Rotavirus potency assay by $\text{CCID}_{50}$ method

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Rotavirus assay (CCID_{50})

- Objectives
- Titration method
- Validation data
- Batch release testing
- Additional comments
Rotavirus assay (CCID$_{50}$)

- Objectives
  - Titration method
  - Validation data
  - Batch release testing
  - Additional comments
Rotavirus assay (CCID$_{50}$)

Objectives

1. To determine the viral titre in rotavirus in the batch submitted for lot release

2. To determine the identity G1 of the vaccine strain
Rotavirus assay (CCID\textsubscript{50})

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Rotavirus assay (CCID\textsubscript{50})

Titration method principle
To determine the potency of Rotavirus by assessing the dose infecting 50% of MA-104 cell monolayer (monkey kidney -by end-point dilution) followed by revelation with MoAb 2C9 (anti-VP7) and immunoperoxidase staining or indirect immunofluorescence
Rotavirus assay (CCID\textsubscript{50})

1. Preparation of cell substrate
2. Virus activation step (30min) dil 1/10 & Successive dilutions in activation medium
3. Inoculation virus suspension & incubation at 37\(\pm\) 1\(^\circ\) C for 7 \(\pm\) 1 days
Rotavirus assay (CCID$_{50}$)

4. Revelation: MoAb + immunoperoxydase or indirect immunofluorescence staining

5. Reading & Calculation of titres by Spearman-Kärber method (or Reed-Muench)(logCCID$_{50}$/ml)

6. Validity of the assay (criteria)

7. Acceptance of the batch (criteria)
1. Preparation of cell substrate

- MA-104 foetal monkey kidney cells
- Cell growth medium:
  EMEM - 1% L-Glu - 10% FCS
- 50,000 ± 10,000 cells/ml
- 200 µl/well (microplates 96-wells)
- Incubation 4 days at 37 ± 1°C - 5 ± 1% CO₂
- Monolayer confluent (100%)
2. Virus activation step & dilutions

- Reconstitution of vaccines (3 vials + 3 “stability” and reference preparation (1 vial in triplicate) with 1ml WFI

- dilution 1/10 in activation medium
  EMEM - 1% L-Glu -1% Ab - 0,32% trypsine

- 20-30 min at RT

- successive dilutions (1/10 - 1/4) - same medium
3. Inoculation of virus suspensions

- Microplates washed 2 times (150 µl/well)
- Carefully - multichannels
- Inoculation: 100 µl/well - 10 wells/dilution - x dilutions/vial

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</table>

- Incubation 7 days - 37± 1°C - 5 ± 1 % CO₂ - HR
Washing before virus inoculation
4. Revelation

- To wash microplates (150 µl/well)
- Fixation: iced acetone solution (80%) - 20 min at -20°C - 150µl/well
- Acetone discarded (kleenex) and let dry
- 100µl MoAb 2C9/well (1/250 in PBS w/o Ca++ and Mg++ with 5% skimmed milk = “blotto”)
- Incubation 1 hour at 37± 1°C
- To wash µplates 4 times with PBS w/o Ca++ and Mg++
Distribution of MoAb anti-VP7
4a. Revelation by immunoperoxidase

- Ab Anti-mouse IgG conjugated to peroxidase (1/400 in blotto) - 50µl/well - incubation 1 h - 37°C ± 1°C.
- To empty µplates & wash 4 times /PBSw/o
- Fresh solution : 1 tablet DAB + 1 tablet urea in 30 ml distilled water
- 50 µl/well incubation 10 min at RT (25± 5°C).
- To wash µpls 3 times in tap water
- To let dry (kleenex wypall)
4b. Revelation by indirect immunofluorescence staining

- anti-mouse IgG conjugated to FITC (1/200 in blotto + Evans Blue 1/300)
- 100µl/well - Incubation 1 hour at 37± 1°C
- To wash µpls 4 times in tap water
- To let dry (kleenex wypall)
5. Reading & calculations

- reading: wells with stained cells = rotavirus positive
- calculation of titre by Reed-Muench or Spearman-Kärber
- estimation of the precision by Irwin-Cheesman
<table>
<thead>
<tr>
<th>Frac</th>
<th>7,2</th>
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<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
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<td>37°C</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
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<tr>
<td>Specification :</td>
<td>6,2 logCCID 50/1,0 ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limites</td>
<td>-</td>
<td>#DIV/0!</td>
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</tbody>
</table>

**Frais**

| Titre moyen: | => | #DIV/0! |
| 95%:         | => | #DIV/0! |

**Stabilité**

| Titre moyen: | => | #DIV/0! |
| 95%:         | => | #DIV/0! |

**Perte de titre :**

| => | #DIV/0! |

**Spécification:**

| 0,5 logCCID 50/1,0 ml |

**REFERENCE**

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<th>Gsk</th>
<th>VRC021A44/Q</th>
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**Gsk**

<table>
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<tr>
<th>Titre :</th>
<th>6,5 logCCID 50/ml</th>
</tr>
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<tbody>
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<td>=&gt;</td>
<td>#DIV/0!</td>
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<tr>
<td>95%:</td>
<td>#DIV/0!</td>
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</table>

**Limites:**

| => | #DIV/0! - #DIV/0! |

n* = 0

**Date du test:**

-2004
Spearman-Kärber

\[-\log_{CCID_{50}} = -\log_{10}(d1) - [\log_{10}(d) \times (Sp-0,5)]\]

Where

\(d1\) = highest dilution with 100% CPE

\(d\) = dilution step (i.e. 0,6log)

\(Sp\) = sum of proportion of all positive wells
Spearman-Kärber

<table>
<thead>
<tr>
<th>N° lot</th>
<th>Dilution</th>
<th>Positive recorded</th>
</tr>
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<tbody>
<tr>
<td>-4,6</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>-5,2</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>-5,8</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>-6,4</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

- \( \log_{10} \text{CCID}_{50} = -4,6 - [0,6 \times \frac{(10 + 8 + 2 + 0)}{10 - 0,5}] \)
- \( \log_{10} \text{CCID}_{50} = -4,6 - 0,9 \)
- \( \log_{10} \text{CCID}_{50} = -5,5 \)
- \( \log_{10} \text{CCID}_{50} = + 5,5 \)

Correction factor: \( \log_{10} 1000 \, \mu l / 100 \mu l = 1,0 \)

Vaccine titre: \( 5,5 + 1,0 = 6,5 \, \log \text{CCID}_{50}/\text{ml} \)
Precision by Irwin–Cheesman

\[ s = \log d \times \sqrt{\sum \left[ \frac{p \times (1-p)}{n - 1} \right]} \]

- \( s \) = std deviation
- \( d \) = dilution step
- \( \sum \) = sum of all dilutions
- \( p \) = proportion of infected (positive) wells for one specific dilution
- \( 1 - p \) = proportion of non-infected (negative) wells
- \( n \) = nr of inoculated well/dilution
Precision by Irwin-Cheesman

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Positive recorded</th>
<th>p</th>
<th>1-p</th>
<th>p x (1-p)</th>
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</thead>
<tbody>
<tr>
<td>-4,6</td>
<td>10</td>
<td>10/10</td>
<td>0/10</td>
<td>0</td>
</tr>
<tr>
<td>-5,2</td>
<td>8</td>
<td>8/10</td>
<td>2/10</td>
<td>16/100</td>
</tr>
<tr>
<td>-5,8</td>
<td>2</td>
<td>2/10</td>
<td>8/10</td>
<td>16/100</td>
</tr>
<tr>
<td>-6,4</td>
<td>0</td>
<td>0/10</td>
<td>10/10</td>
<td>0</td>
</tr>
</tbody>
</table>

\[ s = 0.6 \times \sqrt{\frac{32}{100}} / 9 \]
\[ = 0.6 \times 0.1886 \]
\[ = 0.1132 \]

Confidence interval (P=0.95) : \( 0.1132 \times 1.96 = 0.22 = 0.2 \)

Virus titre : \( 6.5 \pm 0.2 \log \text{CCID}_{50}/ \text{dose} \)
6. Validity of the assay

- negative control cells: free of toxicity or abnormalities and do not show any fluorescent/coloured cells

- Homogeneous distribution of CPE vs dilutions (10-90% of CPE on 3 dilutions)

- the confidence interval (P=0.95) of the vaccine virus titre is ≤ 0.3\( \log * \)

- max diff. 0.5\( \log * \) between 2 vials/3 (*)
Reference titre: Observed reference titre within 0.5 log of its established mean titre - Company: 6.5 ± 0.5 logCCID50/ml or IPH titre: 6.4/1ml - LCL: 6.0/1ml - UCL: 6.9/1ml - n=30
7. Acceptance of the batch (criteria)

- The estimated titre of the batch meets the release specification: \( \geq 6.2 \log_{10} \text{CCID}_{50}/\text{ml} \)
- The maximum loss of titre is \( 0.5 \log_{10} \text{CCID}_{50}/\text{ml} \)
Rotavirus assay (CCID_{50})

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Rotavirus assay (CCID$_{50}$)

Validation data

1. The test specificity
2. The range of titration
3. The limit of detection
4. The precision (intra-assay repeatability)
5. The reproducibility (inter-assay repeatability)
Rotavirus assay ($\text{CCID}_{50}$)

1. The test specificity

- MA-104 cells
- Trypsin activation step
- MoAb anti-VP7 & Validation data provided by Manufacturer (each new MoAb batch)
- File: demonstrated in identity test performed by seroneutralisation
Rotavirus assay (CCID$_{50}$)

- Validation on MA-104 cells
- cell bank (nr of passages)
- cell concentration in culture flasks

MA104 cells in confluent culture flasks (175cm$^2$) - (June2005- April2006)
Rotavirus assay \((\text{CCID}_{50})\)

- MA-104 cells
- Cell concentration inoculated (target 50.000 +/- 10.000)

Graph showing MA104 cell concentration (50,000 +/- 10,000 cell/ml)
Rotavirus assay (CCID$_{50}$)

2. The range of titration
   In practice: choice of range of dilution

3. The limit of detection
   End-point dilution method (0–100% CPE)
### Rotavirus assay (CCID<sub>50</sub>)

#### 4. The precision (intra-assay repeatability)

One sample – x replicates – same day

<table>
<thead>
<tr>
<th>Date</th>
<th>Replicate 1</th>
<th>Replicate 2</th>
<th>Replicate 3</th>
<th>Mean</th>
<th>Max Diff</th>
<th>Stdev</th>
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<tr>
<td>14/03/2006</td>
<td>6.2</td>
<td>6.5</td>
<td>6.3</td>
<td>6.3</td>
<td>0.3</td>
<td>0.15</td>
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<td>28/03/2006</td>
<td>6.2</td>
<td>6.3</td>
<td>6.3</td>
<td>6.3</td>
<td>0.1</td>
<td>0.06</td>
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<td>04/04/2006</td>
<td>6.6</td>
<td>6.6</td>
<td>6.7</td>
<td>6.6</td>
<td>0.1</td>
<td>0.06</td>
</tr>
<tr>
<td><strong>Mean</strong></td>
<td><strong>6.4</strong></td>
<td></td>
<td></td>
<td><strong>6.4</strong></td>
<td><strong>0.2</strong></td>
<td><strong>0.09</strong></td>
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</table>
Rotavirus assay (CCID$_{50}$)

5. The reproducibility (inter-assay repeatability) – time – operators

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<tr>
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<tr>
<td>Mean</td>
<td>6,4</td>
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<tr>
<td>Stdev</td>
<td>0,2</td>
</tr>
<tr>
<td>cv (%):</td>
<td>2,4%</td>
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<tr>
<td>min</td>
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<td>max</td>
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<td>m± 3s = action limits</td>
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Rotavirus assay (CCID$_{50}$)

- Objectives
- Titration method
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Rotavirus batch release testing

1. Appearance
2. pH
3. Identity (by titration)
4. Potency
5. Thermal stability (7d - 37±1°C)
# Rotavirus batch release testing

<table>
<thead>
<tr>
<th>Test</th>
<th>Specification</th>
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<tbody>
<tr>
<td>Appearance before reconstitution</td>
<td>Whitish cake or powder</td>
</tr>
<tr>
<td>Appearance after reconstitution</td>
<td>Clear colourless solution</td>
</tr>
<tr>
<td>pH after reconstitution with WFI</td>
<td>Between 7.5 and 8.5</td>
</tr>
<tr>
<td>Identity</td>
<td>Positive by titration</td>
</tr>
<tr>
<td>Titre</td>
<td>$\geq 6.2 \log_{10} CCID_{50}/\text{ml}$</td>
</tr>
<tr>
<td>Heated titre (7d, 37°C)</td>
<td>To be determined $(\log_{10} CCID_{50}/\text{ml})$</td>
</tr>
<tr>
<td>Titre loss</td>
<td>Not more than $0.5 \log_{10} CCID_{50}$/vial from the release titre</td>
</tr>
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</table>
Rotavirus batch release testing

Rotarix Lots 2005 - GSK & IPH results - 2005

logCCID50/ml

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

GSK Titre
GSK Heated titer
IPH Titre
IPH Heated titre
Rotavirus batch release testing

Rotarix Lots - loss of titre - 2005

-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
0.4
0.5

logCCID50/ml

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

GSK loss titre

IPH loss titre

Scientific Institute of Public Health
BIOLOGICAL STANDARDISATION
Rotavirus assay (CCID₅₀)

Objectives

- Titration method
- Validation data
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- Additional comments
Additional comments

- Availability of qualified
  - MA-104 cell bank
  - Stock of MoAb 2C9
- Expensive equipment (Epi-fluorescence)
- Questions?
Rotavirus assay ($\text{CCID}_{50}$)

MUCHAS GRACIAS POR SU ATTENCION

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BIOLOGICAL STANDARDISATION