

ABSTRACT

TITLE: Densitometric analysis of point-of-care adenoviral chromatographic immunoassays (AdenoPlus™) in individuals with acute conjunctivitis

Purpose: The AdenoPlus™ test is a point-of-care rapid chromatographic immunoassay for the qualitative detection of adenoviral conjunctivitis (Ad-Cs). It yields a bivariate “positive/negative” result for adenovirus antigen detection based on the presence or absence of a red visible line in the test’s result window. As a procedural control, a blue line will appear in the control window to indicate validity of the test. In this analysis, we quantify positive AdenoPlus™ test line intensities of individuals with conjunctivitis and correlate positive test line intensities to PCR-determined adenoviral titers.

Methods: Patients with presumed Ad-Cs, age ≥ 18 and symptom onset ≤ 4 days were screened at 9 centers for eligibility for inclusion in the Reducing Adenoviral Patient Infected Days (RAPID) study. The AdenoPlus™ test was performed on anesthetized eyes of 212 participants by clinical examiners. 142 of the AdenoPlus™ tests were photographed by examiners within 4 hours of the visit and mean pixel brightness intensity of the test result lines in these images were quantified in Photoshop (Adobe, San Jose, CA) by two masked graders. The densitometry ratio of the red test line to the blue control line was calculated for each test, with a value of 1.0 signifying that the red line had the same pixel intensity as the blue line. Conjunctival swab samples were analyzed using an adenovirus-specific primer set and an Integrated qPCR Cyclor (DiaSorin Molecular, Cypress CA).

Results: The median densitometry ratio was 0.85 (25%=0.70; 75%=1.43) for positive Ad-Cs immunoassays that were confirmed by PCR to be Ad-Cs (n=26). This was not significantly different ($P=1.0$) than for positive Ad-Cs immunoassays (n=16) that were determined negative for Ad-Cs by PCR (median=0.84; 25%=0.67; 75%=0.92). Densitometry ratios for both of these groups were significantly ($P<0.05$) higher than those for the tests (n=82) determined to be negative for Ad-Cs by the immunoassay test and by PCR (median=0.68; 25%=0.51; 75%=0.82). There was a significant positive correlation ($P=0.002$; $R^2=0.32$) between the densitometry ratios and adenoviral titers in the group that was positive for Ad-Cs for both the immunoassay and PCR.

Conclusions: Although brighter intensities of the red test line on the AdenoPlus™ correlated to higher PCR adenoviral titers in tears, there was no significant difference in the line intensities between the true positive and false positive groups. These results highlight the challenge for clinicians in identifying true Ad-Cs at the initial visit, even with the use of point-of-care chromatographic immunoassay testing.