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Short communication

Diagnostic yield and clinical impact of routine cell culture for respiratory viruses among children with a negative multiplex RT-PCR result

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ABSTRACT

Background: Polymerase chain reaction (PCR) is the reference standard for respiratory virus testing. However, cell culture may still have added value in identifying viruses not detected by PCR.

Objectives: We aimed to estimate the yield and clinical impact of routine respiratory virus culture among children with a negative PCR result.

Study design: A retrospective cohort study was performed from Jan. 2013 to Sept. 2015. Respiratory samples from hospitalized or immunocompromised patients < 18 years old were routinely inoculated on traditional tube cell culture monolayers if they tested negative by a PCR assay for 12 respiratory viruses. We studied patients with a respiratory specimen negative by PCR and positive by culture. Duplicates and samples of sold services were excluded. Data on demographics, clinical history, laboratory findings, and patient management were collected from patients' charts. Descriptive and multivariate statistics were performed.

Results: Overall, 4638 PCR-negative samples were inoculated in cell culture. Of those, 196 (4.2%) were cell culture positive, and 144 met study inclusion criteria. Most subjects (81.9%) were hospitalized. Mean age was 2.4 ± 3.4 years. The viruses most frequently isolated were cytomegalovirus (33.3%) and enteroviruses (19.4%). Cell culture results prompted a change in management in 5 patients (3.5%), all of whom had acyclovir initiated for localized HSV-1 infection. Four of these had skin or mucosal lesions that could be sampled to establish a diagnosis.

Conclusion: In children, routine viral culture on respiratory specimens that were negative by PCR has low yield and minimal clinical impact.

1. Background

Viral respiratory infection is a leading cause of hospitalizations, acute care visits, and antibiotic overuse in children [1]. The diagnosis is confirmed by identification of a respiratory virus in a respiratory specimen [2]. Traditional diagnostic methods include viral isolation by cell culture and detection of viral antigens by immunofluorescence. The superior sensitivity of molecular methods such as reverse-transcriptase polymerase chain reaction (RT-PCR) has led to their increased use in clinical laboratories [3,4]. Still, some authors suggest performing viral culture in a backup role in respiratory specimens from high-risk populations to detect viruses that are not part of the RT-PCR assay or whose genomes have mutations that may lead to false-negative RT-PCR results [5,6]. However, the implementation of a second diagnostic step,

i.e., viral culture after a negative RT-PCR result, is associated with additional costs and unknown effect on clinical management.

2. Objectives

We aimed to estimate the yield of routine respiratory virus culture among children with a negative PCR and to describe the impact of a positive cell culture result on their care.

3. Study design

We performed a single-center retrospective cohort study at the Montreal Children's Hospital of the McGill University Health Centre (Montreal, Quebec, Canada) from Jan. 2013 to Sept. 2015. The study

population included all patients < 18 years old with a viral culture performed on a respiratory specimen that had tested negative by multiplex PCR. Duplicate samples were excluded (> 1 viral culture for the same patient within 7 days or during the same illness episode).

All respiratory virus specimens submitted to the clinical virology laboratory were tested using a laboratory-developed multiplex real-time PCR assay for 12 viruses (Influenza A/B, Parainfluenza 1/2/3, RSV, Adenovirus, Coronavirus 229E/OC43, Human Metapneumovirus, Enterovirus, and Rhinovirus) [7,8]. The mean turnaround time for the PCR assay was ~9 h [8]. Respiratory samples from hospitalized or immunocompromised patients that tested negative by PCR were routinely inoculated on traditional tube cell culture monolayers (RMK, A-549, and MRC-5 cell lines; Quidel Corporation, San Diego, CA). Cell culture tubes were observed for cytopathic effect three times per week and incubated for a total of 16 days. Hemadsorption was performed in one of the two RMK tubes between days 6 and 8.

Patient charts were reviewed to collect data on patient demographics, clinical history, significant underlying chronic co-morbidities, and medical management.

Data were analyzed using descriptive statistics. In addition, clinical factors associated with the isolation of a respiratory virus (compared to other viruses) were assessed by logistic regression. Multivariate logistic regression model selection was guided by Akaike information criterion.

4. Results

During the study period, 4638 respiratory specimens were processed for viral culture (Fig. 1). Of those, 196 (4.2%) specimens were culture +/PCR- and 144 (3.1%) specimens were included in the analysis. Excluded specimens included 39 duplicate samples and 13 sold service specimens (medical record unavailable). Subjects were mostly male (63.2%) and 118 (81.9%) were hospitalized (Table 1). Sixty-two patients (43.1%) were previously healthy and without known comorbidities. The mean age was 2.35 ± 3.4 years. Most specimens were nasopharyngeal samples (95%). The working diagnoses that most frequently prompted respiratory virus testing were unspecified febrile illness (30.6%) and lower respiratory tract infection (24.3%). There were 14 specimens (9.7%) that were processed for viral culture without clear indication.

The mean turnaround time for a positive viral culture result was 9 days (Table 2). Viruses that were not part of the PCR assay comprised 53.5% of culture-positive specimens. The most frequently isolated viruses were cytomegalovirus (33.3%) and enteroviruses (19.4%).

In multivariate logistic regression analysis, factors independently associated with recovering a respiratory virus in cell culture (compared to other viruses) were a working diagnosis of upper or lower respiratory tract infection (adjusted odds ratio [aOR] 4.88, 95% CI 1.87–12.76), malignancy comorbidity (aOR 5.71, 95% CI 1.38–23.71) and lymphopenia (aOR 2.91, 95% CI 1.13–7.52).

Cell culture results prompted a change in management in only 5 patients (3.5% of positive cultures, 0.1% of all cultures done), all of
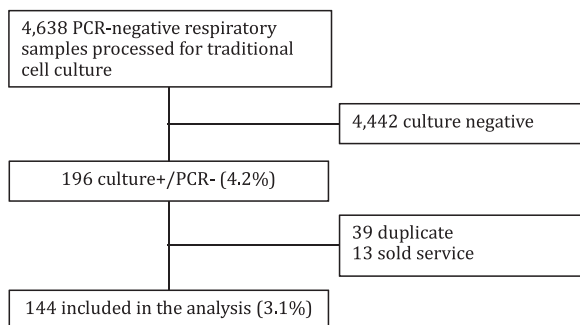
Table 1
Demographic and clinical description of children with a negative PCR result and a positive viral culture.

	n = 144
Gender	
Male	91 (63.2%)
Female	53 (36.8%)
Age (years)	
Mean ± SD	2.35 ± 3.42
Median (IQR)	0.92 (0.24–2.64)
Sample type	
Nasopharyngeal aspirate or swab	137 (95.14%)
Other (BAL and ETT aspirate)	7 (4.86%)
Setting specimen collected in	
Medical and surgical inpatient units	72 (50.0%)
Hematology-Oncology clinic or inpatient unit	6 (4.16%)
Intensive care units	29 (20.14%)
Emergency room	24 (16.67%)
Other*	13 (9.03%)
Admitted to hospital	118 (81.94%)
Comorbidities	
Healthy	62 (43.06%)
Malignancy	11 (7.64%)
Immunosuppressed state	8 (5.56%)
Preterm	14 (9.72%)
Sickle cell disease	2 (1.39%)
Neuromuscular disease	12 (8.33%)
Pulmonary disease	17 (11.81%)
Cardiovascular disease	15 (10.42%)
Renal disease	1 (0.69%)
Recurrent pneumonia	6 (4.17%)
Gastroesophageal reflux	9 (6.25%)
Other chronic diseases	22 (15.28%)
Working diagnosis	
Upper respiratory infection	23 (15.97%)
Lower respiratory infection	35 (24.31%)
Febrile neonate	12 (8.33%)
Unspecified febrile illness	44 (30.56%)
Meningitis	7 (4.86%)
Elective bronchoalveolar lavage	5 (3.47%)
Gastrointestinal infection	7 (4.86%)
Other	11 (7.64%)
Chest x-ray findings	n = 77
Normal chest x-ray	16 (20.78%)
Consolidation	18 (23.38%)
Pleural effusion	5 (6.49%)
Perihiliar thickening	38 (49.35%)
Air leak	1 (1.30%)
Atelectasis	14 (18.18%)
Hyperinflation	6 (7.79%)
Pulmonary edema	2 (2.60%)
Ground glass opacities	1 (1.30%)
Lymphocyte count (References [9,10])	n = 112
Low	54 (37.5%)
Normal	55 (38.19%)
High	3 (2.08%)
Neutrophil count (References [9,10])	n = 112
Low	12 (8.33%)
Normal	75 (52.08%)
High	25 (17.36%)

*Other: Day hospital, Day surgery, Respiratory clinic, Gastroenterology clinic, Infectious Diseases clinic.
SD = Standard deviation.
IQR = Interquartile range.

whom had acyclovir initiated for localized HSV-1 infection. Four of these had skin or mucosal lesions that could have been or were sampled to establish a diagnosis. One of the 5 patients was immunosuppressed (receiving cytotoxic chemotherapy), and none were neonates. Moreover, mumps, a reportable disease, was isolated in one patient in which mumps infection was not suspected. Communication with a patient's family regarding culture results was documented in 4 instances where management had not been otherwise affected. No instances of cessation of antibiotics, discharge from hospital or initiation of

Fig. 1. Flow diagram of patient samples.

Flow diagram:
- 4,638 PCR-negative respiratory samples processed for traditional cell culture
  - 4,442 culture negative
- 196 culture+/PCR- (4.2%)
  - 39 duplicate
  - 13 sold service
- 144 included in the analysis (3.1%)

Table 2
Viruses isolated and mean result turnaround time (TAT) for cell culture.

Viruses	*n* (%)	Mean TAT (days)
CMV	48 (33.3%)	8.0
Enteroviruses	28 (19.4%)	7.9
HSV-1	24 (16.7%)	5.7
Mumps	1 (0.7%)	6.0
Respiratory viruses*	43 (29.9%)	12.8

*Adenovirus (1), Influenza B (1), Parainfluenza virus 1 (1), Parainfluenza virus 2 (3), Parainfluenza virus 3 (15), Parainfluenza virus 4 (4), Rhinovirus (15), and RSV (3).

antivirals for influenza or other respiratory viruses were observed in association with positive viral culture results.

5. Discussion

Molecular testing for respiratory viruses is highly sensitive [2,11]. We observed that only 2% of PCR-negative pediatric respiratory samples were positive by cell culture for a virus in our RT-PCR panel. Moreover, positive cell culture results for any virus had little or no influence on clinical management.

The diagnostic yield of viral culture depends on several factors including the viral load, the quality of the respiratory specimen and cell lines, and the laboratory staff expertise [4]. A major drawback of traditional tube cell cultures is the long turnaround time, which can take between 2 days to 2 weeks to produce a result. For this reason, the clinical usefulness of the technique is guarded as the result may arrive too late to impact physician decision making [12]. In our study, only patients with HSV infection had their management changed due to viral culture. This could be explained in part by the shorter time to positivity for HSV and the availability of specific antiviral treatment (acyclovir). Another limitation of viral culture is that some respiratory viruses, e.g., group C rhinovirus, some group A coxsackieviruses, coronaviruses, and polyomaviruses KIV and WUV, neither replicate nor produce identifiable cytopathic effect in standard cell culture lines [13–15].

Viral culture has the advantage of isolating and identifying a wide range of viruses rather than detecting specific viral genetic targets by RT-PCR. This may be important in the immunocompromised and newborn populations where viral infections not typically included in respiratory PCR assays (CMV and HSV) may be of clinical importance [3,16]. Moreover, infections caused by viruses with genetic mutations may be falsely negative by PCR [3,6,17]. Viral culture may have additional advantages at the public health level for monitoring phenotypic antiviral susceptibilities, informing vaccine development, and identifying rare or novel strains. Because of the limitations and advantages of different diagnostic methods for respiratory viruses, a combination of methods is still recommended by some authors [5]. However, in our clinical laboratory, routine viral cultures from PCR-negative respiratory specimens had minimal impact on patient care.

While viral culture may rarely alter management, it can aid in identifying the causative viral agent in certain clinical scenarios. In our study, seven patients had a working diagnosis of meningitis; 3 of 7 grew enterovirus from the nasopharyngeal specimen (2 Echovirus and 1 Coxsackievirus B5). In those three patients without microbiological evidence of bacterial meningitis, one can infer that the likely cause of the aseptic meningitis was enterovirus.

To our knowledge, this is the first study to evaluate the impact of routine viral culture in respiratory specimens that were negative by multiplex PCR. This single-center study is limited by its retrospective nature; changes in patient management associated with viral culture results may not have been well documented in medical charts. Moreover, our findings may not be applicable to shell vial methods, which have a more rapid turnaround time compared to traditional culture techniques [18]. Nevertheless, over nearly three years, we observed that routine traditional viral cell culture on specimens negative

by multiplex PCR had very low yield and minimal clinical impact in the pediatric population. Our findings can aid microbiology labs to optimize the utilization of respiratory virus testing. We have since modified our laboratory practice to restrict cell culture to respiratory specimens from high-risk immunocompromised children or upon special request.

Funding

Competing interests

Ethical approval

Approved by the McGill University Health Centre pediatric research ethics board (study 15-380-MUHC).

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