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BioFire FilmArray Respiratory Panel for Detection of Enterovirus D68

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During the enterovirus D68 (EV-D68) outbreak of 2014, the BioFire FilmArray (FA) respiratory panel was used to detect rhinovirus/enterovirus in respiratory specimens; suspected EV-D68-positive specimens were sent to CDC for confirmation. Positive rhinovirus/enterovirus FA targets revealed patterns loosely associated with EV-D68 that may be useful for confirmation triaging.

In 2014, an outbreak of enterovirus D68 (EV-D68) spread through the United States. Cases of severe respiratory illness due to EV-D68 in pediatric patients were reported in many states, particularly in Missouri and Illinois. Generally, enteroviruses are associated with mild respiratory illnesses, but these reported cases were associated with increased complications, such as hospitalization and admission into pediatric intensive care units, especially in patients with underlying asthma (1). No treatment is available for EV-D68. Previous clusters of EV-D68 have been documented but have not been as widespread as in the 2014 season (2).

Enterovirus is a member of the family *Picornaviridae*. It is a small, nonenveloped, single-stranded, positive-sense RNA virus (3). Enteroviruses have been classified as belonging to four species, A, B, C, and D (4). Currently there are 5 serotypes of enterovirus D, i.e., D68, D70, D94, D111, and D120, with the last two having been identified in primates (2). Characteristics of EV-D68 exhibit similarities to those of rhinovirus, such as acid lability and preference for growth at 33°C (2, 3). Genetically, enterovirus and rhinovirus have conserved structures in the 5′ untranslated region (UTR) (5). The similarities between the sequences of rhinoviruses and enteroviruses allow cross-amplification in molecular assays (6).

During the outbreak, respiratory pathogen testing at The University of Chicago was performed on the BioFire FilmArray respiratory panel (BioFire Diagnostics, Salt Lake City, UT). This panel is a multiplex PCR assay with melting curve analysis that detects 20 respiratory pathogens, including human rhinovirus/enterovirus. The package insert states that for the human rhinovirus/enterovirus targets, there are 6 different assays that amplify the following targets: human rhinovirus targets HRV 1, HRV 2, HRV 3, and HRV 4 and enterovirus targets Entero 1 and Entero 2 (7, 8). The final result of the FilmArray is based on the compilation of the assays for rhinovirus and enterovirus. Due to similarity between the viruses, a distinction between rhinovirus and enterovirus cannot be made based on which targets are positive, so a positive result for any of the targets would indicate a final reportable result of "human rhinovirus/enterovirus detected." The manufacturer recommends follow-up of a positive rhinovirus/enterovirus test with sequence analysis or viral culture (7). The GenMark eSensor (GenMark Dx, Carlsbad, CA) is another respiratory viral panel capable of detecting rhinovirus. EV-D68 is now known to cross-react with the rhinovirus targets on the GenMark eSensor, leading to a positive rhinovirus result (9, 10). Given the similarities of EV-D68 and rhinovirus, none of the commercially available respiratory virus panels could reliably differentiate the two viruses during the outbreak. This was problematic, because clinical laboratories were required to send specimens to a reference laboratory or a public health laboratory for confirmatory testing, delaying the final results. An investigation into the positive target patterns on the FilmArray respiratory panel and EV-D68 status was initiated.

TABLE 1 BioFire FilmArray respiratory panel targets for clinical specimens tested for the presence of EV-D68 by the CDC

FilmArray target	No. (%) of specimens		
	With target	Confirmed EV-D68 positive	EV-D68 negative
Human rhinovirus 1,4	22	20 (41)	2 (5)
Human rhinovirus 1,2,4	19	17 (34)	2 (5)
Human rhinovirus 4	13	7 (15)	6 (15)
Human rhinovirus 1,2,3,4	27	5 (10)	22 (55)
Human rhinovirus 3,4	4	0 (0)	4 (10)
Human rhinovirus 1,2	2	0 (0)	2 (5)
Human rhinovirus 1	1	0 (0)	1 (2.5)
Human rhinovirus 2,4	1	0 (0)	1 (2.5)
Enterovirus 1	0	0 (0)	0 (0)
Enterovirus 2	0	0 (0)	0 (0)
Total	89	49 (100)	40 (100)

Ninety-one frozen nasopharyngeal and bronchoalveolar lavage specimens from 65 children and 26 adults with suspected EV-D68 infection were positive for rhinovirus/enterovirus by the FilmArray. These specimens were sent to the Centers for Disease Control and Prevention (Atlanta, GA) for confirmatory testing. The CDC performed enterovirus sequencing but later switched to an EV-D68-specific real-time reverse transcription-PCR (RT-PCR) and panenterovirus detection (11–14). Two pediatric specimens were confirmed as being negative for rhinovirus/enterovirus by the CDC assay. Forty-nine of the remaining 89 specimens (55%) were positive for EV-D68; 43 were from pediatric patients (88% of positives; 48.3% of total specimens), and 6 were from adults (12% of positives, 6.7% of total specimens). There were 40

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TABLE 2 Predictability of BioFire FilmArray patterns for the presence of EV-D68[a]

Pattern	EV-D68 positive	EV-D68 negative	OR+	OR−	% SE (95% CI)	% SP (95% CI)
HRV 1,4	20	2				
All others	29	38	13.10	0.08	40.82 (27.00, 55.79)	95.00 (83.08, 99.39)
HRV 1,4 or HRV 1,2,4	37	4				
All others	12	36	27.75	0.04	75.51 (61.13, 86.66)	90.00 (76.34, 97.21)
HRV 1,4 or HRV 1,2,4 or HRV 1,2,3,4	42	26				
All others	7	14	3.23	0.31	85.71 (72.76, 94.06)	35.00 (20.63, 51.68)
HRV 1,2,3,4 or HRV 3,4	5	26				
All others	44	14	0.06	16.34	10.20 (3.40, 22.23)	35.00 (20.63, 51.68)

[a] HRV, human rhinovirus; OR+, odds ratio in favor of EV-D68; OR−, odds ratio in favor of not having EV-D68; SE, sensitivity; SP, specificity; CI, confidence interval.

EV-D68-negative specimens, 20 from pediatric patients (50% of negatives; 22.5% of total specimens) and 20 from adults.

A retrospective review of the positive targets detected by the FilmArray respiratory panel was performed to determine if a particular pattern could predict EV-D68 positivity. The following observations were noted. Both positive and negative EV-D68 specimens signaled for various combinations of human rhinovirus (HRV) targets; interestingly, none of the specimens were positive for the enterovirus 1 or enterovirus 2 targets (Table 1). Of the EV-D68-positive specimens ($n = 49$) (Table 1), the majority ($n = 44$; 90%) included some combination of targets 1, 2, and 4, though the combination of HRV 1 and HRV 4 was the most prevalent ($n = 20$; 41%). HRV 3 was seen in 5 (10%) of the EV-D68 positive specimens, and only in combination with all of the other human rhinovirus targets. Twenty-six (65%) of the 40 EV-D68 negative specimens were positive for HRV 3, also usually in combination with other HRV targets (Table 1). Four of the 40 (10%) EV-D68-negative specimens had patterns more commonly associated with EV-D68 positive specimens, i.e., HRV 1 and 4 and HRV 1, 2, and 4.

A statistical analysis of the data is shown in Table 2. The combination of HRV targets 1 and 4 was 13.1 times more likely to be associated with an EV-D68-positive specimen. Including the pattern HRV 1, 2, and 4 increased the odds ratio (OR) to 27.8, and 90% of patients who tested positive for these patterns were confirmed as being EV-D68 positive (specificity = 90%). Adding the HRV pattern 1, 2, 3, and 4 increased the sensitivity to 85% at the cost of specificity. Conversely, the EV-D68 negative specimens were 16.3 times more likely to have HRV 3 than the positives.

Further classification of the 40 EV-D68-negative specimens by the CDC revealed that the initial 11 specimens contained 5 strains of human rhinovirus A101, 2 strains of human rhinovirus C, 1 strains of coxsackievirus B5, 1 strain of human rhinovirus 49, 1 strain of human rhinovirus 59, 1 strain of human rhinovirus 83, and no other enterovirus strains as determined by sequencing. During the outbreak, the CDC assay changed from sequencing to an EV-D68 specific real-time RT-PCR and panenterovirus detection assay, so the remainder of the EV-D68-negative specimens ($n = 29$) were classified as 21 panenterovirus-negative specimens and 8 specimens containing rhinovirus/enterovirus other than D68.

In conclusion, though no particular pattern on the FilmArray RP could point to the definitive presence of EV-D68, the following patterns emerged: the pattern HRV 1 and 4 and the pattern HRV 1, 2, and 4 were 27.8 times more likely to be associated with an EV-D68-positive specimen, with a specificity of 90%. The EV-D68-negative specimens were 16.3 times more likely to have HRV 3 than the positives, making HRV 3 a point of distinction in this assay.

These findings suggest that the presence of HRV 1 and 4 or of HRV 1, 2, and 4 might be useful in identifying EV-D68 infection. Public health laboratories might choose to use these targets to prioritize confirmatory testing in severely ill pediatric patients. The additional presence of HRV 3 (as in the pattern HRV 1, 2, 3, and 4) suggests a lack of EV-D68 infection; however, the analysis does not support it as a definitive marker for ruling out the virus. Perhaps if HRV 3 existed in more patterns other than in combination with HRV 1, 2, and 4, its use in ruling out EV-D68 would be more robust.

It is possible that our observations were limited by the particular strains of non-EV-D68 viruses circulating in our region. Larger studies involving specimens from different geographic regions over time would be needed to further assess the validity and significance of these findings. Nonetheless, these observations, in conjunction with the degree of clinical suspicion, could potentially influence which specimens are triaged or prioritized for further confirmatory testing. These results might also influence hospital practice regarding allocation of resources to care for patients with a "likely" EV-D68 infection, pending confirmatory testing.

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