Adenovirus has been implicated in diseases affecting the respiratory, ocular, and gastrointestinal systems. 1,2

Adenovirus is a frequent cause of infectious conjunctivitis. Human Adenoviruses are classified into 6 subgroups and 53 serotypes. 3,4 Approximately one third of the human Adenovirus serotypes have been associated with common forms of Adenovirus related eye infections but the most common causes of acute conjunctivitis are related to serotypes 3, 4, 8, 11, 19 and 37. The serotypes have the following associations: serotypes 8, 19, and 37 are associated with pharyngitis; serotypes 1-3 and 11 are associated with epidermal keratoconjunctivitis; 5,6 serotypes 3, 4, 5, and 7 tend to cause pharyngeal-conjunctival fever, which usually affects children,7,8 serotypes 1-11 and 19 are the primary cause of nonspecific follicular conjunctivitis. 9,10,11 However, the other serotypes can also produce clinically indistinguishable episodes of acute follicular conjunctivitis. 1,12

Cell culture in combination with immunofluorescence is the historical "gold standard" for identifying Adenovirus in conjunctival specimens. 13,14 Virus isolation requires an intensive process, technical expertise and may take up to 3 weeks to complete. The polymerase chain reaction (PCR) is increasingly used in place of cell culture to detect Adenovirus. 15,16 In addition, the differential diagnosis of various forms of conjunctivitis (viral, bacterial, allergy) is often difficult because they manifest similar symptoms.

PRINCIPLES OF THE PROCEDURE

AdenoPlus utilizes Direct Sampling Microfiltration technology. Adenoviral antigen, the conserved Adenovirus hexon protein, when present in the patient sample is captured between two antigen specific monoclonal antibodies. One antibody is immobilized in the detection zone of the device. The second antibody is labeled with colloidal gold. The detector is a disposable, rapid test requiring 10 minutes for a result.

REAGENTS AND MATERIALS

Materials Provided

The AdenoPlus test kit includes two foil pouches containing the following materials and a buffer vial:

- Sample Collector (A)
- Test Cassette (B)
- Absorbent Tip (C)
- Protective Cap (D)
- Result Window (E)
- Sample Transfer Window (F)
- Test Cassette Body (G)

The sample collector (A) is a separately packaged sterile component that can be assembled easily onto the test cassette (B). Additionally, the test cassette (B) guarantees correct sample transfer onto the lateral flow assay strip.

Materials Recommended but Not Provided:

- Timer
- Gloves
- Quality Control materials (see section on external controls)

TEST PROCEDURE

I. PREPARING THE TEST

1. Check the expiration date on all packaging. Make sure there is no damage to the foil pouches. Do not use if foil pouches are damaged.
2. Tear open each foil pouch at the indicated perforation and remove the contents. Remove the protective cap (D) from the test cassette body (D). Do not touch the sterile sampling fleece (C) prior to collecting the patient sample.

II. TAKING A SAMPLE

1. Locate the sampling fleece (C) on the underside of the sample collector (A).
2. If ocular architecture is applied to the eye, wait at least 5 minutes prior to collecting a sample. Gently lever the patient’s eyelids to expose the inside of the lower lid (palpebral conjunctiva).
3. Gently dab and drag the sampling fleece (C) in multiple locations along the palpebral conjunctiva 6-8 times and then allow it to rest against the conjunctiva for an additional 5 seconds. This will moisten the sampling fleece.

NOTE: Do not discard this package insert.

The presence of both a BLUE line in the control zone and a RED line in the result zone indicates a positive result. An uneven or indistinct RED line is due to an uneven distribution of eye fluid on the sampling fleece (C). Even if the RED line is faint in color, it must be interpreted as positive. A positive result indicates the presence of Adenovirus antigens in the tear fluid.

If the test runs and the reagents work, a blue line will appear in the control zone. This is indicative of the functionality of the test.

If the control line does not appear, the test must be interpreted as invalid and has to be repeated by resampling the eye using a new AdenoPlus test kit. A purple fluid wave is observed moving across the result window (H) while the test is running. Once a streaky-fluid wave in the background after 10 minutes, allow an additional 5-10 minutes of running time prior to interpretation. The clearing of the background color from the result window (H) is a negative backround control.

IV. RUNNING THE TEST

NOTE: The sample should be collected, the test assembled, and buffer applied within one (1) hour of opening the test cassette.

1. Open the buffer vial. Do not allow any portion of the test cassette to touch the absorbent tip or the buffer vial.
2. Immerse the absorbent tip (C) into the buffer vial for a minimum of 20 seconds.
3. Remove the absorbent tip (E) from the buffer vial and place the absorbent tip (E) in the buffer vial for an additional 10 seconds. If a BLUE line still does not appear after 10 minutes, the test must be discarded and the subject retested by resampling the eye using a new AdenoPlus test kit. Do not repeat invalid test result your patient. Although the test requires only 10 minutes of total running time, repeat samples may reveal reduced eye fluid available for collecting an adequate sample. Each additional sampling may reduce the Adenoviral antigen load transferred to the test. The test should always be performed on the eye that is more severely affected.

If both eyes are equally affected, it is recommended that the second sample be taken from the other eye. If only one eye is affected, the sample may be repeated 30 minutes later.

If the test runs and the reagents work, a blue line will appear in the control zone. This is indicative of the functionality of the test.

If the control line does not appear, the test must be interpreted as invalid and has to be repeated by resampling the eye using a new AdenoPlus test kit. A purple fluid wave is observed moving across the result window (H) while the test is running. Once a streaky-fluid wave in the background after 10 minutes, allow an additional 5-10 minutes of running time prior to interpretation. The clearing of the background color from the result window (H) is a negative backround control.

QUALITY CONTROL

AdenoPlus has built-in procedural controls (see below). For daily quality control, RPS recommends documenting that these in-tensional and external controls were checked for the first sample tested each day.

Procedural Controls

An unused AdenoPlus device has a purple fluid wave observed moving across the sample transfer window (G). The unused device also has faint orange lines in the result window (H).

NOTE: Do not discard this package insert. There is only one package insert per dispenser box. Additional copies of this package insert can be found at: RPSdetectors.com

AdenoPlus is a rapid immunoassay test for the visual, qualitative in vitro detection of Adenoviral antigens (hexon protein) directly from human eye fluid. The test is intended for professional use as an aid in the rapid differential diagnosis of acute conjunctivitis.

Negative results do not exclude Adenovirus infection nor are they intended to rule out other microbial-caused infections of the conjunctiva, and should not be used as the sole basis for treatment or other management decisions. Store between 77ºF/25ºC and 39ºF/4ºC. Do not use AdenoPlus past the expiration date.

PREREQUISITE

Read this package insert completely before performing a test. Failure to follow the instructions or modification to the test system instructions will result in the test no longer meeting the requirements for waived classification.

SUMMARY AND EXPLANATION OF THE TEST

Morphologically, Adenoviruses are non-enveloped DNA viruses with an icosahedral structure about 80 nm in diameter. 2
The performance of this test has not in conjunction with other clinical information, particularly in the case of weak test lines that may not rule out false negative results.

3. Inadequate specimen collection or transport in the specimen zone and may not result in a true negative result.

Any problems with the device should be checked RPS at 1.941.556.1850 before testing patients.

INTERFERING SUBSTANCES
A prospective, multicenter, masked, time-blinded, clinical trial was performed in order to evaluate the performance of AdenoPlus when used by operators in clinical settings. The following agreement was observed between AdenoPlus and viral cell culture.

Sensitivity: 90% (28/31) 95% CI (74.9-98.0)
Specificity: 96% (97/99) 95% CI (89.8-98.9)

The following table summarizes the results when tested by untrained users is presented below.

N=189

<table>
<thead>
<tr>
<th>Sample</th>
<th>Cell Culture</th>
<th>AdenoPlus</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=189</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

LIMITATIONS OF THE TEST
All human adenoviruses serotypes contain the hexon protein that is detected by AdenoPlus. The antibody target a conserved domain of the hexon protein universal to all adenoviruses serotypes. In the laboratory, RPS tested serotypes 1, 4, 5, 7, 8, 11, 13, 14, 15, 31, and 37 and confirmed a positive antibody-antigen reaction. The AdenoPlus detection limit was determined by serial dilutions of the adenovirus hexon protein and found to be 6 ng/ml or 60 pg per test is estimated to be equivalent to 40-50 Adenoviruses.

Cross Reactivities
Various infectious pathogens generated in cell culture and important for conjunctivitis were applied in the laboratory to determine potential cross-reactivities with AdenoPlus:
- Adenovirus Type 1A
- Adenovirus Type 1B
- Adenovirus Type 2
- Adenovirus Type 3
- Adenovirus Type 4
- Adenovirus Type 5
- Parainfluenza Type 1
- Parainfluenza Type 3
- Pseudomonas aeruginosa
- Streptococcus pneumoniae
- Staphylococcus aureus
- Staphylococcus epidermidis
- Human lactoferrin

The specificity of the test was tested with positive, partially positive, and negative controls consisting of inactivated adenovirus in human tears and human tears pre-exposed with positive and negative tests.

The table below depicts the positive and negative agreement of AdenoPlus with known positive and negative external controls, when tested by untrained operators in 3 clinical sites.

No external control

Positive Percent Agreement 97% (120/124) 95% CI (90.9-99.0)
Negative Percent Agreement 99% (98/81) 95% CI (93.3-98.5)

There were no invalid results.

Study Near the Array Cut-off: This study evaluated the performance of the AdenoPlus test with weak reactive samples when used by untrained operators at 3 CLIA-waived sites. Twelve (12) untrained intended users were required to assemble, interpret and test interpret results from 12 unknown samples. The samples were collected in tear matrix spiked with adenovirus hexon protein and consisted of 60 weak positives (at the limit of detection (LOD) or assay cutoff) and 60 weak negatives. The results for each clinical site, the samples were blinded, randomized and tested. The agreement of testing with the external controls, the results when tested by untrained users is presented below.

Sample

<table>
<thead>
<tr>
<th>Agreement with expected result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weak Positive* (at LOD)</td>
</tr>
<tr>
<td>Weak Positive* (below LOD)</td>
</tr>
</tbody>
</table>

*The expected results for "Weak Positive" samples are "Positive," while the expected results for "Weak Negative" samples are "Negative."

There were no invalid results.

flex studies were conducted. Using risk analysis as a guide, the results were used to develop a clinical decision algorithm which defined the acceptable range of results when tested by untrained operators are presented below.

Sample

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<td>Weak Positive* (at LOD)</td>
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</table>

*The expected results for "Weak Positive" samples are "Positive," while the expected results for "Weak Negative" samples are "Negative."

There were no invalid results.


ORDERING INFORMATION

Ordering Information

RPS-AD – AdenoPlus

RPS-AD – AdenoPlus

NIC-AD – AdenoPlus (Canada only)

At-Home Detection Kit

AdenoPlus – External Controls

Contact Information and Technical Support

Manufacturier and United States Representative

RPS-AD – AdenoPlus

Reagent Development, Inc.

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www.mt-procons.com

U.S. patents 5,614,773 and 7,272,124, international patents, and other patents pending. www.mt-procons.com

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