Development of reference reagents for RSV assays

WHO Consultation on RSV Vaccine Development
March 23-24, 2015, Geneva

Deborah Higgins
Director
RSV Vaccine Project
Vaccine Development
Global Program

PATH/Deborah Phillips
RSV antibody assays – where are we now?

The good news

• RSV-specific functional antibody is associated with protection against RSV infection.1-4

• Many RSV vaccine strategies in development target induction of protective antibodies.

• Virus neutralization is a long accepted assay method for quantifying the protective ability of antibody.

The problem

• There are ~10 different neutralization assay methods currently in use.

RSV neutralization assay methods

Classic plaque reduction neutralization test (PRNT)

- Considered the gold standard.
- Antibody dilutions mixed with a consistent amount of virus, allowing antibody to bind to virus.
- Antibody/virus mixture added to susceptible cells (HEp2, Vero).
- Reduction of virus infectivity in cell culture as measured by plaque formation compared to control.
- Multiple rounds of viral replication required to visualize plaques.
- Including complement in cell culture media increases titer results in some instances.
- Labor intense and lengthy (5-7 days).
Microneutralization (MN)

- Similar to PRNT, but after virus/cell culture period, presence of virus is detected using higher throughput methods to assess reduction of infectivity compared to controls.
- Complement also included in some assay formats.
- Virus detection methods
  - Allow automated reading and recording of results.
  - Can allow reduced cell culture timeframe and well size since readout doesn’t require plaque visualization.
  - Include ELISA and PCR to detect viral proteins, or use of reporter viruses containing fluorescent proteins.
A look at neutralization titers across PATH’s RSV research collaborations

<table>
<thead>
<tr>
<th>Neutralization Assay Format</th>
<th>BEI Repository</th>
<th>Karron (JHU) PRNT with guinea pig complement</th>
<th>Englund (UW) MN - ELISA readout, no complement</th>
<th>Nokes (KEMRI Kilifi) PRNT, no complement</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEI RSV Antibody Sample*</td>
<td>Expected log2 neut titer range</td>
<td>Mean log2 titer (±1SD)</td>
<td>Mean log2 titer (95% CI)</td>
<td>Mean log2 titer (95% CI)</td>
</tr>
<tr>
<td>BEI NR-4020 Wyeth lot 6594</td>
<td>8.79 (±6.48)</td>
<td>9.72 (8.86-10.58)</td>
<td>11.57 (10.78 – 12.36)</td>
<td>8.93 (8.61-9.20)</td>
</tr>
<tr>
<td>BEI NR-4021 Wyeth lot 6937 - high</td>
<td>10.88 (±9.75)</td>
<td>11.13 (10.52-11.74)</td>
<td>13.57 (13.17-13.97)</td>
<td>10.29 (9.91-10.60)</td>
</tr>
<tr>
<td>BEI NR-4022 Wyeth lot 6938 - medium</td>
<td>7.81 (±5.81)</td>
<td>8.87 (8.32-9.41)</td>
<td>10.69 (10.05- 11.34)</td>
<td>8.75 (8.12-9.19)</td>
</tr>
<tr>
<td>BEI NR-4023 Wyeth lot 6939 - low</td>
<td>8.28 (±2.81)</td>
<td>8.87 (8.04-9.70)</td>
<td>10.94 (10.66 – 11.23)</td>
<td>8.73 (8.25-9.08)</td>
</tr>
<tr>
<td>BEI NR-21973 CBER reference Ig lot RSV-1</td>
<td>7.56 (±5.21)</td>
<td>10.48 (9.72-11.24)</td>
<td>14.13 (13.78-14.49)</td>
<td>8.59 (8.09-8.96)</td>
</tr>
</tbody>
</table>

* BEI RSV antibody panel catalog #NR-32832 contains 5 different pooled human polyclonal antisera to RSV.
Harmonizing results across assay formats

- Reference reagents can be used to harmonize results across assay formats to facilitate:
  - comparison and prioritization of vaccine candidates.
  - establishing a correlate of protection.

- Reference reagents could include:
  - International antibody standard(s).
  - Virus stock(s).
  - Cell line(s).

- WHO | NIBSC | PATH are coordinating efforts to develop reference reagents and strengthen RSV antibody assay capabilities.
Towards an International Standard for antibody to RSV

Othmar G Engelhardt

Learnings from development of other global harmonized assays

David Wood
NIBSC’s role in standardisation

• NIBSC has been making International Standards for decades
  – More than 90% of International Standards developed and produced by NIBSC
• NIBSC is a WHO Collaborating Centre and International Laboratory for Biological Standards
• NIBSC also makes working reagents and other reference materials
• NIBSC contributes to international guidelines and method standardisation
Standardisation of biologicals

- SI units based on physical properties and do not apply to biological activities.
- Biological activity can only be measured in a biological assay.
- International Unit
  - A fraction of a vial of a biologically active substance, arbitrarily defined as having the biological activity of 1 IU
- Method not defined
  - Use of standard permits measurement in IU, irrespective of method (within limits)
Generation of an International Standard

- Source material that has biological activity that we want to measure.
- Fill material into vials (consistency of fill and stability of material).
- Evaluate candidate material in international collaborative study.
  - Use of different methods
  - Inclusion of candidate standard(s) and other samples (e.g. clinical samples)
  - Statistical evaluation of all results

  ➢ Does use of standard improve agreement between laboratories?
Commutability – What is it?

- The WHO guidelines for preparation of International Standards (TRS 932) state -

- “The behaviour of the reference standard should resemble as closely as possible the behaviour of test samples in the assay systems used to test them”
  - General Considerations

- “The concept of commutability seeks to establish the extent to which the reference standard is suitable to serve as a standard for the variety of samples being assayed.”
  - Glossary
Commutability of a standard

- Standard should work in as many assays as possible, but may not work for all assays.
- Standard should behave in all/most assays the same way.

Prediction interval used to determine commutability

“Commutable”

“Not commutable”
What causes non-commutability?

Noncommutability of a reference material can be caused by

- **Matrix effects**
  - The influence of a property of the sample (interference), independent of the presence of the analyte, on the measured value of the analyte, often caused by
    - processing in the preparation of a reference material e.g. pooling serum or plasma samples, purification, freezing, lyophilisation, adding preservatives etc.

- **Analyte-specific effects**
  - Presence of surrogate analyte e.g. animal derived substitute, different viral strain.
  - Denaturation or degradation products of the analyte.
Establishment of an International Standard

- RSV IS project has been endorsed by WHO Expert Committee for Biological Standardisation (ECBS).
- Project to be reviewed at various stages.
- Final review at WHO ECBS.
  - ECBS is a standard setting body through the UN system.
  - Responsible for the establishment of WHO International Biological Reference Preparations.
  - Responsible for the adoption of the WHO Recommendations and Guidelines.
- If found suitable, the International Standard will be established and a unitage assigned (International Units).
  - Unitage is arbitrary and not linked to SI units.
Use of an International Standard

- IS is precious and should not be used regularly in assays.
  - Intended for periodic use

- IS is used to calibrate secondary reference materials or assay controls which can be used freely.

- Antibody standard for serology
  - Titres can be converted to IU: ratio between measured titre for test sample and titre of IS, multiplied by IU value of IS.
Lessons learned

- Establishing a global international standard is best done early in the lifecycle of a product.
- Requires collaboration between product developers, academia, regulators, public health authorities, metrologists.
- Requires long-term commitments,
- But provides tangible benefits in terms of access to products.
Questions to be answered to define next steps

• Will neutralization assays support vaccine development throughout the pipeline?

• Are additional reagents needed beyond an IS?
  – Virus stocks
  – Cell lines

• Are other antibody characterization assays needed?
  – IgG isotype
  – Palivizumab competing antibody (PCA) assay
  – Site Ø competition assays
  – Others?

• Is another IS, or assignment of IUs for alternate assay use required?
Towards an RSV antibody IS – Next steps

1. Conduct broad survey across assay formats using a common specimen panel (no assay modifications).
   - ~10 assay formats represented among lab participants.
   - Sample panel to include:
     - Convalescent sera/plasma (pooled and individual)
     - Post-vaccination sera/plasma (pooled)
     - IgG preparation(s)
     - mAb(s)
     - Negative control
   - Results to be made publicly available.
     - Show need for assay harmonization and establishment of IS.
     - Inform on sample types that may be appropriate for IS.
   - Study timeframe: Q2-Q3 2015
Develop international standard

2. NIBSC to conduct formal study with potential IS materials. (start Q4 2015?)
   - Identify labs interested in participating in collaborative study.
     - Use of different methods.
   - Inclusion of candidate standard(s) and other samples (e.g. clinical samples).
     - Identify large volume of, ideally, high-titre human serum or plasma to be used for standard.
       - Volume calculation based on estimated use over 10 years.
       - Convalescent sera collected for common panel could be used for this purpose (60 sera x ~250 ml/sera available).
   - Statistical evaluation of all results.
     - Does use of standard improve agreement between laboratories?
Strengthen antibody assays

3. Conduct assay characterization/qualification studies across a broad range of neutralization assay formats.
   • Identify assay formats to facilitate RSV vaccine development.
     – Robust, higher throughput assay with objective readout and reasonable cost.

4. Facilitate establishment of one or more of these neutralization assays to be commercially available.

5. Facilitate establishing alternate assays to characterize antibody responses.
Acknowledgements

PATH RSV Assay Subteam
- Deborah Higgins
- Kutub Mahmood
- Carrie Trujillo
- John Donnelly

WHO
- David Wood
- Ivana Knezevic
- Tiequn Zhou
- Birgitte Giersing

NIBSC
- Othmar Engelhardt

Funder
- Bill & Melinda Gates Foundation