Enterovirus D68-US Outbreak 2014
Laboratory Perspective

Allan Nix
Team Lead
Picornavirus Laboratory
Polio and Picornavirus Laboratory Branch

PAHO / SARI-net Webinar
November 6, 2014
Genus *Enterovirus* Taxonomy*

**Old taxonomy:** by disease in humans and animal model systems

- Polioviruses
  - PV1-3
- Coxsackie A viruses
  - CAV1-22, 24
- Coxsackie B viruses
  - CBV1-6
- Echoviruses
  - E1-7, 9, 11-21, 24-27, 29-33
- Enteroviruses
  - EV68-120
- *Rhinovirus* (genus)

**Current taxonomy:** by molecular, as well as biological properties

- Enterovirus A (EV-A)
  - 25 types
- Enterovirus B (EV-B)
  - 61 types
- Enterovirus C (EV-C)
  - 23 types
- Enterovirus D (EV-D)
  - 5 types
- Rhinovirus A (RV-A)
  - 80 types
- Rhinovirus B (RV-B)
  - 32 types
- Rhinovirus C (RV-C)
  - 54 types

*Does not include animal EV-E, -F, -G, -H, -J and non-classified
Rhinoviruses (RV) and Enteroviruses (EV)

- EV and RV “specific” real-time assays both target the same conserved nucleotide sequences in the 5’ NTR IRES region
  - These assays are referred to in the literature as pan-EV or pan-RV
  - The pan-EV and the pan-RV assays are, in fact, **NOT SPECIFIC**
  - Recently, ICTV eliminated the genus *Rhinovirus* and reclassified all RV as members of the genus *Enterovirus*
- US CDC pan-EV real-time assay detects 66% of RV species A and B and detects an unknown number of species RV-C
- Conversely, published pan-RV assays detect an unknown percentage of species EV-A, -B, -C and -D
- Reporting to clinicians has been problematic, since they are not aware of virus taxonomy or well acquainted with the assays
  - A positive pan-EV or pan-RV 5’ NTR assay should be reported as RV/EV/; RV or EV
  - Typing is required to assign EV or RV species and (sero) type
EV Species A-D—Rhinoviruses Excluded

**Neighbor-Joining Method ; MEGA 5.0**

**EV Species A**

**EV Species B**

**EV Species C**

**EV Species D**

Amino-acid; complete coding sequences
Enterovirus Species D

- **EV-D68**
  - First isolated in 1962 from children with respiratory disease
  - HRV87 isolated in 1963 in the same lab (Corn strain)
  - HRV87 reclassified around 2002 as an EV-D68
  - Associated with severe respiratory disease (since 1962)

- **EV-D70**
  - Associated with acute hemorrhagic conjunctivitis (AHC)
  - AHC first reported in Ghana in 1969; first isolated 1971 in Japan

- **EV-D94**
  - Isolated from stool of AFP patients on RD cells (stools from 2001, Democratic Republic of Congo)
  - Isolated from sewage samples on RD cells (Egypt)

- **EV-D111**
  - First detected and identified in Cameroon in chimpanzees
  - Subsequently isolated from stool of AFP patients on RD cells (Cameroon, DRC, Central African Republic)

- **EV-D120**
  - First detected and identified in Cameroon in 4 gorillas and 1 chimpanzee
EV-D68 Cell Culture

• EV-D68 grows to highest titer at 33°C
• Primary monkey kidney cells support growth
  • CMK  *Cynomolgus* primary kidney
  • RMK  *Rhesus* primary kidney
• Cell lines derived from human lung tissue support growth
  • A549  human embryonic lung epithelium
  • HLF  human embryonic lung fibroblast
  • WI-38  human embryonic lung fibroblast
  • MRC-5  human embryonic lung fibroblast
• Others cell lines
  • RD  human rhabdomyosarcoma
  • HFKD  human fetal diploid kidney
Cytopathic Effect in Rhabdomyosarcoma Cells

Photo Credit: Yiting Zhang
EV-D68 Thin Section EM; RD Cells

Photo Credit: Cynthia S. Goldsmith and Yiting Zhang

EV-D68 Status
- None
- Confirmed
- Pending

*If known
Enterovirus Genome / Typing Target

- Antigenic (serotype) identity
- Conserved amino acid sequences flank variable sites of interest
- By design amplifies all EV-A, -B, -C and -D (some recent types-only in silico analyses)
- Also amplifies many RV-A, -B, and some -C

Virus Identification

- Serotype-specific target for diagnostic sequencing-molecular typing ("serotyping")
- First draft of molecular epidemiology, i.e., phylogenies

2014 US Predominant Outbreak Strain

- 544 Patients (92.4%)
- This EV-D68 strain is closely related to EV-D68 strains detected in the United States in 2011, 2012, and 2013
- Also closely related to China (2012), Italy (2012) and Thailand (2011)

2014 US Minor Outbreak Strain

- 44 Patients (7.4%)
- This EV-D68 strain is closely related to EV-D68 strains detected in Spain (2012) and Italy (2012)

- 1 Patient (0.2%)
## 2014 EV-D68-Specific Real Time RT-PCR Assay

<table>
<thead>
<tr>
<th>Identification #</th>
<th>Orientation</th>
<th>Gene</th>
<th>Location (5’-3’ Sense Strand)</th>
<th>Amino-Acid Motif (NH-COOH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AN887</td>
<td>Sense</td>
<td>VP1</td>
<td>2518-2543</td>
<td>QTRTVINQH</td>
</tr>
<tr>
<td>AN893</td>
<td>Antisense</td>
<td>VP1</td>
<td>2761-2789</td>
<td>VAVNGSSNNT</td>
</tr>
<tr>
<td>AN890 (Probe)</td>
<td>Antisense</td>
<td>VP1</td>
<td>2647-2669</td>
<td>DKNFFKWT</td>
</tr>
</tbody>
</table>

### EV VP1 Standard Typing Assay

<table>
<thead>
<tr>
<th>EV-D68-Specific Assay</th>
<th>+</th>
<th>-</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(True Positive)</td>
<td>(False Positive)</td>
<td>69</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>(False Negative)</td>
<td>(True Negative)</td>
<td>0</td>
<td>65</td>
</tr>
</tbody>
</table>

- **Sensitivity**: 100%
- **Specificity**: 96%

Amplification Plot: Respiratory Clinical Specimens
Thanks for the invitation to speak with you today.

PPLB Contacts:
Allan Nix  WNix@cdc.gov
Steve Oberste  SOberste@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: http://www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.