Evaluation of multiple commercial molecular and conventional diagnostic assays for the detection of respiratory viruses in children

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Abstract

This study compares the performance of four commercial multiplex PCR assays (Resplex II Panel v2.0, Seeplex RV15, xTAG RVP and xTAG RVP Fast) and direct fluorescent antibody (DFA) staining and viral isolation. Seven hundred and fifty nasopharyngeal swabs were tested for 17 viral agents. In each assay, the sensitivity and specificity for each target were determined against a composite reference standard. Two hundred and eighty-eight out of 750 (38.4%) specimens were positive by DFA or viral isolation, while an additional 214 (28.5%) were positive by multiplex PCR, for a total positivity rate of 66.9%. Of 502 positive specimens, one virus was detected in 420 specimens (83.7%), two in 77 (15.3%), three in four (0.8%) and four in one case (0.2%). Compared with a composite reference standard, the inter-assay accuracy of the multiplex PCR assays varied, but all were superior to conventional diagnostic methods in detecting a broad range of respiratory viral agents in children. In addition, the sensitivity of two commercial assays, Resplex II Plus PRE and Seeplex Influenza A/B Subtyping, was determined relative to the Astra influenza Screen & Type assay for detection of influenza A viruses, including seasonal influenzas and pandemic H1N1 2009 influenza A virus. Using 75 positive and 55 negative nasopharyngeal swabs for influenza A by the Astra assay, the sensitivity of Seeplex and Resplex was 95.9% and 91.8%, respectively, with a specificity of 100% for both.

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Introduction

Acute viral respiratory tract infections are a significant cause of morbidity and mortality in children, particularly those with compromised immune systems [1–3]. Nucleic acid amplification tests have shown their superiority over classical diagnostic methods, such as direct fluorescent antibody detection (DFA) and viral isolation, in identifying a broader range of viruses, with higher sensitivity and specificity [4–6]. Recently, several studies have demonstrated the advantages of multiplex PCR for simultaneous detection of a panel of viruses in one assay [7–14]. In this study we compared (i) the sensitivity and specificity of four commercial multiplex PCR assays with DFA and viral isolation for detection of respiratory viruses in children, and (ii) the sensitivity of two assays, Resplex II Plus PRE and Seeplex Influenza A/B Subtyping, with the Astra influenza Screen & Type assay and DFA for detection of H1 and H3 seasonal and pandemic H1N1 2009 influenza A virus.

Materials and Methods

Specimens

Multiplex assays. Seven hundred and fifty nasopharyngeal (NP) swabs were selected from children (birth to 17 years) with suspected respiratory tract infection seen at The Hospital for Sick Children (Toronto, Canada). The first 25 specimens received each week for a 24-week period (November 2007 to April 2008), were selected, for a total of 600 specimens,

without knowing results of DFA or viral isolation. An additional 150 specimens were chosen in the same manner (January 2009 to March 2009). Nasopharyngeal swabs (flocked swabs; COPAN Diagnostics, Murrieta, CA, USA) were inoculated into 3 mL of Universal Transport Medium (UTM-RT COPAN Diagnostics, Murrieta, CA, USA). After vortexing, specimens from 2007/2008 (n = 600) were dispensed into four 400-μL aliquots and stored at −80°C until nucleic acid extraction, whereas specimens from 2009 (n = 150) were submitted to nucleic acid extraction before aliquoting and freezing at −80°C.

Specimen selection for comparison of assays to detect seasonal influenza A and pandemic H1N1 2009 influenza. A total of 130 NP swab specimens were selected from aliquots of the original specimens frozen at −80°C on receipt: 75 influenza A positive specimens by Astra Screen and Type assay (13 seasonal influenza A (INFA)-H1, 12 INFA-H3 and 50 pandemic H1N1 2009 influenza A (INFA)-H1N1), and 55 influenza A/B negative specimens by the Astra assay. All specimens were made anonymous and de-linked from all personal health identifiers.

DFA

DFA was performed using fluorophore-labelled monoclonal antibodies against respiratory syncytial virus (RSV), parainfluenza viruses 1–3 (PIV), adenovirus (ADV), INFA/B (SimulFluor®; Millipore, Temecula, CA, USA) and human metapneumovirus (hMPV) (Diagnostic HYBRIDS, Athens, OH, USA). DFA was carried out for each target as per the manufacturer's protocol.

Cell culture

All NP swabs from 2007/2008 underwent both DFA and culture. During the 2009 pandemic, specimens that were positive for INFA by DFA were not cultured. Specimens were inoculated in duplicate into R-Mix™ (Diagnostic HYBRIDS) shell vials as per the manufacturer's instructions. A shell vial coverslip was stained with SimulFluor® Respiratory Screen Reagents (Millipore) containing monoclonal antibodies for ADV, PIV 1–3, INFA/B and RSV as per the manufacturer's instructions. Staining for anti-hMPV was carried out using a separate shell vial coverslip and reagents (Diagnostic HYBRIDS) as per the manufacturer's instructions.

Nucleic acid extraction

Nucleic acid was extracted from 400 μL of specimen using the biorobot M48 workstation/MagAttract Virus Mini M48 kit (Qiagen, Mississauga, ON, Canada) and eluted in 100 μL of elution buffer.

Multiplex RT-PCR

Respiratory viral panels. The extracted nucleic acid was amplified by four commercial multiplex assays: Resplex II Panel v2.0 (Qiagen), Seeplex RV15 (Seegene Inc., Seoul, Korea), xTAG® Respiratory Viral Panel (RVP) and xTAG® RVP Fast (Luminex Molecular Diagnostics, Toronto, ON, Canada). cDNA and amplification steps were carried out in a single-tube format for Resplex II v2.0, RVP and Fast assays whereas Seeplex RV15 required a separate cDNA synthesis step using a RevertAid H Minus First Strand cDNA Synthesis kit (Fermentas, Burlington, ON, Canada) before performing the multiplex PCR step.

Amplification products were detected using the LiquiChip 200 (Luminex 200) (Austin, TX, USA) for Resplex II v2.0, and the Luminex 100 for RVP and RVP Fast. Qiagen instructs each user to determine appropriate cut-off values for their testing platform and patient population. Cut-off values for Resplex II v2.0 were determined empirically to be the sum of the mean MFI plus ≥5 times the standard deviation of negative specimens (negative in all assays). The cut-off values for RVP and RVP Fast were predetermined by the manufacturer. Each assay had an internal control (IC) included to rule out PCR inhibition. Table 1 shows the targets and characteristics of each multiplex assay.

To detect amplification products, the Seeplex RV15 assay was coupled with capillary electrophoresis technology (Lab901Screen Tape system; Lab901 Ltd, Loanhead, UK).

Specific detection of seasonal influenza A and pandemic H1N1 2009 influenza A virus. Amplification from a selected subpopulation of specimens was performed using the Astra influenza Screen and Type (Astra Diagnostics, Hamburg, Germany), Resplex II Plus Panel PRE (Qiagen) and Seeplex Influenza A/B Subtyping (Seegene) assays. The Astra influenza Screen & Type triplex assay was designed to detect seasonal INFA and pandemic H1N1 2009 INFA in a real-time RT-PCR format and used the Rotor-Gene 3000 instrument (Corbett Research, Mortlake, NSW, Australia). This assay was chosen as the reference standard due to its performance in a proficiency panel for the detection of H1N1 2009 INFA, in which 22 laboratories participated, using 18 different assays. The Astra kit was found to have the highest sensitivity of detection compared with all other assays, including the CDC H1N1 2009 assay (26th Annual Clinical Virology Symposium, abstract S35). Specimens were also tested by the Resplex II Plus Panel PRE (same targets as Resplex II Panel v2.0 plus pandemic H1N1 2009 INFA) and the Seeplex Influenza A/B Subtyping assay (INFA, INFA-H1, pandemic H1N1 2009 INFA, INFA-H3 and INFB). Amplicons from the Resplex II Plus Panel PRE and Seeplex Influenza A/B Subtyping assays

TABLE 1. Targets and characteristics of multiplex assays for detection of respiratory viruses

Assay	Resplex II v2.0	Seeplex RV15	xTAG RVP	xTAG RVP Fast
Target	Influenza A, B	Influenza A, B	Influenza A (H1, H3, H5), B	Influenza A (H1, H3), B
	Respiratory syncytial virus A, B	Respiratory syncytial virus A, B	Respiratory syncytial virus A, B	Respiratory syncytial virus A, B
	Parainfluenza 1–4	Parainfluenza 1–4	Parainfluenza 1–4	Parainfluenza 1–4
	Human metapneumovirus	Human metapneumovirus	Human metapneumovirus	Human metapneumovirus
	Adenovirus B,E	Adenovirus B,C,E, some A,D	Adenovirus A–F	Adenovirus A–F
	Bocavirus	Bocavirus	–	Bocavirus
	Coronavirus OC43	Coronavirus OC43/HKU1	Coronavirus OC43	Coronavirus OC43
	Coronavirus HKU1		Coronavirus HKU1	Coronavirus HKU1
	Coronavirus 229E	Coronavirus 229E/NL63	Coronavirus 229E	Coronavirus 229E
	Coronavirus NL63		Coronavirus NL63	Coronavirus NL63
			SARS coronavirus	
	–	–	–	–
	Enterovirus	Enterovirus	Enterovirus/Rhinovirus	Enterovirus/Rhinovirus
	Rhinovirus	Rhinovirus A/B/C		
Technology	End-point RT-PCR	End-point RT-PCR	End-point RT-PCR	End-point RT-PCR
	Microsphere-based detection	Dual priming oligo (DPO)	Microsphere-based detection	Microsphere-based detection
Equipment	LiquiChip (Luminex 200 system)	Lab901 ScreenTape system	Luminex 100 system	Luminex 100 system
Amplification/detection[a]	310 min	520 min	450 min	220 min

[a]For 24 specimens and excluding the nucleic acid extraction. Times are approximate.

were detected using the LiquiChip 200 and the MultiNA instrument, a microchip electrophoresis system for DNA/RNA analysis (Shimadzu Biotech, Tokyo, Japan), respectively. Each assay had an IC to detect inhibition of amplification.

Ethics

This study was approved by the Research Ethics Board of the Hospital for Sick Children.

Definitions

True positive.   For all targets except PIV4, BoV, coronaviruses, enterovirus and rhinovirus, any positive viral culture or a positive result for a single target from any two of DFA and the four molecular assays was considered true positive. Because PIV4, BoV, coronaviruses, enterovirus and rhinovirus could not be detected by DFA or viral isolation, a true positive for these agents was defined as a positive result in at least two of the three or four multiplex PCR assays.

False positive.   A single positive result in any assay, with the exception of viral culture, was considered false positive.

Results

Respiratory viral panels

Distribution of respiratory viruses.   Virus isolation was performed on 684 specimens. Of 750 specimens tested, 502 (66.9%) were considered true positive results for at least one virus by DFA, culture or PCR; 288/750 (38.4%) specimens were positive by DFA or viral isolation, while an additional 214 (28.5%) were positive by multiplex PCR. A single virus was detected in 420 specimens (83.7%), two viruses in 77 (15.3%), three in four specimens (0.8%)

TABLE 2. Distribution of respiratory viruses in paediatric nasopharyngeal samples

Virus	Virus subtype	Number	Single-infection number (%)	Dual-infection number (%)
Enterovirus/rhinovirus	–	173	128 (21.7)	45 (7.6)
RSV	A	86	108 (18.3)	34 (5.8)
	B	56		
INFA	H1	40	58 (9.74)	6 (1.02)
	H3	22		
	Unidentified	2		
INFB	–	37	34 (5.8)	3 (0.51)
Parainfluenza	1	14	25 (4.23)	12 (2.04)
	2	9		
	3	6		
	4	8		
hMPV	–	39	27 (4.6)	12 (2.0)
Coronavirus	NL63	13	20 (3.28)	22 (3.69)
	OC43	15		
	HKU1	12		
	229E	2		
BoV	–	21	7 (1.2)	14 (2.4)
ADV	–	19	13 (2.2)	6 (1.0)
Total	–	574	420 (71.2)	154 (26.1)

and four viruses in one (0.2%). Table 2 shows the overall distribution of respiratory viral pathogens, indicating the predominance of enteroviruses/rhinoviruses, influenza viruses and RSV. It also shows that parainfluenza viruses, hMPV and coronaviruses accounted for about 7% each, followed by bocavirus and adenovirus at around 4% each. Among dual infections (*n* = 77) entero/rhinoviruses were most commonly associated with other viruses. Triple virus-infected specimens included ADV + enterovirus/rhinovirus + BoV (*n* = 1), PIV4 + ADV + enterovirus/rhinovirus (*n* = 1), RSVB + NL63 + BoV (*n* = 1) and RSVB + PIV4 + enterovirus/rhinovirus (*n* = 1). The unique quadruple-agent infected specimen was PIV3 + NL63 + enterovirus/rhinovirus + BoV (*n* = 1).

Comparison of sensitivity and specificity among assays.   Sensitivity and specificity were calculated for each target and

TABLE 3. Sensitivity of direct fluorescent antibody (DFA), culture and four multiplex assays for detection and identification of respiratory viruses. Number of positives (within brackets)

Target	DFA	Culture	Resplex II Panel v2.0	Seeplex RV15	xTAG® RVP	xTAG® RVP Fast
INFA	76.7% (46)	60.3% (35)	96.9% (62)	96.9% (62)	98.4%[a] (63)	93.7%[b] (60)
INFB	78.4% (29)	75.0% (21)	100% (37)	100% (37)	100% (36)	64.9% (24)
PIV (1–4)	72.4% (21)	61.5% (16)	82.9% (34)	97.6% (40)	85.4% (35)	65.8% (26)
PIV1	76.9%	66.7%	86.7%	93.3%	71.4%	46.7%
PIV2	55.5%	44.4%	88.9%	100%	100%	77.8%
PIV3	100%	66.7%	100%	85.7%	71.4%	42.8%
PIV4	–	–	60.0%	100%	100%	100%
hMPV	68.6% (24)	43.3% (13)	82.0% (32)	97.4% (38)	97.4% (38)	92.3% (36)
RSV (A/B)	93.5% (130)	86.5% (96)	84.0% (121)	100% (144)	88.2% (127)	91.7% (132)
RSVA	–	–	90.4%	100%	85.5%	92.5%
RSVB	–	–	79.3%	100%	98.3%	94.8%
ADV	38.1% (8)	44.4% (8)	71.4% (15)	100% (21)	85.7% (18)	52.4% (11)
BoV	–	–	75.0% (18)	100% (24)	–	100% (24)
CoV OC43/HKU1	–	–	92.6% (25)	100% (27)	48.1% (13)	59.3% (16)
CoV 229E/NL63	–	–	100% (17)	100% (17)	88.2% (15)	88.2% (15)
Enterovirus/rhinovirus	–	–	96.7% (172)	71.7% (127)	93.8% (167)	97.7% (174)

[a]Based on combination of INFA + H1 + H3 + H5.
[b]Based on combination of INFA + H1 + H3.

TABLE 4. Specificity of direct fluorescent antibody (DFA), culture and four multiplex assays for detection and identification of respiratory viruses

Target	DFA (%)	Culture (%)	Resplex II Panel v2.0 (%)	Seeplex RV15 (%)	xTAG® RVP (%)	xTAG® RVP Fast (%)
INFA	99.7	100	100	98.8	100	100
INFB	99.8	100	100	100	100	100
PIV (1–4)	99.8	100	100	99.0	99.6	97.6
hMPV	99.4	100	100	99.7	99.7	100
RSV (A/B)	99.6	100	100	97.7	100	100
ADV	100	100	99.9	98.1	99.9	100
BoV	–	–	100	100	–	99.6
CoV OC43/HKU1	–	–	100	99.3	99.9	100
CoV 229E/NL63	–	–	100	98.8	99.9	100
Entero/rhinovirus	–	–	99.3	99.1	96.0	99.8

assay according to our definition of a true and false positive (Tables 3 and 4). Some viral targets were lumped together as necessary to perform a sensitivity and specificity analysis (i.e. entero/rhinovirus, coronaviruses). Table 3 indicates that DFA and culture were, as expected, less sensitive than PCR for most targets. However, DFA was more sensitive than PCR for a small number of specific targets and assays (i.e. influenza B and parainfluenza 1–4 by RVP Fast and RSV A, B by Resplex, RVP and RVP Fast).

Seeplex RV15 demonstrated sensitivity of ≥96.9% for all targets except entero/rhinovirus (71.7%). Resplex II v2.0 had good sensitivity for influenza A and B (≥96.9%), but lower sensitivity in the detection of hMPV, PIV and RSV (82.0–84.0%). Resplex II v2.0 sensitivity fell further for ADV and BoV (71.4% and 75.0%, respectively). RVP showed high sensitivity for INFA/B and hMPV (≥98.4%), but lower sensitivity for PIV, ADV and RSV (85.4–88.2%), and lowest sensitivity for CoV OC43/HKU1 (48.1%). RVP Fast had good sensitivity for INFA, hMPV, RSV, BoV and entero/rhinoviruses, but demonstrated significantly reduced sensitivity

in the detection of ADV, CoV OC43/HKU1, INFB and PIV (52.4–65.8%).

The specificity of DFA and the multiplex kits was generally very high (Table 4), with all multiplex assays showing a specificity of >98% for all targets except for RSV A/B for Seegene (97.7%), entero/rhinovirus for RVP (96%) and parainfluenza virus 1–4 (97.6%) for RVP Fast.

Comparison of commercial assays for the detection of seasonal influenza A subtypes and pandemic H1N1 2009 influenza A virus

The Seeplex influenza A/B Subtyping and Resplex II Plus Panel PRE assays showed good sensitivity (95.9% and 91.8%, respectively) in detecting pandemic H1N1 2009 INFA compared with the Astra influenza Screen & Type assay (Table 5). Resplex II Plus Panel PRE and Seeplex influenza A/B Subtyping assays detected all 25 seasonal INFA-H1 and INFA-H3 positives, even though the Seeplex subtyping assay was unable to subtype two INFA-H1 and one INFA-H3 positive specimens. Fifty-five negative specimens remained negative in all assays.

TABLE 5. Performance of two commercial multiplex PCR assays vs. Astra Influenza Screen and Type assay for detection of the pandemic H1N1 2009 influenza A virus and seasonal influenza A strains

Target	Number	DFA # (% positive)	Astra influenza Screen & Type # (% positive)	Resplex II Plus Panel PRE[a] # (% positive)	Seeplex influenza A/B Subtyping # (% positive)
Pandemic H1N1 2009 INFA	50	32/46 (69.6%)	50 (100%)	46 (92%)	48 (96%)
INFA (seasonal H1 and H3)	25	14 (56%)	25 (100%)	25 (100%)	25 (100%)
Seasonal H1N1 INFA	13	4/10 (40%)	13 (100%)	13 (100%)	11 (84.6%)
Seasonal H3N2 INFA	12	10 (83.3%)	12 (100%)	12 (100%)	11 (91.7%)
Negative	55	0	0	0	0

[a]Resplex II Plus Panel PRE assay does not differentiate INFA subtypes (except for pandemic H1N1 2009 influenza A subtype).

Discussion

In a head to head comparison of four multiplex PCR assays with DFA and culture in children, multiplex PCR offered significantly improved sensitivity in the detection of the traditionally diagnosed respiratory viral agents (INFA, INFB, PIV (1–3), RSVA, RSVB, ADV and hMV), in addition to detecting coronaviruses, BoV, enteroviruses and rhinoviruses, which increased the overall positivity rate from 38.4% to 66.9%.

Among all the multiplex assays tested, Seeplex RV15 was the most sensitive for detecting all targets except for enteroviruses and rhinoviruses. All multiplex assays had good sensitivity for the detection of influenza A (93.7–98.4%). Influenza B sensitivity was good in all multiplex assays (100%) except for RVP Fast (64.9%). The superior performance of Seeplex RV15 for RSV (100% sensitivity) reflected strong performance for both RSVA and RSVB, whereas the decreased sensitivity of other assays reflected a weaker performance for either target (i.e. Resplex II v2.0: RSVA 90.4%, RSVB 79.3%, and RVP: RSVA 85.5%, RSVB 98.3%). Similarly, variability in the sensitivity of individual targets of the four parainfluenza viruses resulted in variation in the overall sensitivity. Again, Seeplex RV15 showed good sensitivity for all four types (85.7–100%), while Resplex II v2.0 had reduced sensitivity for PIV4 (60%), RVP had reduced sensitivity for PIV1 (71.4%) and 3 (71.4%), and RVP Fast had reduced sensitivity for PIV1 (46.7%), 2 (77.8%) and 3 (42.8%). Sensitivity for detecting hMPV was good for Seeplex RV15, RVP and RVP Fast (92.3–97.4%), and acceptable for Resplex II v2.0 (82%). However, performance for adenovirus, an important respiratory pathogen, was very variable, ranging from 52.4% (RVP Fast) to 100% (Seeplex RV15), probably reflecting the variation in serotype coverage among the assays.

Of the additional viral agents tested in the multiplex assays, the coronaviruses were consistently detected across all assays except for CoV OC43 by RVP (53.8%) and CoV HKU1 by RVP Fast (16.7%). Seeplex RV15 and RVP Fast detected 100% of bocavirus infections, while the sensitivity

of Resplex II v2.0 was only 75%. Detection of enterovirus and rhinovirus was the most inconsistent. Although the specific targets for each multiplex assay are proprietary, it is known that the highly conserved regions of the 5′NTR region of either rhinoviruses or enteroviruses, will also amplify members of the other genus. Thus, some assays, such as the RVP and RVP Fast assays, have combined the enterovirus and rhinovirus targets, because developing specific targets for each genus outside of the 5′NTR region may compromise sensitivity of detection, especially of the rhinoviruses. This is possibly the case with the Seeplex RV15 assay, which separates enteroviruses and rhinoviruses, but has a lower sensitivity than the other assays. Though the Resplex II v2.0 assay differentiates between enteroviruses and rhinoviruses, the occurrence of 38.4% of positive specimens testing positive for both targets, suggests that there may be cross-reactivity between them.

Specificity was excellent for all assays, using our composite reference standard. Without using individual single-plex assays to adjudicate the single test positives, we cannot determine whether the slightly lower specificity observed for a few targets in several assays was due to higher sensitivity of detection or detection of false positives.

Multiplexed respiratory panels provide clinicians with more diagnostic and treatment information for managing patients. In the case of influenza A, knowledge of the subtype is important with respect to predicting the activity of antiviral agents such as the adamantanes and neuraminidase inhibitors. In addition to increased sensitivity and number of viruses detected, multiplex assays permit the improved identification of cases of infection with multiple agents, which may be clinically significant, especially in immune compromised individuals. In our study, we found that two or more viruses were present in 10.9% of specimens (16.3% of positive specimens). Bocavirus and coronaviruses were the viruses most commonly associated with multiple agent infection, followed by human metapneumovirus, the parainfluenza viruses, adenovirus and the entero/rhinoviruses. Influenza A/B and RSV were the least likely to be detected in the

presence of another virus. The role of multiple viral agents in affecting the clinical course of disease is at present unknown and worthy of further study.

With respect to the technical performance of the different multiplex assays, the following issues were identified: Seeplex RV15 was designed as a two-step RT-PCR format necessitating a separate RT (cDNA) assay, though a new one-step procedure has been developed. It was the only assay that required three PCR master mixes with five targets in each one plus the internal control. It was also the only assay that incorporated positive controls for all 15 viral targets, which is considered an additional quality control feature of the assay. Seeplex RV15 was the assay with the shortest post-PCR step, especially for a small number of specimens when using the Lab 901 Screen Tape® system (maximum five specimens per run). In contrast, the Resplex II v2.0, RVP and RVP Fast use a 96-well microtitre plate format on the Luminex platform, permitting high throughput analysis.

Practical considerations in most laboratories regarding the feasibility and the direct and indirect costs of introducing multiplex molecular testing for respiratory viruses have led to a relatively slow routine implementation of this methodology. Mahony *et al.* [15] have shown that RVP employed as the first-line diagnostic tool in children was the least costly strategy, compared with DFA and culture, DFA alone or DFA plus RVP, when the prevalence of infection was ≥11%. The cost of molecular testing is offset by its more efficient use of labour than conventional DFA and culture and by savings to the healthcare system when additional testing and hospitalization can be avoided by knowledge of a test result with high sensitivity and specificity. Operationally, molecular methods also allow virology laboratories to continue to function, even in the event that viral culture cannot be carried out due to biosafety issues.

In our study, newer assays or versions of the multiplex assays (Resplex II Plus Panel PRE (21 targets) and Seeplex Influenza A/B Subtyping (six targets)) showed good sensitivity and specificity relative to a tri-plex influenza real-time RT-PCR (Astra influenza Screen & Type) for pandemic H1N1 2009 INFA virus detection. This is important, as traditional seasonal H1 subtyping molecular assays will not react with the pandemic strain and it is expected that the 2009 pandemic strain may become the predominant circulating seasonal H1 strain in the immediate post-pandemic period.

We have shown that multiplex PCR increases the sensitivity of detection of respiratory viruses in children by 74.3% over DFA and viral isolation, while maintaining excellent specificity. However, it will be important to develop more effective clinical and laboratory algorithms for their timely and optimal use and to study their impact on patient care in different populations in different clinical settings. Influenza, RSV, parainfluenza virus, adenovirus and hMPV have been well established as leading causes of respiratory infection among infants and children [16–18]. However, the role of rhinoviruses, enteroviruses, bocavirus and coronaviruses as co-pathogens in upper respiratory tract infection or as agents of lower respiratory tract infection, has been less well investigated, and will be aided by studies using this technology [19].

Acknowledgements

Transparency Declaration

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