Issues related to providing reference materials during an outbreak scenario

Mark Page
Standards for an outbreak scenario
Ebola/Zika experience

• Issues
  • WHO priority pathogen list includes viruses requiring high laboratory containment; hampers development work to establish assay reagents
  • Assuring safety of inactivated pathogens can not be guaranteed (also requires extensive validation)
  • Sourcing antibodies and pathogens is confounded by shipping regulations from source countries, ethical issues, biosafety of reagents, governmental and institutional approvals etc.
  • Validation of diagnostics for WHO Emergency Use Assessment and Listing hampered in absence of suitable reagent panels
Solutions

• **Nucleic amplification technology standards**
  - Synthetic RNA packaged in lentiviral vectors
    - Safe, no high containment
    - Expeditious
    - Freeze drying precludes cold chain requirement
    - Established precedent with Ebola

• **Antibody standards**
  - Tc bovines immunised with DNA/recombinant immunogens (SAB Biotherapeutics)
    - Safe, non infectious
    - Large volumes of high titre antibody suitable for producing >1000 ampoules of standard
    - Human IgG = commutable
  - Human Mabs = commutable (Prof. Alan Townsend, Oxford University)

• **Pseudotyped viruses**
  - Antibody characterisation by neutralisation assays
    - Safe, non infectious, BSL1 laboratory, stable, amenable to lyophilisation
    - Established methodology for many viruses (flu, rabies, Ebola, MERS)
    - Candidate vaccine characterisation
Chimaeric HIV-outbreak virus RNA particles

Advantages:
- Safe: non-replicative HIV VLP, non-infectious (lack of Env) no expression of outbreak virus genes (no promoter and added stop codons)
- Easy and fast production
- HIV-1 ΔU3 LTR allows for genome quantification

Mattiuzzo et al., PLoS One, 2015

Patent filed October 2015
PV production in 48 hr

The Ebola Example…

**In-house characterisation**

Pseudotyped virus:

- Replication defective viral particles
- Study functions related to the envelope protein without the need for live virus

**Diagram:**

1. **Plasmid co-transfection of producer cells**
   - HIV gag-pol
   - En
   - Reporter

2. **Harvest lentiviral pseudotype**

3. **Inoculate rVSV onto cells expressing foreign envelope glycoprotein**

4. **Harvest pseudotyped rVSV**

**References:**

PV neutralization

FIGURE 7 | Example of a pseudotype neutralization assay (pMN). Serum or antibodies are serially diluted across a 96-well plate, a known quantity of pseudotype is added and the plate is centrifuged and incubated to allow antibody binding. A set quantity of cells are added and plates are incubated for 48 h. Output is measured in a manner depending on reporter used.

Carnell et al. Front Immunol, 2015
Tc Bovine Immunoglobulin Production Process

Tc Bovine *
Cows genetically designed to produce human antibodies.

Immunization
Tc Bovine are vaccinated with target disease antigen.

Human Antibody Production
Cows begin to produce specific human antibodies after immunization that circulate in the blood stream.

Plasma Collection
Antibodies are harvested from cows in the form of plasma.

Purification
The purification process separates antibodies from the plasma.

Fully Human Antibodies
Product is fully human polyclonal antibody.

Testing
Each lot of material is further tested to ensure safety for use as a human therapeutic.

Antibody Therapeutic
Therapeutic used for treatment of human patient.

Antibody Reagent
Targeted antibodies ready for use either purified or spiked in human plasma.

EBOV RNA reference material in Collaborative Study

Wide spread of 3-5 logs reported for individual laboratory assays
Harmonisation of results expressed relative to WHO reference material

LVV-NP VP35 GP LOW CALIBRATED ON LVV-NP VP35 GP HIGH
Outcome

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Geneva, 12 to 16 October 2015

Establishment of WHO reference reagents for use in the calibration of Ebola RNA NAT assays

- Sample 1003 (Ebola NP-VP35-GP-LVV high) with an assigned potency of \(7.5 \log_{10} \text{units/mL}\) when reconstituted in 1 mL of nuclease-free water.
- Sample 1012 (Ebola VP40-L-LVV high) with an assigned potency of \(7.7 \log_{10} \text{units/mL}\) when reconstituted in 1 mL of nuclease-free water.

Establishment of WHO reference reagents for use as in-run controls for Ebola RNA NAT assays

- Sample 1081 (Ebola NP-VP35-GP-LVV low) with a potency of \(3.5 \log_{10} \text{units/mL}\) as calibrated against Sample 1003.
- Sample 1089 (Ebola VP40-L-LVV low) with a potency of \(3.7 \log_{10} \text{units/mL}\) as calibrated against Sample 1012.

Each when reconstituted in 1 mL of nuclease-free water.
### Ebola antibody Collaborative study samples.

<table>
<thead>
<tr>
<th>EBOV Ab Sample Code</th>
<th>Sample Name</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>Purified Tc Bovine IgG Pre-Bleed Negative control</td>
<td>1 mg/mL in sterile buffer#</td>
</tr>
<tr>
<td>28</td>
<td>NHSBT EBOV Convalescent Plasma</td>
<td>SD-extracted</td>
</tr>
<tr>
<td>31</td>
<td>Purified EBOV139 Tc Bovine IgG rGPZaire2014</td>
<td>1 mg/mL in sterile buffer#</td>
</tr>
<tr>
<td>36</td>
<td>NHSBT Negative Plasma</td>
<td>SD-extracted</td>
</tr>
<tr>
<td>43</td>
<td>Norwegian EBOV Convalescent Plasma</td>
<td>SD-extracted</td>
</tr>
<tr>
<td>58</td>
<td>Vaccinees Plasma Pool (high)</td>
<td>Plasma pool not SD-extracted</td>
</tr>
<tr>
<td>64</td>
<td>Vaccinees Plasma Pool (low)</td>
<td>Plasma pool not SD-extracted</td>
</tr>
<tr>
<td>79</td>
<td>American Red Cross EBOV Convalescent Plasma</td>
<td>SD-extracted</td>
</tr>
<tr>
<td>88</td>
<td>Purified EBOV132 Tc Bovine IgG Zaire95+Sudan GP DNA</td>
<td>1 mg/mL in sterile buffer#</td>
</tr>
</tbody>
</table>

Abbreviations: NHSBT= National Health Service Blood and Transplant; SD= Solvent-detergent; # PBS-Ca2+-Mg2+; 5% human serum albumin.
Outcome

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION

Geneva, 12 to 16 October 2015

American Red Cross EBOV Convalescent Ab (Sample code 79) was established as the WHO reference reagent for use in Neutralisation, Pseudotype Neutralisation and Enzyme Immuno assays with an assigned unitage of 1 unit/mL.
1st International standard for Ebola antibody

Candidate material:
Pool of plasma from Sierra Leone patients recovered from EVD

Reference panel of convalescent plasma

Existing reference reagent for calibration into International Units

Establishment by WHO ECBS at October 2017 meeting
assigned unitage of 1.5 units/mL
### 1st International Standard for Ebola antibody and reference panel

<table>
<thead>
<tr>
<th>EBOV Ab Collaborative Study Sample Code</th>
<th>NIBSC Product Code</th>
<th>Sample Name and description</th>
<th>Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>79*</td>
<td>15/220</td>
<td>WHO 1st IRR for Ebola antibodies ARC convalescent plasma</td>
<td>100 μL frozen plasma</td>
</tr>
<tr>
<td>38</td>
<td>15/284</td>
<td>Candidate Panel Member 3: NOR Anti-EBOV Convalescent Plasma</td>
<td>0.25 mL plasma Freeze-dried</td>
</tr>
<tr>
<td>39</td>
<td>15/288</td>
<td>Candidate panel member Negative Human Plasma (anti-EBOV)</td>
<td>0.25 mL plasma Freeze-dried</td>
</tr>
<tr>
<td>66</td>
<td>15/282</td>
<td>Candidate Panel Member 2: NHSBT Anti-EBOV Convalescent Plasma</td>
<td>0.25 mL plasma Freeze-dried</td>
</tr>
<tr>
<td>85</td>
<td>15/286</td>
<td>Candidate Panel Member 4: INMI Anti-EBOV Convalescent Plasma</td>
<td>0.25 mL plasma Freeze-dried</td>
</tr>
<tr>
<td>92</td>
<td>15/262</td>
<td>Candidate WHO 1st International Standard Anti-EBOV Convalescent Plasma Pool Sierra Leone</td>
<td>0.5 mL plasma Freeze-dried</td>
</tr>
<tr>
<td>95</td>
<td>15/280</td>
<td>Candidate Panel Member 1: ARC Anti-EBOV Convalescent Plasma</td>
<td>0.25 mL plasma Freeze-dried</td>
</tr>
</tbody>
</table>

* Sample Code 79 was established as the WHO 1st International Reference Reagent for Ebola antibodies (NIBSC 15/220) by ECBS in 2015
1st IS Ebola antibody and reference panel

Product Number: 15/262
Product Description: Anti-EBOV Convalescent Plasma Pool - Sierra Leone (International Reference Reagent)
Type of Standard: International Reference Reagent
Category: Vaccines > Ebola Virus
Instructions for Use: 15-262.pdf
Keywords: Ebola virus EBOV, Ebola virus convalescent plasma EBOV anti-EBOV
Related Products: 16/344, 15/220, 15/222, 15/224, 15/136, 15/138
Minimum Quantity: 1
Unit Price: £95.00

Product Number: 18/344
Product Description: WHO Anti-EBOV Convalescent Plasma (International Reference Panel)
Type of Standard: International Reference Preparation
Category: Vaccines > Ebola Virus
Instructions for Use: 18-344.pdf
Keywords: Ebola virus EBOV, Ebola virus convalescent plasma EBOV anti-EBOV
Related Products: 15/262, 15/220, 15/222, 15/224, 15/136, 15/138
Minimum Quantity: 1
Unit Price: £220.00
Final report

WHO collaborative study to assess the suitability of an interim standard for antibodies to Ebola virus

Dianna E. Wilkinson1, Mark Page1, Neil Almond1, Robert Anderson1, Neil Berry1, Thomas Dougall2, Stacey Elsthatiou1, Ruth Harvey1, Mark Hassall1, Giada Mattiuzzo1, Peter Rigby2, Nicola Rose1, Silke Schepelmann1, Lindsay Stone1, Philip D. Minor1 and the Collaborative Study Group*

1Division of Virology and 2Biostatistics
National Institute for Biological Standards and Control, South Mimms, Potters Bar, Herts., EN6 3QG, UK

2Study Coordinator; Tel +44 1707 641000, Fax +44 1707 641050,
E-mail: Dianna.Wilkinson@nibsc.org

* See Appendix 1
Zika response

- **Serology standard**
  - Infected patients
    - IgG for vaccine
  - Transchromosomal cows (SAB Biotherapeutics)
    - immunised with inactivated virus (IgG only)
  - Need for reference panels to improve assays performance over cross reactivity problems (Esp DENV)

- **NAT standard**
  - PEI conducting collaborative study
    - Timeline, endorsement for October ECBS meeting
  - Inactivated virus preparations of Polynesian/African lineages
  - NIBSC has produced ~5000 vials of African strain
    - Potential use as in-run control
## Collaborative study materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Donating Organisation</th>
<th>Volume</th>
<th>Origin</th>
<th>PRNT50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>NHSBT</td>
<td>500ml (4 donors)</td>
<td>UK</td>
<td>160-1150</td>
</tr>
<tr>
<td>Serum</td>
<td>Vaccine Research Centre, NIH</td>
<td>50ml (8 donors)</td>
<td>Puerto Rico</td>
<td>340-1280</td>
</tr>
<tr>
<td>Plasma</td>
<td>Boca Biolistics</td>
<td>500ml (2 donors)</td>
<td>Dominican Republic</td>
<td>320, &gt;640</td>
</tr>
<tr>
<td>Serum</td>
<td>CARPHA</td>
<td>~100ml (100+ donors)</td>
<td>Caribbean</td>
<td>Ongoing</td>
</tr>
<tr>
<td>Purified IgG (Inactivated virus immunogen)</td>
<td>SAB Biotherapeutics</td>
<td>5ml</td>
<td>Transchromosomal Bovine</td>
<td>503</td>
</tr>
<tr>
<td>Purified IgG (pDNA immunogen)</td>
<td>SAB Biotherapeutics</td>
<td>5ml</td>
<td>Transchromosomal Bovine</td>
<td>412</td>
</tr>
<tr>
<td>Negative purified IgG</td>
<td>SAB Biotherapeutics</td>
<td>5ml</td>
<td>Transchromosomal Bovine</td>
<td>NEG</td>
</tr>
<tr>
<td>Negative serum</td>
<td>NIBSC</td>
<td>200ml (</td>
<td>UK</td>
<td>NEG</td>
</tr>
<tr>
<td>Dengue serotype 1</td>
<td>NIBSC</td>
<td>Reference reagent</td>
<td>Thailand</td>
<td>NEG</td>
</tr>
<tr>
<td>Dengue serotype 2</td>
<td>NIBSC</td>
<td>Reference reagent</td>
<td>Thailand</td>
<td>NEG</td>
</tr>
<tr>
<td>Dengue serotype 3</td>
<td>NIBSC</td>
<td>Reference reagent</td>
<td>Thailand</td>
<td>NEG</td>
</tr>
</tbody>
</table>
Harmonization of Zika neutralization assays by using the WHO International Standard for anti-Zika virus antibody

Giada Mattiuzzo,1,2 Ivana Knezevic,2 Mark Hassall,1 James Ashall,1 Sophie Myhill,1 Valwynne Faulkner,1 Jason Hockley,3 Peter Rigsby,3 Dianna F. Wilkinson,1 Mark Page,1 and the collaborative study participants

World Health Organization

EXPERT COMMITTEE ON BIOLOGICAL STANDARDIZATION
Geneva, 29 October to 2 November 2018

WHO collaborative study to assess the suitability of the 1st International Standard for antibody to Zika virus

Mark Page1,2, Giada Mattiuzzo1, Mark Hassall1, James Ashall1, Sophie Myhill1, Valwynne Faulkner1, Jason Hockley3, Peter Rigsby3, Dianna F. Wilkinson1, Mark Page1, Stacey Efstathiou1 and the Collaborative Study Group*

1Division of Virology and 2Biostatistics
National Institute for Biological Standards and Control,
South Mimms, Potters Bar, Herts, EN6 3QG, UK
Future outbreaks?

- **NIBSC response options**
  - Contemporaneous
    - as for Ebola/Zika – unlikely to be available at the time of outbreak
  
- **Pre-outbreak**
  - Prepare standards before outbreaks occur
    - Commitment of resources?
    - CEPI

<table>
<thead>
<tr>
<th>Priority pathogen</th>
<th>Genome size (kb)</th>
<th>Hazard group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crimean-Congo haemorrhagic fever virus</td>
<td>19.2</td>
<td>4</td>
</tr>
<tr>
<td>Marburg virus</td>
<td>19</td>
<td>4</td>
</tr>
<tr>
<td>Lassa fever virus</td>
<td>10-19</td>
<td>4</td>
</tr>
<tr>
<td>Middle East respiratory syndrome coronavirus</td>
<td>30.1</td>
<td>3</td>
</tr>
<tr>
<td>Severe acute respiratory syndrome coronavirus</td>
<td>29.7</td>
<td>3</td>
</tr>
<tr>
<td>Nipah virus</td>
<td>18.2</td>
<td>4</td>
</tr>
<tr>
<td>Rift valley fever virus</td>
<td>11.9kb</td>
<td>3</td>
</tr>
</tbody>
</table>
Concluding remarks

Global framework of networked labs required to respond rapidly to an outbreak to source reagents, sequences

Real time response difficult

Or be prepared – eg CEPI

Can never predict disease X!
Acknowledgements

Wellcome Trust
- Mike Turner

PHE
- Maria Zambon
- Angie Lackenby
- Simon Carne
- Miles Caroll

Collaborative study participants
- USAMRID/FDA/NIH/PHE

HCWs
- Adrian Hill
- Katie Ewer
- Tess Lambe

WHO
- David Wood
- Pat Fast
- Robyn Meurant
- Micha Neubling

NHSBT
- Sheila McClellan
  University of Liverpool
- Callum Semple

NIBSC project team
- Mark Page
- Phil Minor
- Dianna Wilkinson
- Stacey Efstathiou
- Giada Mattiuzzo
- Sophie Myhill
- James Ashall
- Mark Hassall
- Neil Almond
- Neil Berry
- Rob Anderson
- Claire Ham
- Ruth Harvey
- Carolyn Nicolson
- Silke Schepelmann
- Nicola Rose

American Red Cross
- Susan Stramer

Emory University
- Annie Winkler
- Scott Koepsell