Sunday-05

Comparison of Three Adenovirus Quantitative PCR Assays with ATCC Reference Strains and Clinical Samples

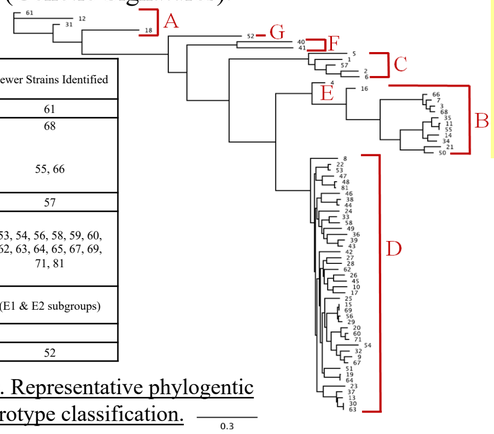
[1]Kimberly Starr, [2]Richard Smith, [2]Rohan Baker, [2]Douglas Millar, [1]Alex Greninger, [1]Keith Jerome, [1]Linda Cook

[1]Microbiology and Virology Divisions, Department of Laboratory Medicine, University of Washington Medical Center, Seattle, Washington

[2]Genetic Signatures, Newtown, Australia

Background

Adenoviruses (AdVs) have been associated with a wide variety of human diseases and are recognized as pathogens causing significant morbidity and mortality in immunocompromised or transplant patients. Quantification of AdV DNA in plasma is useful for monitoring patients at risk for invasive AdV disease. There is a paucity of quantitative reference material and no WHO standard available to harmonize quantitative data so results between labs vary widely. Also, sequences are highly divergent between the currently defined 71 human Adenovirus (HAdV) strains making primer design difficult and HAdV strains are still being identified. In this study we compared an in-house, multiplex PCR assay with primers and probes specific for each subgroup (A-G) (Octaplex) to one with degenerate primer and probe bases (Jothikumar) and one utilizing bisulfite pre-treatment of DNA to reduce variation prior to amplification allowing for a single primer set and probe (Genetic Signatures).



Adenovirus Type	Previously Identified Strain #	Newer Strains Identified
A	12, 18, 31	61
B	B1: 7, 16, 21 B2: 11, 14, 34, 35 B3: 3 Unclassed: 50, B72, B79	68 55, 66
C	1, 2, 5, 6	57
D	8, 9, 10, 13, 15, 17, 19, 20, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 33, 36, 37, 38, 39, 42, 43, 44, 45, 46, 47, 48, 49, 51,	53, 54, 56, 58, 59, 60, 62, 63, 64, 65, 67, 69, 71, 81
E	4	(E1 & E2 subgroups)
F	40, 41	
G	52	

Table 1. Adenovirus Types and strains. Representative phylogentic tree showing grouping of AdVs and serotype classification.

Methods

Samples – All available ATCC Adenovirus strains (types 1-51), 69 previous positive patient samples, 8 samples from QCMD proficiency surveys, the ATCC Adenovirus quantitative reference material, and the AcroMetrix AdV quantitative panel. Extractions for the Octaplex and Jothikumar assays were on the Roche MagnaPure 96 and on the GS-mini for the Genetic Signatures assay.

PCR Assays – We compared our new and improved multiplex assay (Octaplex) to a degenerate primer/probe assay utilizing a low annealing temperature (Jothikumar) and a new "3base™" assay (Genetic Signatures) that employs bisulfite to convert cytosine into thymine in the sample, allowing for the use of a single primer set and probe while greatly reducing the need for degenerate bases.

UW Octaplex Assay Design – We aligned >400 HAdv genomes, including sequences for types 52-71 which are not available from ATCC. Our existing multiplex assay primer sets had multiple mismatches to some aligned sequences with the potential to prevent amplification. To improve the assay, we added new primer sets for D, E and G types, and modified sequences for the B and C primer sets, resulting in an 8 primer/probe sets assay.

Controls- We used commercial reference materials ATCC VR-1516 and AcroMetrix Plasma Panel for generating standard curves/quantification.

ATCC VR-1516 reference material

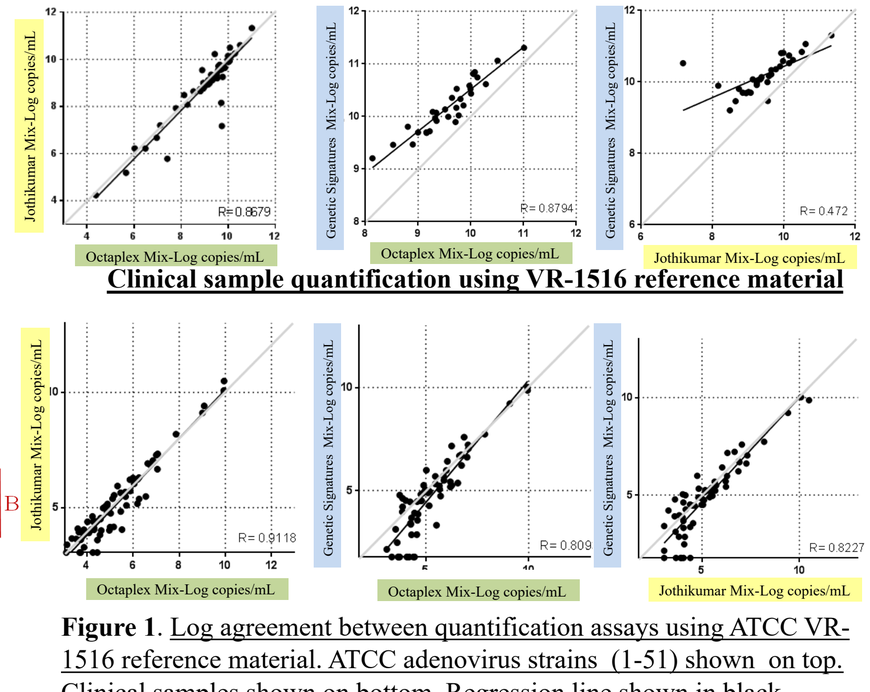


Figure 1. Log agreement between quantification assays using ATCC VR-1516 reference material. ATCC adenovirus strains (1-51) shown on top. Clinical samples shown on bottom. Regression line shown in black.

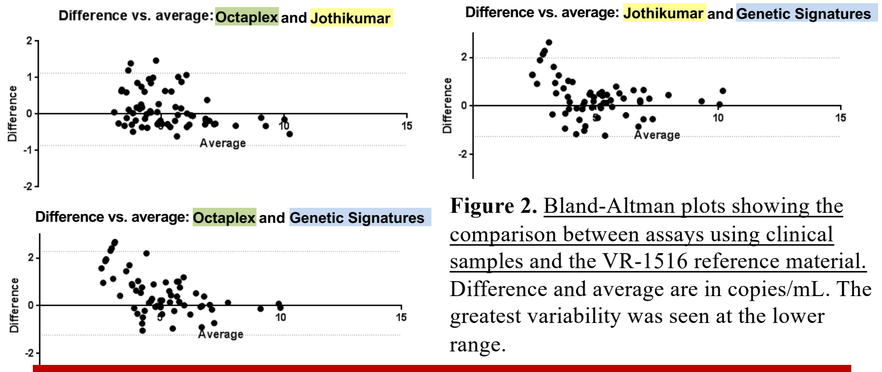


Figure 2. Bland-Altman plots showing the comparison between assays using clinical samples and the VR-1516 reference material. Difference and average are in copies/mL. The greatest variability was seen at the lower range.

AcroMetrix quantitative panel

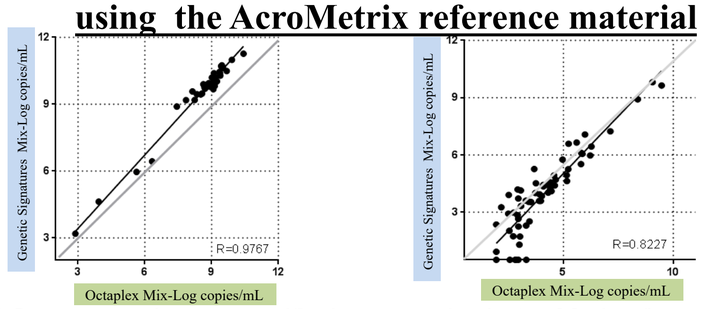


Figure 3. Log agreement between quantification assays using the AcroMetrix reference material. ATCC adenovirus strains (1-51) shown on left. Clinical samples shown on right. Regression line in black. The Jothikumar assay did not efficiently amplify this reference material.

Proficiency Testing Results

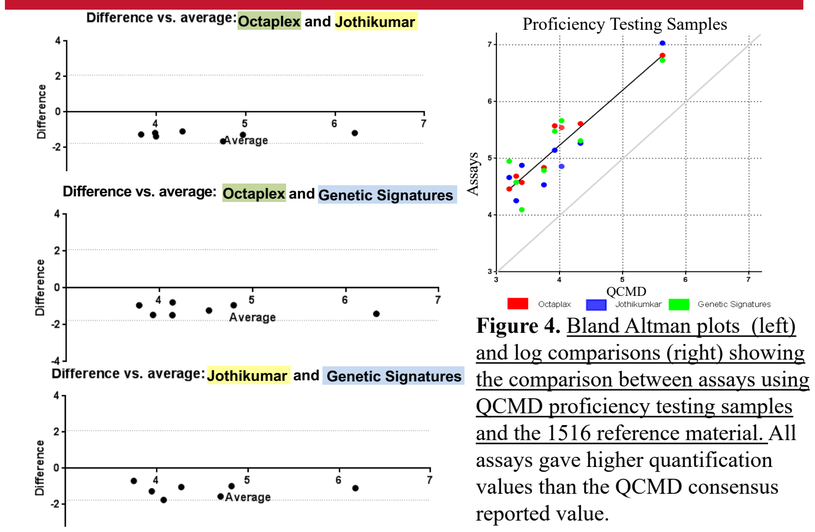


Figure 4. Bland Altman plots (left) and log comparisons (right) showing the comparison between assays using QCMD proficiency testing samples and the 1516 reference material. All assays gave higher quantification values than the QCMD consensus reported value.

Jothikumar Assay Failures – Strains 3, 34, 41

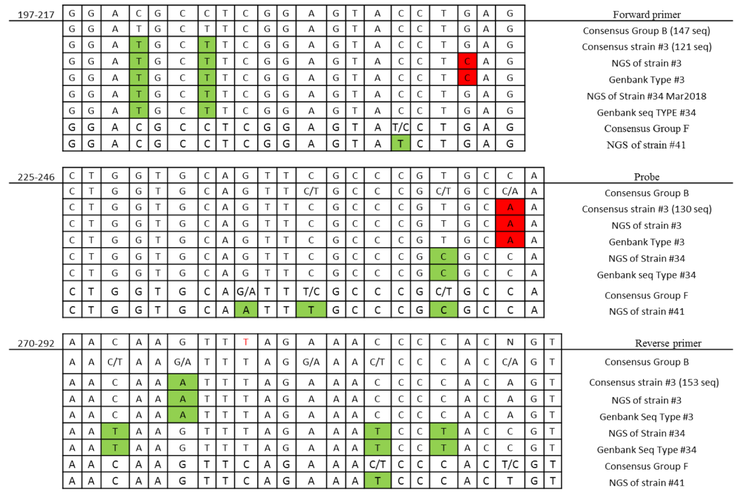


Figure 5. Sequence comparison for ADV strains that gave partial failures with the Jothikumar assay compared to the octaplex assay. ADV strain #3 amplified 10 cycles later in the Jothikumar assay. ADV #34 and #41 amplified 7 cycles later in the Jothikumar assay. Mismatches present in the 3' region of both the forward primer and probe may account for the late amplification for ADV #3, while 2 additional C/T mismatches in the reverse primer may account for late amplification in ADV #34. Three mismatches in the probe may be responsible for partial failure of ADV #41.

Conclusions

The new and improved UW assay (Octaplex) performed slightly better on low-titered clinical samples than the other two assays which were somewhat less sensitive and had subsets of samples that did not amplify. The Jothikumar assay failed to efficiently amplify 3 of the high-titered cultured strains whereas the Genetic Signatures 3base™ assay resulted in higher copies/mL for all culture fluids tested, suggesting that either the PCR was more efficient or the GS-mini extraction was better for culture fluids. The 3base™, single primer set/probe assay shows promise and is available as an analyte specific reagent (ASR) in the USA.