |
| Max | 10.1% |

| Sample B | |
|---|---|
| Overall GM (IU/mL) | 4.915 |
| 95% CL | 4.441-5.439 |
| Overall %GCV | 11.6% |

*For laboratory 6b, the combined operator results were used to determine overall GM and %GCV.

** Assays for laboratory 9a invalid due to confidence intervals for individual assay falling outside the 80%-120% range.

# ELISA Results – Samples A & B combined

Combined Overall GM Potencies for blinded duplicates of the Candidate 7th IS Rabies Vaccine (16/204) [Samples A and B] in ELISA.

| | | LCL | | UCL |
|---|---|---|---|---|
| Samples A and B N=14 | overall GM Potency (IU/mL) | 4.650 | 4.937 | 5.242 |
| | Overall %GCV | | 10.5% | |
| | Max | | 5.647 | |
| | Min | | 4.222 | |

| Lab Code | Assay | LCL | Potency (IU/mL) | UCL | Intra-laboratory %GCV | LCL | Laboratory GM Potency (IU/mL) | UCL |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 5.794 | 6.164 | 6.550 | | | | |
| 1 | 2 | 3.678 | 3.904 | 4.134 | 28.6% | 4.395 | 5.212 | 6.181 |
| 1 | 3 | 5.442 | 5.895 | 6.368 | | | | |
| 3c | 1 | 4.269 ~~0.000~~ | 5.251 ~~4.786~~ | 5.315 ~~6.076~~ | | | | |
| 3c | 2 | 5.245 | 6.113 | 6.721 | n/a | 5.223 ~~5.174~~ | 5.715 ~~5.736~~ | 6.254 ~~6.361~~ |
| 3c | 3 | ~ ~~3.282~~ | Invalid* ~~4.610~~ | ~ ~~5.271~~ | | | | |
| 7c | 1 | 5.056 | 5.559 | 6.077 | | | | |
| 7c | 2 | 4.576 | 5.069 | 5.561 | 4.9% | 5.062 | 5.289 | 5.527 |
| 7c | 3 | 4.684 | 5.212 | 5.744 | | | | |
| 8 | 1 | 4.805 | 5.743 | 6.770 | | | | |
| 8 | 2 | 5.527 | 6.763 | 8.309 | 8.5% | 5.635 | 6.203 | 6.828 |
| 8 | 3 | 5.036 | 6.247 | 7.684 | | | | |
| 13a | 1 | 4.626 | 5.654 | 6.779 | | | | |
| 13a | 2 | 5.750 | 6.430 | 7.183 | 9.3% | 5.562 | 5.970 | 6.408 |
| 13a | 3 | 4.641 | 5.415 | 6.230 | | | | |
| 13b | 1 | 5.200 | 5.785 | 6.403 | | | | |
| 13b | 2 | 5.514 | 6.163 | 6.869 | 5.6% | 5.555 | 5.918 | 6.305 |
| 13b | 3 | 4.234 | 5.532 | 6.853 | | | | |

Highlighted: post-public consultation corrections
*Invalid due to 95% CL falling outside 80%-120%

| Sample A | |
|---|---|
| **Laboratory GM Potency** | |
| Min | 5.212 |
| Max | 6.203 |
| **Intra-laboratory %GCV** | |
| Min | 4.9% |
| Max | 28.6% |

| Sample A | |
|---|---|
| Overall GM (IU/mL) | 5.706 ~~5.710~~ |
| 95% CL | 5.3074– ~~5.30~~ 6.1440 ~~6.14~~ |
| Overall %GCV | 7.2% |

# SRD Results – Sample B (16/204)

Blinded Duplicate

| Lab Code | Assay | LCL | Potency (IU/mL) | UCL | Intra-laboratory %GCV | LCL | Laboratory GM Potency (IU/mL) | UCL |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 5.560 | 6.022 | 6.508 | | | | |
| 1 | 2 | 6.453 | 6.739 | 7.052 | 8.1% | 5.852 | 6.217 | 6.605 |
| 1 | 3 | 5.348 | 5.799 | 6.267 | | | | |
| 3c | 1 | 4.649 | 6.300 | 7.209 | | | | |
| 3c | 2 | 6.087 | 6.375 | 6.632 | 0.6% | 6.172 | 6.349 | 6.532 |
| 3c | 3 | 6.043 | 6.324 | 6.588 | | | | |
| 7c | 1 | 4.113 | 4.630 | 5.133 | | | | |
| 7c | 2 | 4.417 | 4.913 | 5.403 | 8.4% | 4.783 | 5.011 | 5.250 |
| 7c | 3 | 4.906 | 5.432 | 5.969 | | | | |
| 8 | 1 | 5.312 | 6.346 | 7.549 | | | | |
| 8 | 2 | 5.277 | 6.499 | 7.982 | 4.4% | 5.709 | 6.267 | 6.879 |
| 8 | 3 | 4.959 | 5.981 | 7.133 | | | | |
| 13a | 1 | 4.598 | 5.640 | 6.781 | | | | |
| 13a | 2 | 5.093 | 5.894 | 6.768 | 5.6% | 5.173 | 5.595 | 6.050 |
| 13a | 3 | 4.563 | 5.292 | 6.050 | | | | |
| 13b | 1 | 5.305 | 6.188 | 7.182 | | | | |
| 13b | 2 | 4.843 | 6.150 | 7.707 | 6.8% | 5.436 | 5.973 | 6.563 |
| 13b | 3 | 4.399 | 5.506 | 6.625 | | | | |

| Sample B | |
|---|---|
| Laboratory GM Potency | |
| Min | 5.011 |
| Max | 6.349 |
| Intra-laboratory %GCV | |
| Min | 0.6% |
| Max | 8.4% |

| Sample B | |
|---|---|
| Overall GM (IU/mL) | 5.882 |
| 95% CL | 5.347-6.471 |
| Overall %GCV | 9.5% |

# SRD Results – Samples A & B combined

Combined Overall GM Potencies for blinded duplicates of the Candidate 7th IS Rabies Vaccine (16/204) [Samples A and B] in SRD.

| | | LCL | | UCL |
|---|---|---|---|---|
| Samples A and B N=12 | overall GM Potency (IU/mL) | 5.510 5.490 | 5.794 5.773 | 6.091 6.071 |
| | Overall %GCV | | 8.2% | |
| | Max | | 6.349 | |
| | Min | | 5.011 | |

Highlighted: post-public consultation corrections

# Stability of Candidate 7th IS (16/204)

| Temperature (°C) | Combined Potency by ELISA n=3 (IU/mL) | 95% CI | Predicted % Monthly Loss (UCL) | Predicted % Yearly Loss (UCL) |
|---|---|---|---|---|
| -20 | 97.92 | 0.93-1.03 | 0 (0) | 0.002 (0.037) |
| +4 | 99.07 | 0.96-1.01 | 0.009 (0.094) | 0.111 (1.164) |
| +20 | 99.05 | 0.97-1.01 | 0.087 (0.486) | 1.042 (5.708) |
| +37 | 90.87 | 0.87-0.95 | 0.73 (1.634) | 8.416 (17.93) |

- The predicted loss of potency is 0.002% per year when stored at -20 °C
- The candidate 7th IS Rabies vaccine is stable for long-term storage at -20 °C
- Given that the predicated loss of potency at 37 °C is 0.73 % per month, the 7th IS may be shipped at ambient temperature.

# Comments/Issues/Summary

- Ten participating laboratories responded to the report. There were no disagreements with the suitability of the candidate (NIBSC code 16/204) to serve as the 7th WHO IS for Rabies Vaccine.

- 16/204 should be assigned different unitages for the 3 assay methods.

- Some respondents had queries or suggestions for editorial changes and these have been addressed.

- One participant indicated that Huber's robust means should be calculated for the three assay methods to account for outliers. This has been added to the report.

| Assay | NIH | ELISA | SRD |
|---|---|---|---|
| 16/204 Overall GM Potency (IU/mL) | 8.79 | 4.94 | 5.79 |
| 16/204 Overall %GCV | 51% | 10.5% | 8.2% |
| 95% Confidence Interval | 7.21-10.72 | 4.65-5.24 | 5.51-6.09 |
| 16/204 Huber's Robust Mean (IU/mL) | 8.86 | 4.89 | 5.84 |

# Accepted Proposal

**Table 25.** Proposed unitage (IU/ampoule) and recommended volumes for reconstitution of the candidate 7$^{th}$ IS (16/204) for use in the NIH test, ELISA or SRD assay. The unitage is derived from the Huber's robust mean for each assay method.

| Assay | NIH | ELISA | SRD |
|---|---|---|---|
| Proposed unitage | 8.9 IU/ampoule | 2.5 IU/ampoule | 2.9 IU/ampoule |
| Recommended volume for reconstitution | 1 mL | 0.5 mL | 0.5mL |

# Participants

| Name | Laboratory | Country |
|------|-----------|---------|
| Alejandro Parola, Analia De Nichilo | Fundación Pablo Cassará, (Pablo Cassara Foundation) | Argentina |
| Maria Luisa Brero, Silvina Gil, Javier Espeche, Silvana Deluchi, Carlos Atzori | Servicio Vacunas Bacterianas<br>Centro Nacional de Control de Calidad de Biológicos (CNCCB)<br>Administración Nacional de Laboratorios e Institutos de Salud. (ANLIS) "Dr Carlos G Malbrán" | Argentina |
| Cynthia M. Allen, Gayle Pulle | Viral Vaccines Division, Center for Biologics Evaluation (CBE)<br>Biologics and Genetic Therapies Directorate (BGTD), Health Canada | Canada |
| Stéphane MAISONNEUVE | Agence Nationale de Sécurité du Médicament et des produits de santé (ANSM) | France |
| Marie-Paule Furnion, Delphine Painblanc; Nicolas Bardy, Audrey Toinon | SANOFI PASTEUR | France |
| Kristina Scheerer, Andre Noll, Heidi Scholz | GSK Vaccines GmbH | Germany |
| Beate Krämer, Constanze Göpfert | Paul-Ehrlich-Institut (PEI), Federal Institute for Vaccines and Biomedicines | Germany |
| Sai D. Prasad, Dipankar Das, Gopal Singh | Bharat Biotech International Limited, Hyderabad | India |
| Sunil Gairola | Serum Institute of India PVt. Ltd. | India |
| Guillermo Vega Rodriguez | Commission for Analytical Control and Coverage Extension (CCAYAC) COFEPRIS | Mexico |
| Nadezhda Alekseeva | Krasnoyarsk branch of the Federal State Budgetary Institution 'Information and Methodological Centre for Expertise, Stocktaking and Analysis of Circulation of Medical Products' of the Federal Service on Surveillance in Healthcare | Russian Federation |
| Srđan Stankov, Dusan Lalosevic | Department for Microbiology, Pasteur Institute Novi Sad | Serbia |
| Derek Litthauer, Yolandi Roodt, Quinton Meyer | National Control Laboratory of South Africa University of the Free State | South Africa |
| Supaporn Phumiamorn, Koraphong Pinyosukhee | Institute of Biological Products, Department of Medical Sciences Ministry of Public Health | Thailand |
| Judith Prince | National Institute for Biological Standardisation (NIBSC) | UK |
| Alethea Fry | Center for Veterinary Biologics, Virology, Policy, Evaluation, and Licensing USDA/APHIS/VS/CVB | USA |