EVALUATION OF A COMMERCIAL QUANTITATIVE REAL-TIME PCR ASSAY FOR ADENOVIRUS IN PLASMA SPECIMENS

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ABSTRACT

Background:
Adenoviruses (AdV) cause a wide range of illnesses, from mild respiratory infections in young children to life-threatening multi-organ disease in people with a weakened immune system including transplant recipients. The implementation of a quantitative real-time PCR assay to monitor AdV viral load is expected to improve prognosis and management of AdV infection in transplant patients.
Materials and Methods:
Plasma specimens were separated from whole blood collected in EDTA containing tubes drawn from transplant patients and stored at -70°C until analysis. Additional viral isolates from nasal specimens were obtained from North Shore-Long Island Jewish Health System Laboratories and spiked into plasma. AdV Reference Material: AcroMetrix Adenovirus Plasma Panel containing specific concentrations of Adenovirus (Type 3) in a human plasma matrix ($1x10^3$ to $1x10^7$ copies/mL) was obtained from Life Technologies and utilized in determining the limit of quantification. Specimens were extracted on the QIAcube and quantified using the Altona Diagnostics RealStar® Adenovirus PCR Research Use Only Kit on the Rotor-Gene Q instrument.
Results:
Viral loads for 44 clinical specimens, 22 of which were reported positive, were determined using the Altona real-time PCR kit and compared to values obtained by a reference laboratory. A good correlation between observed and expected values (± approximately 0.5log) was obtained over at least 6 logs of detection with a coefficient of determination (R2 value) of 0.98 when compared to the reference laboratory. For the AdV-positive reported samples viral load ranged in concentration from 273 to 1,437,688,203 copies/mL (mean= 77,645,149 and median= 11,982), and was on average within 0.17 log with the Altona real-time PCR assay. The assay was linear (R^2=1) over 4 logs of detection (2.7-7.0 log10 copies/ml) using a commercially available AdV quantification panel and assay precision ranged from approximately 1% to 3% CV. The specificity was 100% with all 22 samples having viral loads below the limit of quantification of 200 copies/mL.
Conclusions:
The Altona Diagnostics RealStar® Adenovirus real-time PCR assay permitted rapid, sensitive and specific detection and quantification of Adenovirus and may be useful in monitoring the course of infection in transplant patients.

MATERIALS AND METHODS

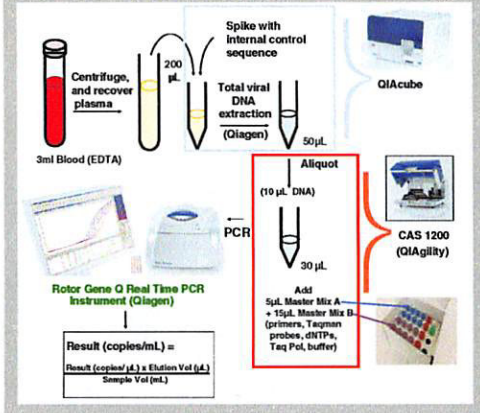
Plasma aliquots of whole blood specimens and viral isolates from nasal specimens spiked into plasma for Adenovirus viral load analysis were collected and stored frozen at -70°C, for up to 6 months before automated DNA extraction using the QIAcube and subsequent Altona Diagnostics RealStar AdV PCR analysis could be performed.

The results obtained with the Altona assay were compared with the results obtained by a reference laboratory. Commercially available AdV controls were obtained from Life Technologies and stored frozen at -70°C until extraction and analysis.

QIAcube DNA Isolation from 200 µl plasma was performed using the QIAamp DNA Extraction Kit. A QIAcube custom protocol program was created with assistance from the QIAGEN Application Group to match the manual extraction steps featuring addition of an Internal Extraction Control DNA to every sample.

The QIAcube extracted sample was tested in the Altona AdV assay by real-time PCR quantification of AdV. The Altona AdV assay was performed in the same tube for target and internal control in a volume of 30 µL.

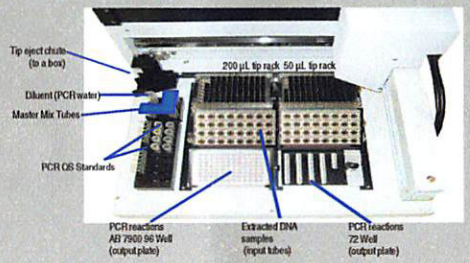
Adenovirus Assay Workflow



RESULTS

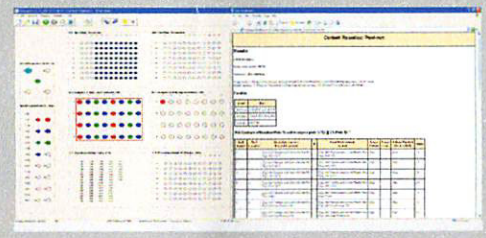
CAS-1200 (QIAgility) PCR Setup

Open tubes containing PCR water, AdV Master Mix, QS standards, and extracted samples with 10ul excess (dead) volume were added to the Master Mix, Reagent, and Sample Blocks, as defined in the corresponding banks of the Robotics-4 Setup program along with Filter Pipet Tips.



Setup Software

A Robotics-4 setup program was created for the Altona Diagnostics Adv assay and the program was modified daily according to varying numbers of samples in the run. The assay was set up using a 72 well block (shown above) and 100µL PCR tubes. PCR setup success was confirmed by the Post-Run Report (below).

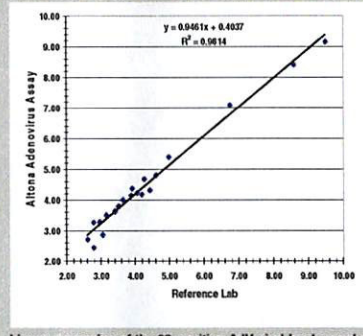


Qualitative Result Summary

There was 100% qualitative agreement between the Altona Diagnostics AdV assay and the reference lab (44 of 44 samples). 22 of the 44 plasma specimens were identified as AdV DNA negative by both the Altona assay and the Reference lab assay.

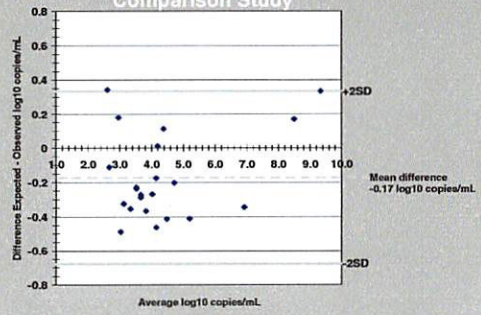
	Reference Lab	
Altona	Positive	Negative
Positive	22	0
Negative	0	22

Correlation Study



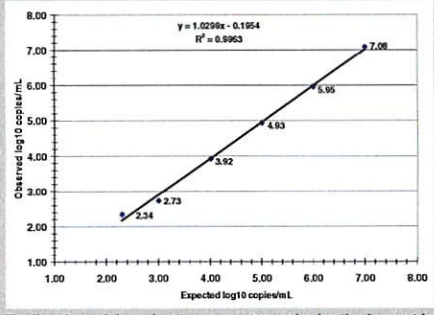
Linear regression of the 22 positive AdV viral load samples tested in parallel yielded a coefficient of determination (R^2 value) of 0.98 with the reference lab assay indicating a high degree of accuracy throughout the assay range.

Comparison Study



22 patient specimens for AdV viral load testing were analyzed by the Altona Diagnostics and reference lab assay and the results compared in a Bland and Altman plot shown above. The average difference was within approximately two fold (on average less than 0.2 log), indicating good agreement with the reference lab method. All results were within 0.5 log of the reference lab method.

Linearity and Dynamic Range



The linearity and dynamic range were measured using the Acrometrix Adenovirus panel. The linearity was determined by plotting the log of the mean measured titers against the log of the mean input titers. The results demonstrate a linear response over four logs of detection with an R^2 value=0.99.

Linearity Panel Results

Nominal Concentration	Measured Concentration $\bar{X}_2$	Log_{10} Difference Expected-Observed
7.00	7.08	0.08
6.00	5.95	0.05
5.00	4.93	0.07
4.00	3.92	0.08
3.00	2.73	0.27

Quantitative results obtained using the Acrometrix AdV panel across the linear range were within 0.3 log of the expected values.

Precision

Mean Quantity $\bar{X}_3$ log_{10} Copies/mL	S.D. (log_{10} Copies/mL)	% C.V.
7.00	0.19	2.7
5.93	0.08	1.3
4.92	0.04	0.8
3.91	0.04	0.9
2.72	0.07	2.5

Standard deviation (SD) and %CV were calculated using Acrometrix AdV panel samples measured on three different days. The Altona AdV assay exhibits good reproducibility throughout the linear range of detection with low inter-assay variation.

Inclusivity

Panel Member	Adenovirus Type	Panel Concentration	Measured Concentration $\bar{X}_2$	Log_{10} Difference Expected-Observed
ADV12-05	1	4.12	4.32	-0.20
ADV12-06	4	4.25	4.32	-0.07
ADV12-01	34	3.98	4.42	-0.44

A QCMD proficiency panel containing AdV types 1, 4, and 34 was analyzed with the Altona AdV assay. The Altona Adv assay results were within 0.5 log of the survey results, similar to the results obtained with Acrometrix AdV type 3 plasma panel (see preceding Linearity Panel Results table).

Specificity

Virus	Viral Concentration	AdV Detected	Internal Control
Human herpesvirus 1 (HSV-1)	1,300,000 copies/mL	Negative	Positive
Human herpesvirus 2 (HSV-2)	1,400,000 copies/mL	Negative	Positive
Human herpesvirus 3 (VZV)	1,100,000 copies/mL	Negative	Positive
Human herpesvirus 4 (EBV)	1,000,000 copies/mL	Negative	Positive
Human herpesvirus 5 (CMV)	3,000,000 I.U./mL	Negative	Positive
Human herpesvirus 6	471,816 copies/mL	Negative	Positive
Human herpesvirus 7	445,000,000 copies/mL	Negative	Positive
Human herpesvirus 8	8,500,000 copies/mL	Negative	Positive
Hepatitis B virus	5,000,000 I.U./mL	Negative	Positive
Hepatitis C virus	5,000,000 I.U./mL	Negative	Positive
Human Immunodeficiency virus	516,417 copies/mL	Negative	Positive
BK virus	5,000,000 copies/mL	Negative	Positive
JC virus	13,000,000 copies/mL	Negative	Positive

Specificity was examined by testing other pathogens potentially found in human plasma. The pathogens listed did not generate any reactivity (FAM signal) for AdV DNA using the Altona AdV assay. The presence of the internal control DNA (JOE signal) confirms that a target nucleic acid is detected. In addition, 22 samples negative by the reference laboratory, have also been confirmed negative by the Altona AdV assay.

SUMMARY

- The Altona Diagnostics RealStar AdV assay of plasma specimens from transplant patients and viral isolates from nasal specimens spiked into plasma correlated well with the reference lab.
- Bland and Altman plots demonstrated that 55% of the positive samples agreed within 0.3log, while 100% agreed within 0.5 log.
- The Altona AdV assay was linear over a range of at least 4 logs from approximately 2.3 to 7log_{10} copies/mL, with a limit of quantitation of 2.3 log_{10} copies/mL. The AdV assay precision ranged from 1 to 3% CV from approximately 2.7 to 7 log_{10} copies/mL.
- The QIAcube greatly reduces the hands-on time for performing the Altona AdV assay by automating the nucleic acid extraction steps.
  QIAcube hands-on time :
  - Instrument maintenance ~5 min.
  - Instrument setup ~15 min.
- The QIAgility (CAS-1200) automates the PCR setup of the Rotor-Gene 0.1ml PCR tubes.
- Instrument run time:
  - Extraction of 12 samples ~ 1 hour (QIAcube).
  - Real-time amplification and detection (Typically 24 samples) ~2 hours Rotor-Gene Q (72 tube rotor option)