Tools for the standardisation of assays

Mark Page
Uses

Reference materials important for calibration of assays
• Ensures comparability of results across a range of assays measuring biological activity
• Interpreting vaccine trial data
• Establishing protective antibody levels
• Diagnostics
• Sero-prevalence
• Vaccine evaluation
  • Vaccine standards
  • Release assays
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

Number of Laboratories

-6 -5 -4 -3 -2 -1 0 1
Log10 units/ml

- Quantitative Assays
- Qualitative Assays
Vaccine evaluation assays

Immune response
   ELISA
   neutralisation
Antibody standards required

Animal challenge
   protection from challenge
Virus standards required
   for challenge (live) and PCR (inactivated)
Assay types

- IgM/IgG ELISA – various formats
  - Coating antigen; virus or recombinant protein
    - Envelope for vaccine
- Neutralisation – PRNT
  - Viruses needed (Virus standard; lineages)
  - Pseudotype/VLP assays/reporter viruses
    - Pseudotyping flaviviruses is challenging
- Cross reactivities with flaviviruses/arboviruses?
  - Dengue
  - Yellow fever
  - West Nile virus
  - JEV
  - Spondweni
Infection diagnosis

Vaccine efficacy
• Confirmation of protection from infection by PCR assays

NAT standard
• PEI conducting collaborative study
  – Timeline, endorsement for October ECBS meeting
• Inactivated virus preparations of Polynesian/African lineages
Antibody sources

• Infected patients (urgently required!)
  • IgG for vaccine
• Transchromosomal cows (SAB Biotherapeutics)
  • immunised with inactivated virus (IgG only)
• Monoclonal antibodies
• Animal sera?
• Need for reference panels

• Commutability issues – human origin preferred, standard must behave closely to the clinical sample
Standards available

• NIBSC has reference antibody standards for:
  • Dengue serotypes 1-4; pan specific
    • Very short supply, needs replacing
  • Japanese Encaphalitis virus
    • Good supply
  • Yellow fever virus
    • No human antibody reagents
      – Monkey serum available

• NIBSC is producing a panel of murine monoclonal antibodies raised against whole inactivated Zika virus
  • May be useful for neutralisation assays?
International Collaborative Studies

- WHO International Standards are the ‘gold standard’ against which regional, national and international laboratories and manufacturers calibrate their own working standards.
- They are calibrated in units of biological activity which are assigned following extensive studies involving multiple international laboratories.
- Assess the suitability of the material preparations to serve as International Standards with an assigned unitage in IU per vial.
- Characterise each candidate International Standard in terms of reactivity/specificity.
- Assess each candidate’s potency i.e. readout in a range of typical assays performed in different laboratories.
- Assess commutability.
- Assess stability.
- Recommend to WHO ECBS for establishment of the International Standard(s).

Requests

- Serum/plasma for
  - Zika antibody IgM/IgG – recently infected
  - Dengue serotypes
  - Yellow fever antibody
  - Others?

- Participants for collaborative study to evaluate candidate materials

- Thanks! Mark.Page@nibsc.org

- Workshop being planned for ~July
  - Design collaborative study
  - Understand assay landscape
  - Invite participants to the collaborative study