Key Activities – CDC Rabies

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WHO Collaborating Center on Rabies Reference and Research
1st Meeting of East African Rabies Network
February 2017

Ethiopia, Kenya, Uganda, Rwanda, Tanzania
CDC Rabies Program

- National reference laboratory
- OIE reference laboratory for rabies
- WHO collaborating center for reference and research on rabies
- ~80 Staff in Branch
  - 3 Laboratory Diagnostic Teams
    - Immunology and proteomics
    - Genomics and nucleic acid
    - Quality and compliance
      - CLIA
      - ISO 17025 (pending)
  - 11 dedicated rabies lab staff
  - 7 epi staff
Activity

- **Domestic**
  - Confirmatory diagnosis for state laboratories
  - Primary diagnosis and serological monitoring for active surveillance

- **International**
  - Diagnostic testing as requested (Human and animal rabies)
  - Surveillance and pathogen discovery projects with partner countries
    - Haiti, Guatemala, Ethiopia, Kenya, Rep. Georgia, India, Bangladesh, Vietnam

- **Latin American Country Support 2017**
  - 7 human rule outs
  - 468 animal samples for confirmatory diagnosis or serological surveillance
Diagnostic Network

- Pre-identify transport networks for shipping diagnostic samples
- Regional laboratories for diagnosis
Real-time RT-PCR Assay (LN34)

- The LN34 assay is comprised of two real-time RT-PCR assays that detect either lyssavirus genomic RNA or host beta actin mRNA.
- In silico analysis (>1 nucleotide mismatch makes detection unpredictable)
  - 538 rabies virus genomic sequences
    - 533 (99%): 100% match
    - 5 (1%): single mismatch
  - 56 Lyssavirus genomic sequences
    - 41 (73%): 100% match
    - 15 (26%): single mismatch

The LN34 assay

**The LN34 assay**

Sample

**LN34 real-time RT-PCR assay**
Amplifies and detects lyssavirus genomic RNA

**Beta actin real-time PCR assay**
Amplifies and detects host beta actin mRNA
Indicator for:
- PCR inhibition
- Failed extraction
- RNA degradation

**Controls for both assays**

**Positive amplification control**
Allows for the standardization and optimization of the LN34 assay across laboratories

**No template/Negative control**
Can identify contamination of buffers
LN34: Small-scale pilot study in US public health laboratories testing US samples

States: CA, GA, MD, NM, NY, PA, WI

2,120 samples passed quality standards

<table>
<thead>
<tr>
<th></th>
<th>LN34 Pos</th>
<th>LN34 Neg</th>
<th>LN34 Inc</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFA Pos</td>
<td>577</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DFA Neg</td>
<td>0</td>
<td>1475</td>
<td>0</td>
</tr>
<tr>
<td>DFA ND</td>
<td>23</td>
<td>45</td>
<td>0</td>
</tr>
</tbody>
</table>

Diagnostic sensitivity and specificity were both 100%*

*This calculation reflects results after samples were re-tested at the participating laboratory or at CDC, Atlanta

Issues Identified (corrected in table):
- 1 false positive was confirmed as contamination by sequencing
- 11 false positive DFA results identified
- 1 false negative DFA result identified
- All LN34 inconclusive results were resolved as either LN34 positive or negative after re-tested or re-extraction at the participating laboratory or CDC
## LN34: Large-scale international evaluation of the LN34 assay

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Country/Region</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maryland Department of Health and Mental Hygiene</td>
<td>United States</td>
</tr>
<tr>
<td>Pennsylvania Department of Health</td>
<td>United States</td>
</tr>
<tr>
<td>New Mexico Department of Health</td>
<td>United States</td>
</tr>
<tr>
<td>California Department of Public Health</td>
<td>United States</td>
</tr>
<tr>
<td>Wisconsin State Laboratory of Hygiene</td>
<td>United States</td>
</tr>
<tr>
<td>Wadsworth Center, New York State Department of Health</td>
<td>United States</td>
</tr>
<tr>
<td>University of Georgia</td>
<td>United States</td>
</tr>
<tr>
<td>United States Army (Germany)</td>
<td>Middle East</td>
</tr>
<tr>
<td>Animal Health Centre, British Colombia</td>
<td>Canada</td>
</tr>
<tr>
<td>Research Institute for Tropical Medicine</td>
<td>Philippines</td>
</tr>
<tr>
<td>Ministère de l'Agriculture</td>
<td>Haiti</td>
</tr>
<tr>
<td>Instituto de Salud Pública de Chile</td>
<td>Chile</td>
</tr>
<tr>
<td>Karnataka Veterinary, Animal and Fisheries Sciences University</td>
<td>India</td>
</tr>
<tr>
<td>Centers for Disease Control and Prevention</td>
<td>Georgia</td>
</tr>
<tr>
<td></td>
<td>Asia</td>
</tr>
<tr>
<td></td>
<td>Ethiopia</td>
</tr>
</tbody>
</table>
LN34: Large-scale international evaluation of the LN34 assay

2,978 samples (1,098 DFA positive) passed quality standards

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<th>LN34 Neg</th>
<th>LN34 Inc</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFA Pos</td>
<td>1044</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>DFA Neg</td>
<td>3</td>
<td>1841</td>
<td>4</td>
</tr>
<tr>
<td>DFA ND</td>
<td>51</td>
<td>29</td>
<td>1</td>
</tr>
</tbody>
</table>

Diagnostic Sensitivity: **99.52%** (98.89 – 99.85)
Diagnostic Specificity: **99.62%** (99.22 – 99.85)

*These estimates are conservative* and consider LN34 inconclusive results as false positive or false negative. Inconclusive results are flagged for re-testing according to CDC guidelines for the LN34 assay. If the result remains inconclusive after re-testing, PEP is recommended if rabies is supported by the case history.
Serologic Assays

- Improving Sero-Surveillance and ORV monitoring
  - Surveillance for rabies in bats in Caribbean
- Evaluating commercial ELISA kits
- High-throughput neutralization assay
  - Modified RFFIT
  - 96well plate
  - Automated reader
Establishing a Framework for Country Engagement

- Declaring rabies a priority

Diagram:

1. Does the country have a list of prioritized zoonotic diseases?
   - Yes
   - No

   **STEP I**
   - Conduct a CDC One Health Zoonotic Disease Prioritization workshop or similar zoonotic disease prioritization process

2. Is Rabies Listed as a Prioritized Zoonotic Disease?
   - Yes
   - No

   **STEP II**
   - Conduct Evaluation Using SARE & GDREP Tool
     - Then
     - Select Priority Activities Based on SARE Output and Country Capacity

3. Is the County Committed to Controlling Rabies?
   - Yes
   - No

   Reconsider engagement. Work with country to improve understanding of the rabies burden
Establishing a Framework for Country Engagement

- Evaluate current status of program, establish a baseline for documenting progress
Establishing a Framework for Country Engagement

- Identify tools and technical support for burden assessment, surveillance and PEP delivery, lab capacity, and canine rabies elimination.
Thank you!

Questions?
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Sathesh Panayampalli (xdv3@cdc.gov)

For more information please contact Centers for Disease Control and Prevention

1600 Clifton Road NE, Atlanta, GA 30333
Telephone, 1-800-CDC-INFO (232-4636)/TTY: 1-888-232-6348
E-mail: cdcinfo@cdc.gov Web: www.cdc.gov
A Pan-Lyssavirus Taqman Real-Time RT-PCR Assay for the Detection of Highly Variable Rabies virus and Other Lyssaviruses

Ashutosh Wadhwa, Kimberly Wilkins, Jinxin Gao, Rene Edgar Condori Condori, Crystal M. Gigante, Hui Zhao, Xiaoyue Ma, James A. Ellison, Lauren Greenberg, Andres Velasco-Villa, Lillian Orciari, Yu Li

Published: January 12, 2017 - http://dx.doi.org/10.1371/journal.pntd.0005258

1 Probe: 3 specific sequences

Using modified nucleotides to archive optimal real-time PCR temperature conditions
probe sequences comparisons against published Rabies virus genomic sequences - 538 genomes by April 2017

<table>
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<tr>
<th>Published assays</th>
<th>Assay name</th>
<th># of probes</th>
<th># of probe seq</th>
<th>GenBank 538 RABV genome sequences</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100% match</td>
</tr>
<tr>
<td>Canada, NY, Belgium Assay I</td>
<td>NY-RABVD (2 probes)</td>
<td></td>
<td>20</td>
<td>276</td>
</tr>
<tr>
<td>UK, German Assay II</td>
<td>M13+M14 (5 probes)</td>
<td></td>
<td>12</td>
<td>309</td>
</tr>
<tr>
<td>France, Ins. Pasteur Assay III</td>
<td>RABV4,V5 (2 probes)</td>
<td></td>
<td>112</td>
<td>370</td>
</tr>
<tr>
<td>US, CDC Assay IV</td>
<td>LN34 (1 probe)</td>
<td></td>
<td>3</td>
<td>533</td>
</tr>
</tbody>
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More than 2 nucleotides differences between probe sequences and a rabies viral genome make the detection of the genome difficult and unpredictable.
### Probe Sequences Comparisons Against Published Other Lyssaviruses Genomic Sequences - 56 Genomes by April 2017

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2 or more nucleotides differences between probe sequences and a rabies viral genome make the detection of the genome difficult and unpredictable.