Scientific basis for the development of virus concentration test for rotavirus vaccines

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General principles of cell based virus assays.

Detection of infectivity in cells

- Cytopathic effect in cells detected visibly or by metabolic markers.
- Production of antigen detected by antibody as a focus in the cell sheet or in the supernatant.
- Production of viral nucleic acid detected by PCR.

Plaque assays

- Establish cell monolayer
- Inoculate with small volume of dilutions of virus covering the range where the total amount of virus inoculated will be 1-100 infectious units.
- Overlay to confine the infection in the cell sheet.
- An infected cell will infect local cells and make a hole in the cell sheet after incubation.
- Count lesions in cell sheet.

Plaque assays

- Needs a cell that is susceptible and gives a good cpe so that visible plaques develop.
- The cell must form a good monolayer which can survive an overlay of agar or carboxy methyl cellulose.
- Incubation time to develop plaques may be long.
- Quality of plaques may be poor (small or fuzzy).

Infectious dose assays

- Infect replicate cell cultures with dilutions of virus.
- Score for cytopathic effect.

Infectious dose assay

(calculated from dose required to infect 50% of cultures)

- Needs a susceptible cell that gives a cytopathic or other detectable effect on infection.
- Does not necessarily need a monolayer.
- Precision determined by number of replicate cultures

Variables in cell culture based assays

- Method: infectious dose assays will give lower figures than plaque assays if all else is the same; detection of infection may vary in sensitivity.
- Statistical analysis and reproducibility (number of replicates, dilution series etc).
- Susceptibility of the cells: varies from cell line to cell line and on passage of the cells.
- Variation in susceptibility to viruses which are nominally the same or similar (e.g. mumps vaccines).
- Unidentified variations in technique.
- Precision of an acceptable assay is $<0.5 \log_{10}$, typically 0.1-0.3 $\log_{10}$.

References

- Homologous references included in an assay monitor assay performance, acting as a control for differences in technique and cell susceptibility. Declining titres of the reference often indicate problems with the assay.
- Reproducibility can be improved by expressing titres in units relative to a reference in some cases (e.g. yellow fever) but not others (e.g. polio). This will be the case if the cell sensitivity is a major systematic variable between laboratories for example.

Plaque forming units per dose

Fig 1a: Sample A

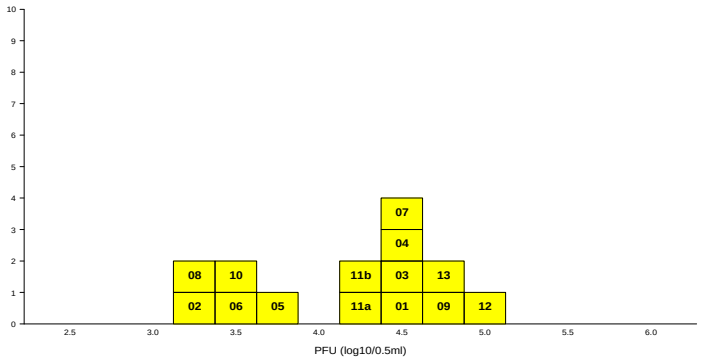


Fig 1b: Sample B

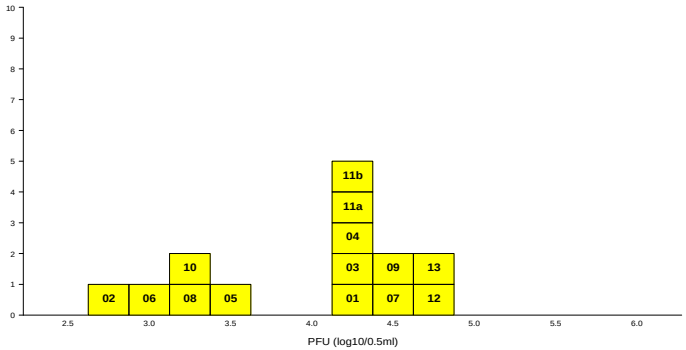


Fig 1c: Sample C

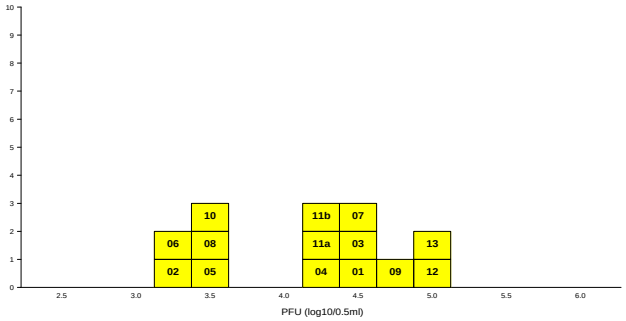


Fig 1d: Sample D

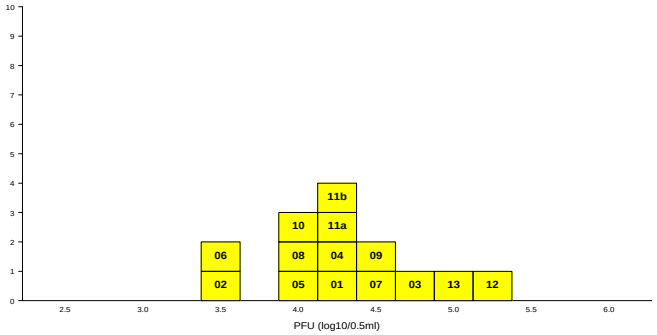


Fig 1e: Sample E

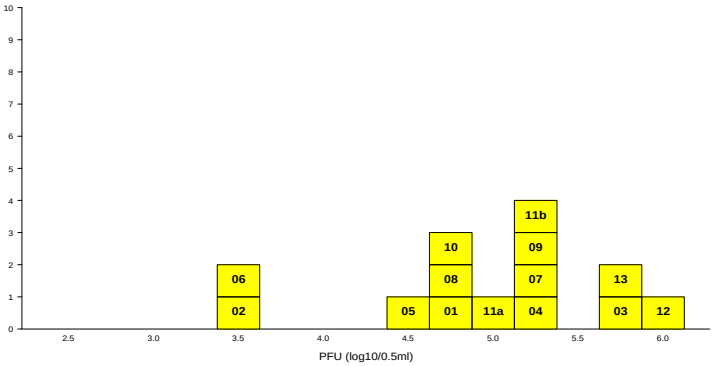
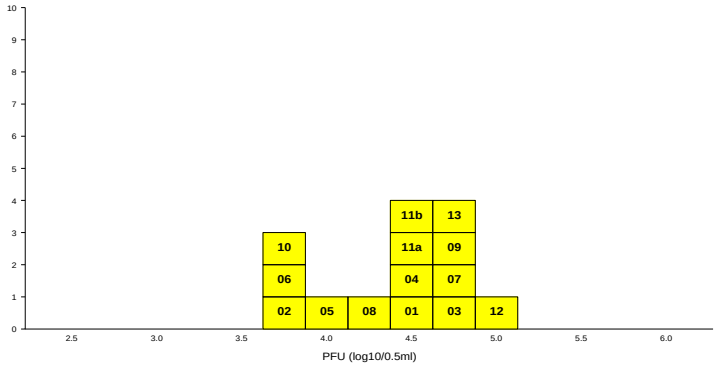


Fig 1f: Sample F



Potencies relative to the IS

Fig 2b: Sample B

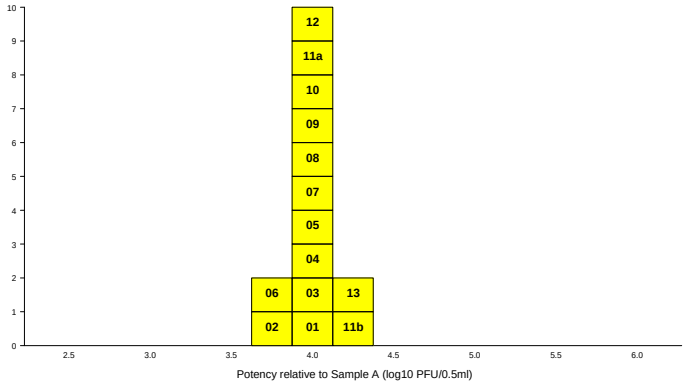


Fig 2c: Sample C

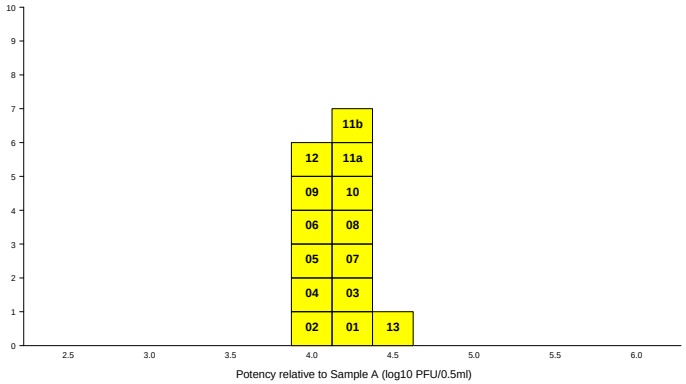


Fig 2d: Sample D

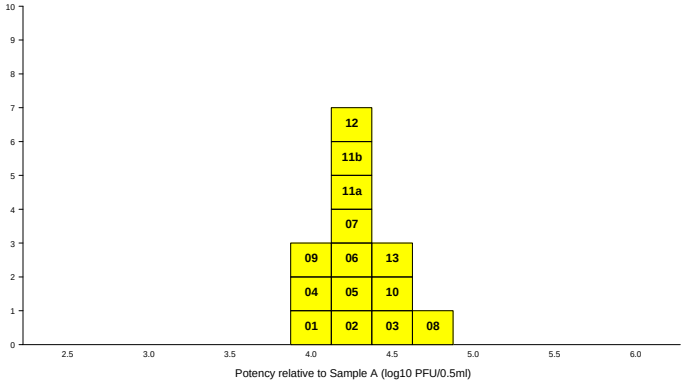


Fig 2e: Sample E

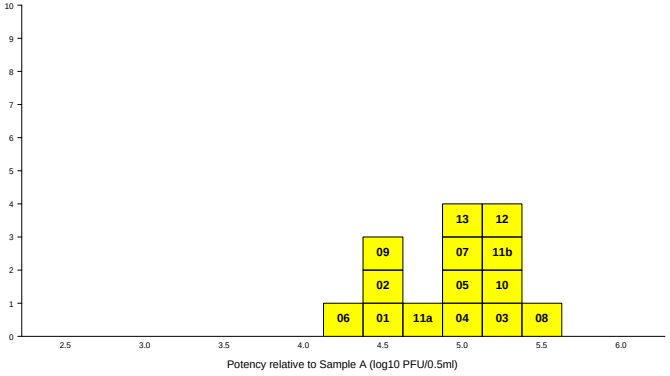
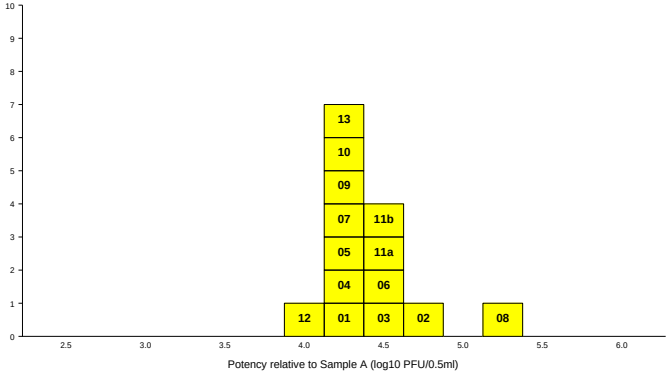


Fig 2f: Sample F



Multivalent vaccines: Oral Polio Vaccine

- Trivalent oral polio vaccine: determine the titres of the individual components by neutralising two of the three.
- Needs specific antisera which do not cross react.
- Needs three infectivity assays.
- Alternatively total virus content can be measured as a less specific check.

- Well established and it works.

Rotavirus assay principles

Rotavirus types

14 G (VP7) serotypes

23 P (VP4) genotypes

Most common human isolates:
P[8]G1; P[4]G2; P[8]G3; P[8]G4.

PLUS
P[8]G9 and P[6]G9

Disease

Not dependent on living conditions although survival is.
The first infection is the worst irrespective of subtype.

Multivalent (Merck) or monovalent (GSK) vaccines?

Types of vaccine

1. Bovine, rhesus and lamb strains (4)
2. Human/bovine, human/rhesus reassortant strains (5)
3. Human strains (5)
4. Inactivated human strains (2)
5. VLPs (1)

Vaccines of interest

1. Merck pentavalent human/bovine reassortant mix: P[5]G1, P[5]G2, P[5]G3, P[5]G4, P[8]G6.

2. GSK monovalent human strain: P[8]G1

The virus

Reovirus; 11 genome segments, no lipid layer. Genome is double stranded RNA transcribed within the core particle. Two surface proteins VP4 (P) and VP7(G) are the target of neutralising antibodies.

VP4 (P) must be cleaved with protease for the virion to be fully infectious.

Particular issues with rotavirus vaccines

1. Products are very different (universal reference materials are difficult).
2. There is likely to be poor cytopathic effect.
3. Trypsin is needed to allow ongoing infection. This is bad for cell sheets.
4. Neutralising type specific antibodies are not easily available (compare to polio)

GSK assay

1. Monovalent vaccine.
2. Poor cpe, detection of viral antigen by antibody reaction.
3. Trypsin in medium to allow ongoing infection.

GSK assay

1. Serial dilutions.
2. Inoculate microtitre plates, incubate with low level of trypsin in the medium to allow infection but not destroy the cell sheet.
3. Detect infected cultures through reaction with monoclonal antibody.
4. Score CCID50 as usual.

Merck vaccine

1. Pentavalent.
2. No specific neutralising antibodies.
3. Detection of infection by quantitative PCR of mRNA. Compare to manufacturer’s standard.
4. Validity and analysis.

Merck

1. Four dilutions of trypsin treated manufacturers standard.
Two dilutions of trypsin treated test. Dilutions chosen so that only a few cells will be infected.
2. Infect cell sheet, four wells per dilution; incubate overnight.
3. Solubilise cells with detergent.
4. Detect RNA by Taqman PCR.
5. Quantitate by number of cycles required to reach threshold of detection; compare to standard.
6. Press the magic button.