Rapid Multiplex PCR Assay To Identify Respiratory Viral Pathogens: Moving Forward Diagnosing The Common Cold

Clifton P. Layman DO; Sarah M. Gordon MD; Diane U. Elegino-Steffens MD; Willie Agee PhD; Jason Barnhill PhD; and Gunther Hsue MD

Abstract

Upper respiratory tract infections (URIs) can be a serious burden to the healthcare system. The majority of URIs are viral in etiology, but definitive diagnosis can prove difficult due to frequently overlapping clinical presentations of viral and bacterial infections, and the variable sensitivity, and lengthy turn-around time of viral culture.

We tested new automated nested multiplex PCR technology, the FilmArray® system, in the TAMC department of clinical investigations, to determine the feasibility of replacing the standard viral culture with a rapid turn-around system. We conducted a feasibility study using a single-blinded comparison study, comparing PCR results with archived viral culture results from a convenience sample of cryopreserved archived nasopharyngeal swabs from acutely ill ED patients who presented with complaints of URI symptoms. A total of 61 archived samples were processed. Viral culture had previously identified 31 positive specimens from these samples. The automated nested multiplex PCR detected 38 positive samples. In total, PCR was 94.5% concordant with the previously positive viral culture results. However, PCR was only 63.4% concordant with the negative viral culture results, owing to PCR detection of 11 additional viral pathogens not recovered on viral culture. The average time to process a sample was 75 minutes. We determined that an automated nested multiplex PCR is a feasible alternative to viral culture in an acute clinical setting. We were able to detect at least 94.5% as many viral pathogens as viral culture is able to identify, with a faster turn-around time.

Introduction

Upper respiratory tract infections (URIs) in the population can have serious economic and public impact. URIs are the leading cause of acute illness in adults, and according to the CDC they are the leading cause of absence from work and school.[1] Each year in the United States, more than 30,000 deaths during flu season are attributed to URIs.[2]

Despite being one of the most common outpatient complaints in the United States and worldwide, pinpointing the etiology and developing an appropriate treatment plan is challenging in the clinical setting. Although the majority of URIs are viral in etiology, differentiating between the bacterial and viral etiologies can prove to be problematic because they often have an overlapping clinical presentation.[3] The overlapping presentation, combined with the genuine desire of the provider to "do something" and the frequent demand for treatment by patients and patient parents, has created a cultural expectation that can lead to the inappropriate use of antibiotics and drive up antibiotic resistance rates.[4]

A major obstacle to fast and accurate etiological diagnosis is the variable sensitivity and response time of the current method for identification of viral etiologies in URIs.[5] The current "gold standard" is viral culture, which can be a cumbersome and lengthy process. At our facility, viral identification begins with limited direct fluorescent antibody assay and viral inoculation into culture media. Some viruses require special conditions for growth, including special media, temperatures, and preparation, which requires additional technician workload and processing. Additionally, the time from media inoculation to viral growth is extremely variable, ranging from 3-14 days for most respiratory pathogens. The time and personnel constraints on viral culture prove to be large economic burdens to facilities such as ours, which runs about 2500 samples annually, the majority of which occur during flu season.

In recent years, advances in PCR methods and techniques have been harnessed in the laboratory to aid in the rapid detection of respiratory pathogens from patient specimens. PCR can differentiate and identify an expanded range of viral and bacterial targets.[6] Additionally, there are multiplex PCR modalities available with the ability to detect multiple targets in a single reaction.[7] However, traditional PCR does come with limitations. In the past, PCR has either been limited in scope of pathogenic targets or by the complexity of the test when targeting more than one pathogen.[8] Additionally, with traditional PCR there is a high risk of contamination due to the process itself, in some cases requiring specialized training and facilities. The risk of contamination and the complexity of the process, often leads to difficulty distinguishing amplification products and traditional multiplex PCR methodologies.

Recently, our facility had the opportunity to try an updated PCR platform, the FilmArray® system. In 2011, the FDA cleared the FilmArray® system, an automated nested multiplex PCR, with a sealed pouch that lowers the risk for contamination. It can run a patient sample from a nasopharyngeal swab in approximately one hour, testing for up to 20 of the most common viral and bacterial URI pathogens (see table 1).

Methods

We evaluated the feasibility of using the FilmArray® PCR in place of viral culture at Tripler Army Medical Center using a

Table 1. Viral and bacterial PCR targets

FilmArray® Automated Nested Multiplex PCR Viral and Bacterial Targets	
Viral Targets (includes multiple subtypes)	Bacterial Targets
• Adenovirus • Bocavirus • Coronavirus • Metapneumovirus • Rhinovirus • Enterovirus • Influenza A • Influenza B • Parainfluenza virus • Respiratory syncytial virus	• *Bordetella pertussis* • *Mycoplasma pneumonia* • *Chlamydophila pneumoniae*

single-blinded comparison study, comparing FilmArray® PCR results with archived viral culture results from a convenience sample of cryopreserved archived nasopharyngeal swabs from a study population. The study population consisted of acutely ill emergency department patients who presented to Tripler Army Medical Center, a tertiary care center, with complaints of URI symptoms.

Unique identification numbers were assigned to each cryopreserved sample. The FilmArray® operators were blinded to the known viral culture results of each sample. Each sample was processed individually by FilmArray® operators. Only after completion of the study were the FilmArray® operators unblinded and allowed to compare the FilmArray® PCR results to the previously known viral culture results for each sample.

Results

A total of 61 archived nasopharyngeal samples were processed by the FilmArray® operators. Of those 61 samples, viral culture had previously identified 31 positive samples and 30 negative samples. The FilmArray® system identified 38 positive samples, and 23 negative samples. Additional testing proved 100% reproducibility among FilmArray® operators. Of note, average time from receipt of a sample to the result was less than 75 minutes. Previously positive viral culture results had shown an array of respiratory viruses including adenovirus, cytomegalovirus (CMV), enterovirus, influenza A and B, metapneumovirus, parainfluenza virus, and respiratory syncytial virus (RSV). The FilmArray® failed to detect 1 out of 4 parainfluenza virus samples. In two other samples, one culture-identified adenovirus and one culture-identified enterovirus, the FilmArray® PCR correctly identified the same pathogen detected by viral culture, but also detected an additional pathogen. Of the 30 negative viral culture samples, the FilmArray® PCR detected 11 additional pathogens.

Overall, the FilmArray® has a 94.5% concordance with previously positive viral culture results when identifying viral targets that are expected to be detected (excluding CMV, which is not a FilmArray® target). If CMV is included in the analysis, there is an 87.1% concordance with previously positive viral culture results. There is only a 63.4% concordance between FilmArray® PCR and previously negative viral culture results.

Table 2. Comparison of Viral Culture and PCR results

Pathogen	Viral Culture	PCR concordance [additional pathogen]	PCR discordance
Adenovirus	4	3 [1]	1
CMV*	2	0 [1]	2
Enterovirus	4	4 [1]	0
Influenza A	6	6	0
Influenza B	2	2	0
Metapneumovirus	5	5	0
Parainfluenza	5	4	1
RSV	3	3	0
Negative	30	19	11

*CMV is not a target for FilmArray®

Table 3. PCR concordance with viral culture

Viral Culture	FilmArray® PCR
Positive [Excluding CMV]	94.5% Concordance
Positive [Including CMV]	87.1% Concordance
Negative	63.4% Concordance (11 additional pathogens detected)

Discussion

Our study demonstrated that the FilmArray® automated nested multiplex PCR system is at least 94.5% as accurate as viral culture when identifying viral targets that are expected to be detected. It is our conclusion that the FilmArray® device is feasible to use in an acute clinical setting, with reproducible results. The implications of being able to identify 95% of intended viral targets in as little as 75 minutes as compared with 3-14 days are exciting. The FilmArray® device has potential to replace viral culture in an acute clinical setting due to its ease of use and rapid turnaround time. Rapid tests have the potential to directly affect clinical management in real-time[9], which may improve antibiotic stewardship and aid in local and national epidemiological surveillance.[10] Limitations of this study include the generally discordant results between culture-negative samples and the FilmArray® system, which may be a result of the enhanced ability of FilmArray® PCR to detect additional respiratory pathogens not recovered in viral culture. Because viral culture is widely accepted to have variable sensitivity, the FilmArray® should be validated in the future against other molecular based testing modalities. Other considerations for future research should be directed at whether real-time results will actually affect clinical management, and a cost-benefit analysis surrounding PCR in relation to cost effectiveness for the patient, the facility, and the payer source.

Disclaimer: The views expressed in this abstract/manuscript are those of the author(s) and do not reflect the official policy or position of the Department of the Army, Department of Defense, or the U.S. Government.

Conflict of Interest

None of the authors identify any conflict of interest.

Authors' Affiliation:
- Tripler Army Medical Center, Honolulu, HI

Correspondence to:
CPT Clifton 'Kip' Layman DO; Tripler Army Medical Center,
Internal Medicine, PGY3, 1 Jarrett White Rd., Honolulu, HI 96859;
Ph: (808) 433-4251; Email: clifton.p.layman@us.army.mil

References

1. Cherry DK, Hing E, Woodwell DA, Rechtsteiner EA. National Ambulatory Medical Care Survey: 2006 summary. *National health statistics reports.* Aug 6 2008(3):1-39.
2. (CDC) CfDCaP. Estimates of Deaths Associated with Seasonal Influenza --- United States, 1976--2007. MMWR. Morbidity and Mortality Weekly Reports. 2010, August 27; http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5933a1.htm.
3. Dasaraju PV, Liu C. Infections of the Respiratory System. In: Baron S, ed. Medical Microbiology. 4th ed. Galveston (TX)1996.
4. Yates RR. New intervention strategies for reducing antibiotic resistance. *Chest.* Mar 1999;115(3Suppl):24S-27S.
5. Falsey AR, Walsh EE. Viral pneumonia in older adults. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* Feb 15 2006;42(4):518-524.
6. Endimiani A, Hujer KM, Hujer AM, et al. Are we ready for novel detection methods to treat respiratory pathogens in hospital-acquired pneumonia? *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* May 2011;52 Suppl 4:S373-383.
7. Choi SM, Kim SH, Kim HJ, et al. Multiplex PCR for the detection of genes encoding aminoglycoside modifying enzymes and methicillin resistance among Staphylococcus species. *Journal of Korean Medical Science.* Oct 2003;18(5):631-636.
8. Nolte FS. Molecular diagnostics for detection of bacterial and viral pathogens in community-acquired pneumonia. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America.* Dec 1 2008;47 Suppl 3:S123-126.
9. Barenfanger J, Drake C, Leon N, Mueller T, Troutt T. Clinical and financial benefits of rapid detection of respiratory viruses: an outcomes study. *Journal of Clinical Microbiology.* Aug 2000;38(8):2824-2828.
10. Petric M, Comanor L, Petti CA. Role of the laboratory in diagnosis of influenza during seasonal epidemics and potential pandemics. *The Journal of Infectious Diseases.* Nov 1 2006;194 Suppl2:S98-110.