Potency assays for Poliovirus Vaccines

Thomas Wilton

29th September 2016, Dubrovnik, Croatia
Poliovirus

- Positive stranded RNA virus
- Non-enveloped pentameric capsid
- Family: Picornaviridae
- Genus: Enterovirus
- Three serotypes; no cross-protection
- Can cause paralytic paralysis (0.1-1% depending on serotype)
Vaccines against poliovirus

**Oral live-attenuated Poliovirus Vaccine (OPV)**
- Live-attenuated vaccine
- Administered orally
- Stimulates local intestinal immunity as well as circulating antibodies
- But... associated with paralytic cases (1:2x10^6 doses)

**Inactivated Poliovirus Vaccine (IPV)**
- Killed vaccine
- Induce high titres of circulating neutralising antibodies
- Inefficient at stopping poliovirus transmission
Global Polio Eradication Initiative (GPEI)

Set up in 1988 by the WHO
Mass immunisation campaigns with OPV:

National Immunisation days (NIDs).
Global Polio Eradication Initiative (GPEI)

1988:
- 350,000 cases
- 125 endemic countries

2016
- 28 cases
- 3 countries
  (x3 endemic)

Serotype 2: Eradicated in 1999
Serotype 3: Eradicated in 2012?
Serotype 1: Still circulating

Data in WHO HQ as of 20 September

Wild Poliovirus & cVDPV Cases¹, 2016
01 January – 20 September

¹Excludes viruses detected from environmental surveillance.
OPV
Potency Assay of OPV: Monovalent
Potency Assay of OPV: Trivalent

- Mixture of type I and type II MAbs added for: assay of Serotype 3
- Mixture of type II and type III MAbs added for: assay of Serotype 1
- Mixture of type I and type III MAbs added for: assay of Serotype 2
Potency Assay of OPV

1) Measures infectious potency of monovalent and trivalent OPV bulk harvests by virus titration in tissue culture

2) Determines dilutions and validates titres of samples tested for neurovirulence
IPV
Potency Assays of IPV

**In vivo Potency Assay**
Rat potency assay

**In vitro Potency Assay**
D-Ag ELISA
In vivo Potency Assay
In vivo potency: Rat Potency Assay

Immunisation

Vaccine

Serum antibodies

Poliovirus

Bleeding

21 days

Rat sera

Neutralization assay

Rat Potency Assay

In vivo

Vaccine

Serum antibodies

Poliovirus

21 days

Rat sera

Neutralization assay
In vivo potency: Rat Potency Assay - ANALYSIS

• Serum antibody titer = highest dilution that protects 50% of the cultures against 100 TCID$_{50}$ of challenge virus
• Number of animals +ve for neutralising antibodies of number inoculated
### In vivo potency: Rat Potency Assay - ANALYSIS

<table>
<thead>
<tr>
<th></th>
<th>Standard</th>
<th>Reference</th>
<th>Sample 1</th>
<th>IPV 01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Id.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(IU/ml)</td>
<td>Lower limit</td>
<td>1.00000</td>
<td>1.00000</td>
<td>0.596042</td>
</tr>
<tr>
<td></td>
<td>Estimate</td>
<td>1.00000</td>
<td>1.00000</td>
<td>2.53798</td>
</tr>
<tr>
<td></td>
<td>Upper limit</td>
<td>1.00000</td>
<td>1.00000</td>
<td></td>
</tr>
<tr>
<td>Potency</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rel. to Ass.</td>
<td>100.0%</td>
<td>100.0%</td>
<td>59.6%</td>
<td>120.7%</td>
</tr>
<tr>
<td>Rel. to Est.</td>
<td>100.0%</td>
<td>100.0%</td>
<td>49.4%</td>
<td>100.0%</td>
</tr>
<tr>
<td>IU/ED50</td>
<td>0.237101</td>
<td>0.394268</td>
<td>0.658600</td>
<td>0.192316</td>
</tr>
<tr>
<td>Rel. to Ass.</td>
<td>151.8%</td>
<td>253.6%</td>
<td>186.3%</td>
<td>306.2%</td>
</tr>
<tr>
<td>Rel. to Est.</td>
<td>59.9%</td>
<td>100.0%</td>
<td>60.9%</td>
<td>100.0%</td>
</tr>
</tbody>
</table>

### Graphs

- **All samples**
- **Standard Reference**
- **Sample 1 IPV 01**
**In vitro potency: D-Ag ELISA**

- Indicator
- Anti-IgG Antibody
- Detection Antibody
- Viral Antigen
- Capture antibody
- Solid support

![Diagram of ELISA assay](image)
**In vitro potency: D-Ag ELISA**

1. Coat 96-well plate with polyclonal serotype specific anti-poliovirus antibodies (capture).
2. Incubate (16 hr, +4 °C) and wash plates.
3. Add diluted IPV (antigen) to the plate coated with capture antibodies.
4. Incubate (120 min, 37 °C) and wash plates.
5. Add serotype specific MAb to plate (detection antibodies).
6. Incubate (60 min, 37 °C) and wash plates.
7. Add anti-mouse IgG antibodies conjugated to peroxidase to the plate.
8. Incubate (60 min, 37 °C) and wash plates.
9. Add phosphate substrate to the plate.
10. Incubate (60 min, 18-20 °C) plates.
11. Add STOP solution to the plate and measure absorbance.
In vitro potency: D-Ag ELISA - ANALYSIS

- Parallel line analysis – potency of test vaccine relative to concurrently tested reference IPV
- Dose response curves of test and reference vaccine linear and parallel = valid D-Ag ELISA
- Vaccine must have D-Ag within given release specification to be recommended for release
Waiving of *in vivo* potency assay of IPV

- Validation study performed on:
  - Final bulk/lot of IPV
  - Two sub-potent batches – Heat treatment or mixing with heat treatment

- Reference standard: homologous production batch

- Batches assayed:
  - The rat potency assay
  - The D-Antigen ELISA

- Acceptable = Final bulk/lot passes
  - Sub-potent batch fails
Alternative potency assays for IPV

1) Immunisation-challenge assay with transgenic mice expressing poliovirus receptor (TgPVR)

2) Biosensor-based analytical system – Surface Plasmon Resonance (SPR) technology
Alternative *In vivo* potency assay of IPV: Immunisation-challenge assay in TgPVR mice
Alternative *In vivo* potency assay of IPV: Immunisation-challenge assay in TgPVR mice
Alternative *In vitro* potency assay of IPV: Biosensor-based analytical system – Surface Plasmon Resonance (SPR)
Alternative *In vitro* potency assay of IPV: Biosensor-based analytical system – Surface Plasmon Resonance (SPR)
International Reference Standards

• International standards = gold standard against which regional, national and global laboratories and manufacturers calibrate in-house standards

• Essential to the batch release process
Summary and conclusion

• Potency assays are required to ensure that poliovirus vaccines are potent enough to be released for use in the Global Poliovirus Eradication Initiative

• Potency of OPV batches is assessed by viral titration

• Potency of IPV is assessed by *in vivo* (rat potency test) and *in vitro* (ELISA) assays

• Alternative *in vivo* and *in vitro* potency assays for IPV are being developed and assessed
Acknowledgements

NIBSC
• Dr Javier Martín
• Gillian Cooper

Intravacc
• Janny Westdijk
• Gideon Kersten