MOLECULAR DETECTION OF ADENOVIRUS IN RESPIRATORY SPECIMENS USING THE PROADENO™ ASSAY

M.J. Bankowski 1, C. Ying 1, L. Tanioka 1, T. Koyamatsu 1, and W. Kim 1,2

1Diagnostic Laboratory Services, Inc. (The Queen’s Medical Center), Aiea, HI and the 2Pathology Department, John A. Burns School of Medicine and the University of Hawaii at Manoa, Honolulu, HI

ABSTRACT

Background
There are 53 recognized serotypes of human adenoviruses contributing to upper and lower respiratory tract infections, acute respiratory disease, hemorrhagic cysts, conjunctivitis, and disseminated disease. A high-performance, rapid molecular assay will serve as a valuable laboratory test in supporting adenovirus disease. The objective of this study was to establish the clinical performance (e.g. Sensitivity, Specificity, and reproducibility) of the ProAdeno™ Assay in respiratory specimens compared to viral culture with IFA staining as the predicate device. The ProAdeno™ Assay detects, but does not differentiate, serotypes 1-61.

Materials and Methods
Specimens (n=307) were prospectively collected between November 2009 to April 2010 from symptomatic patients submitting specimens for routine respiratory virus testing (i.e. Sfeb_visit and IFA testing for Flu A/B, RSV, PV 1-3, and human adenovirus [hAd]). Specimens consisted of nasopharyngeal swabs in viral transport media (MTM) from larynx samples with adequate volume. Molecular testing was performed using the Roche MagnaPlex QC System and the Total Nucleic Acid Isolation kit and the ProAdeno™ Assay (Prodase, Inc./GenProbe) with the Cepheid SmartCycler II. The ProAdeno Assay is based upon the Taqman Real Time assay chemistry. Positive and discordant samples were tested using bidirectional sequencing to determine the genotype. Reproducibility testing consisted of 123 specimens using hAd 3 and 31 and negative samples at various concentrations and replicate testing configurations.

Results
Viral cultures (n=302) resulted in 2 positive adenovirus, 10 influenza type A, 12 parainfluenza type 1, 1 paramyxovirus type 3, 2 RS virus, and one respiratory syncytial virus. The ProAdeno™ Assay detected 16 adenovirus, 14 influenza A, 8 influenza B, 12 parainfluenza, 1 paramyxovirus, 1 respiratory syncytial virus, and 1 adenovirus. The ProAdeno™ Assay reproducibility testing (n=123) revealed a high agreement value (222123 or 99.9%).

Conclusions
The clinical performance of the ProAdeno™ Assay compared to culture was very favorable. This multiplex, real-time PCR qualitative assay detected 50% more adenoviruses than culture. However, additional adenovirus positive samples are necessary to confirm this finding. The ProAdeno™ Assay is required less time to report than culture and showed a high sensitivity (100%), specificity (100%) and reproducibility (99.2%) following dissacntant analysis.

MATERIALS AND METHODS

Specimens and Controls
Study samples were selected in a standardized fashion and included all of the first 15-20 nasopharyngeal specimens in 4 transport media received per shift/day (n=302, 5 dissatisfied). In addition, a verification panel consisting of 18 blinded “samples” split into 3 sets of 6 samples was tested on three separate occasions by the “processing” (technicians) before initiation of the study. This panel was included to ensure that all of the reagents and systems were working properly and the staff was “protocol competent.”

Specimen Processing and Nucleic Acid Extraction
The Roche MagnaPlex QC System with software version 3.0.11 and the Total Nucleic Acid Isolation kit.

ProAdeno Realtime Assay
The ProAdeno™ Real time assay (Prodase, Inc./GenProbe) was performed using the Cepheid SmartCycler II instrument with Ex software version 1.70. An internal control supplied by the manufacturer was added to each sample prior to extraction. The procedure was performed exactly according to the clinical trial protocol instructions.

Reproducibility Testing
Reproducibility was assessed using a panel of 12 simulated clinical samples that included two adenovirus serotypes (hAd 3, 31) at a medium and low positive levels (near the assay limit of detection). 99% positive and two high negative samples (negative at 1.0xLOD, negative at 6.0xLOD). Panels and controls were tested at each site by two operators for 5 days (12 samples and 3 controls X 2 operators X 5 days X 3 sites = 450).

Culture
The reference method was rapid culture using viral culture with IFA stained viral (i.e. R-Mix, Shell Wat, D3 Ultra kit, Diagnostic Hybrids, Inc.)

RESULTS

Table 1: Clinical Trial ProAdeno vs Culture

<table>
<thead>
<tr>
<th>Culture</th>
<th>Positive</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ProAdeno</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Assay</td>
<td>0</td>
<td>289</td>
<td>289</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>302</td>
<td>302</td>
</tr>
</tbody>
</table>

*Adenovirus [1], influenza type A [2], influenzavirus type B [3], parainfluenza type 1 [4], parainfluenza type 2 [5], parainfluenza type 3 [6], parainfluenza type 4 [7], and a combination of 302 and parainfluenza type 3 [8].

Sensitivity = 100%, Specificity = 99.3%

Discussion
Patients were tested from October 2009 to August 2010. Nasopharyngeal swabs specimens were used for this study, which were collected for routine respiratory virus testing for culture, which consisted of the patient population consisted of 49% male and 51% female, with a median age of 1.4 years. The CT values for culture positive and negative were 23.4, 27.5 and 37.2, 31.6 respectively. Even though the adenosin isolated number was low, these values indicate that the lower viral burden specimens were not detected in culture compared to real-time PCR.

Conclusions
The ProAdeno™ Assay is a multiplex, real-time PCR qualitative assay that can detect more adenovirus than culture (i.e. 4 vs 2 respectively).

The ProAdeno™ Assay requires less time to reporting than culture (i.e. Hours vs. Days respectively).

The ProAdeno™ Assay performance for this clinical trial site revealed a high sensitivity (100%) and specificity (100%) following dissacntant analysis (Low # of positives).

The test reproducibility was very high at 99.9% (122123).

The test performance for the ProAdeno Assay was acceptable and suitable for consideration in the laboratory.

REFERENCES