

RAPID, ACCURATE RT-PCR TEST FOR ENTEROVIRUS D68

[Storch, Gregory, Wylie, Kristine, Wylie, Todd](#)

[Hardin, Clyde "Frank"](#)

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Technology Description

A team of researchers at Washington University designed patented primers for a sensitive, specific, real-time reverse transcriptase PCR (RT-PCR) assay to quickly detect enterovirus D68 (EV-D68).

EV-D68 is an emerging, highly contagious respiratory virus that can cause severe illness or death. During previous EV-D68 outbreaks, surveillance and tracking was hindered by the limited testing options – either non-specific assays or time-consuming sequence analysis. In particular, broadly-reactive multiplex commercial tests do not detect EV-D68 alone. Instead they identify panels of enteroviruses and rhinoviruses. This new assay can improve diagnosis and monitoring because it is faster than sequence analysis (hours vs. days) while optimizing sensitivity and specificity for EV-D68. The primers incorporate built-in redundancies so they are extremely effective at identifying all known strains of EV-D68 while excluding other enteroviruses. The resulting test is 100x more sensitive than a CDC-developed assay and 10x more sensitive than FDA-approved multiplex assays. This diagnostic tool could improve epidemiological monitoring and help guide treatment decisions.

Stage of Research

The inventors designed RT-PCR primers to target a 60-bp region and recognize the maximum number of EV-D68 sequences. Using EV-D68-positive clinical samples (n = 35) they demonstrated that assays with these primers:

- had an analytical detection limit of detection of 4 copies per reaction
- were more sensitive than commercially available FDA-approved assays and a CDC assay
- did not detect any other enterovirus strains or rhinoviruses
- detected 100% of divergent strains of EV-D68 (including all known variants and isolates going back to original isolate from 1962)

Applications

- **Clinical diagnostics:** identify patients with EV-D68 to enable decision-making for appropriate treatment and isolation
- **Research and epidemiology:** studies, surveillance and monitoring of EV-D68 outbreaks

Key Advantages

- **Fast:** RT-PCR assay provides results in hours (compared to conventional sequencing analysis which requires days)
- **Sensitive:**
 - 100x more sensitive than CDC assay for EV-D68
 - 10X more sensitive than commercial assays for enterovirus and rhinovirus

- **Specific:** distinguishes EV-D68 from rhinoviruses and other enteroviruses to avoid false positive results

Publications:

- Wylie TN, Wylie KM, Buller RS, Cannella M, Storch GA. [Development and Evaluation of an Enterovirus D68 Real-Time Reverse Transcriptase PCR Assay](#). *J Clin Microbiol*. 2015;53(8):2641-2647.
- [Diagnostic test developed for enterovirus D68](#). *theSource*, July 22, 2015.

Patents: [Methods and compositions for detection of enterovirus d68](#) (U.S. Patent No. 10,752,966; additional patent application pending)

Related Web Links: Storch [Profile](#), [Lab](#); Kristine Wylie [Profile](#), [Lab](#); Todd Wylie [Profile](#)