INTENDED USE
SAS™ Adeno Test is a membrane-based immunogold assay for the detection of adenovirus and adenovirus antigens. The test is a rapid visual test for the qualitative detection of adenovirus serotypes present in eye swabs, nasal and pharyngeal secretions, fecal samples, and cell culture supernatant. This test is for professional use only.

SUMMARY AND EXPLANATION OF THE TEST
Adenovirus has six subgenera and 49 serotype based on DNA sequencing and biological and biochemical properties (1, 3, 4). Morphologically, the adenoviruses are nonenveloped icosahedral structures about 80 nm in diameter (4). Adenovirus has been implicated in diseases affecting the respiratory, the ocular and the gastrointestinal systems (1-3). The monoclonal antibody in the SAS™ Adeno Test is against the group-reactive antigen of human adenovirus.

Conjunctivitis caused by adenovirus is frequently seen in ocular infections. Several studies have confirmed that severe conjunctivitis such as keratoconjunctivitis (EKC), pharyngoconjunctival fever (PCF) and nonspecific follicular conjunctivitis (NFC) are caused predominantly by serotype 3, 4, 6, 11, 19, and 37 in Japan (4).

Adenovirus is also a common cause of upper respiratory tract infections (URT). These infections are manifested in the form of common colds, pharyngitis, or tonsillitis and occur mostly in infants and young children (3). A notable feature of these infections serotypes is the persistence of virus in a latent state in the adenoidal and tonsillar tissues in about 50% of infected children. Another important feature of the infection of this virus is the excretion of virus in the stool for several months without recurrence of symptoms (3).

The gold standard for identifying Adenovirus in conjunctival specimens is culture or electron microscopy (7, 8). Other tests are also available such as immunofluorescence, enzyme immunoassay, and PCR (4, 7, 8). To perform any of these tests, it takes between several hours and a week, in addition, there is a need for sophisticated instruments to obtain the results.

URT and ocular infections frequently manifest similar symptoms of a bacterial infections (6), thus, rapid confirmation of viral infections in patients, often saves on unnecessary antibiotics prescriptions. The SAS™ Adeno Test can be used for the direct testing of eye swab samples, nasal and pharyngeal secretions, fecal samples, and cell culture supernatant, reducing the times required by traditional cell culture isolation.

PRINCIPLE OF THE TEST
The immunochromatographic test utilizes a pair of Adenovirus-specific monoclonal antibodies. An extract is first prepared by suspension of the specimen in the protein extraction buffer solution. The buffer of the extract is placed on the devices well. The reaction between a positive sample and the colored particle-conjugated antibody will form a complex that migrates along the membrane. An immobilized capture antibody will form a colored line at the S (specimen) area upon reacting with the colored complex. An internal control line C (control) area is built in to assure that the test has been carried out correctly.

MATERIALS AND REAGENTS PROVIDED
1. Test devices contain a test strip with a monoclonal anti-adenovirus, colored conjugate, and polyclonal anti-adenovirus, colored conjugate, and polyclonal coated on a membrane. The monoclonal antibody is affinity purified and is specific to the hexon group of the virus.
2. Tubes containing extraction buffer – Buffer contains 0.1% sodium azide
3. Disposable sample transfer pipettes
4. Package insert

MATERIALS NOT PROVIDED
1. Sterile specimen collections swabs
2. Precision micropipette and micropipette tips to deliver 500 µl (optional)
3. Vortex or centrifuge
4. Timer
5. Adenovirus positive control
6. Adenovirus negative control

PRECAUTIONS
1. For in-vitro diagnostic use only.
2. The test device should remain in the sealed pouch until ready for use.
3. Do not mouth pipette samples.
4. Do not smoke, eat or drink in areas where specimens or kit components are handled.
5. All specimens, reagents, and controls should be considered potentially hazardous and handled in the same manner as an infectious agent.
6. Wear disposable gloves while handling samples and wash hands after the assay is complete. Warning: The user should refer to the relevant section of the CDC-NIH manual “Biosafety in Microbiology and Biomedical Laboratories,” 3rd Edition, 1984.
7. Avoid any contact with the eyes, broken skin, or mucous membranes.
8. Avoid splashing or the generation of aerosols.
9. The test device and all materials should be discarded in a proper biohazard container after testing.

STORAGE INSTRUCTIONS
The test kit is to be stored at room temperature (15° - 30°C) for the duration of the shelf life. The test device must remain in the sealed pouch until ready for use.

SPECIMEN HANDLING
Proper sample collection is critical for the isolation and detection of adenovirus. Adenovirus has been recovered from many organ systems; however, it is commonly isolated from respiratory, ophthalmic or rectal samples. The area used for sample collection should be carefully and thoroughly swabbed to insure the best results. If there is a need to culture the collected sample, the swab should be placed in minimum volume of viral transport medium (approximately 1.0 ml). Alternatively, 2-3 ml of nasopharyngeal secretions, aspirates, or washes (in sterile saline) can be collected. In the case of rectal swabs, in order to assure a sufficient rectal sample, the specimen, should be between 40-50mg/swab.

Any samples that are put in viral transport medium should be placed on ice and vortexed properly before testing. Do not freeze samples, unless a delay in testing is expected. In this case, quickly freeze samples using dry ice, and keep sample frozen at -20°C or colder until ready for testing. Tissue cultures should be grown according to guidelines, then samples prepared as described in the sample preparation section. Prior to culturing any sample, it is important that the sample be treated with antibiotics prior to culturing.

SPECIMEN COLLECTION & PREPARATION
Specimens for virus isolation should be collected as soon as possible after the onset of symptoms, preferably within 7 to 10 days. Proper specimen collection is critical for the detection of adenovirus and should only be attempted by experienced personnel. Do not centrifuge specimens as this may remove cellular material and adversely affect test results.
Eye Swabs:
Using a sterile swab, wipe the lower palpebral conjunctiva. Swabs must be extracted using SAS™ extraction buffer provided. Swirl the swab well in the extraction buffer-containing tube provided. Rub the swab thoroughly against the wall of the tube.

2. Nasopharyngeal or Tonsilopharyngeal swabs: A sterile swab is inserted into one or both nostrils to the nasopharyngeal area. The swab is allowed to remain in the nostril for a few seconds to absorb secretions, rotated gently, and then withdrawn. A separate swab used for each nostril may increase the specimen volume. Alternatively, rub the tonsils and the posterior pharynx thoroughly with a sterile swab. Swabs must be extracted in SAS™ extraction buffer. Swirl the swab well in the extraction buffer-containing tube provided. Rub the swab thoroughly against the wall of the tube.

3. Cell Culture Specimens: Grow the cell culture according to the manufacturers guidelines. Aspirate 500 ul of the supernatant fluid for testing. Add sample to the tube containing SAS™ extraction buffer. Shake the mixture well or vortex the tube. Some culture media may contain stabilizers, detergents and animal sera that may adversely affect test results. To qualify cell culture media, seed the media with known positive and negative organisms and test.

4. Fecal Samples: It is recommended that the specimen be collected during the acute phase of gastroenteritis, because a large number of viral particles and viral antigens are excreted during this period. A sample can be collected from a soiled diaper of young children and neonates or an adult stool sample. Alternately, rectal swabs may be used. When using rectal swabs, care should be taken to ensure that a sufficient sample (40-50 mg) is obtained. Both louse and solid stools may be used. Approximately 40-50 mg of raw stool should be collected and added to the extraction buffer. Rub the swab meticulously against the inner wall of the extraction buffer. For best results, vortex the sample, then allow the coarse particles to settle before applying the sample to the test.

TEST PROCEDURE
Allow the pouch (test device), specimen and/or controls to reach room temperature (15° - 30°C) prior to testing. Extract the specimen (eye swabs or samples) using the provided SAS™ extraction buffer. Rub the swab carefully against the tube containing extraction buffer.

1. Remove the test device from the protective pouch and place it on a flat surface. Label the device with expiration date and/or control identifications.
2. Using the sample transfer pipettes provided, dispense 4 drops (approximately 150µl) of the specimen into the round sample well (see illustration below). Wait for colored lines to appear.
3. Read results within 15 minutes. Some positive results may be observed in as short as 30 seconds depending on the concentration of antigen. Do not interpret results after 15 minutes.

INTERPRETATION OF RESULTS

EXPECTED VALUES
The prevalence of adenovirus infection will vary based on many factors such as infection category, geographic location, method of sample collection, sample handling and transportation, and the general health environment of the patient population under study. Normal healthy individuals tested should be negative for adenovirus. Some infected individuals may show symptoms or only minor symptoms, and these patients may test negative.

"The frequency of adenovirus infections will vary with the clinical syndrome and age of the individual. Approximately 5% of acute respiratory disease in children under the age of 5 is due to adenovirus (9). Enteric adenoviruses (types 40 and 41) have been implicated in approximately 10% of pediatric patients suffering from gastroenteritis, and appears most frequently in children under 2 years old (15). Approximately 10% of childhood pneumonia may be of adenovirus etiology (14)."

"Adenovirus has at times been implicated in cervicitis (11) and in acute respiratory disease (12) in adults. Occular infections such as epidemic keratoconjunctivitis due to adenovirus can occur in any age group (13). In a Japanese study of 1105 patients of various ages with viral conjunctivitis, 536 (49%) were determined to be caused by adenovirus. Similarly, studies in three East Asian cities found that 70% of epidemic kerato-conjunctivitis cases were caused by adenovirus (15). Our clinical studies produced similar results, in which conjunctivitis in 178 out of 292 patients tested (61.0%), was confirmed via PCR to be caused by adenovirus."

PERFORMANCE CHARACTERISTICS
The SAS™ Adeno Test was tested in laboratories, clinics, and hospitals in the United States, Japan, and France for tissue culture confirmation, and for the direct testing of stool samples, eye swabs, and nasopharyngeal swabs. Tissue culture analysis was performed in collaboration with the CDC and to Meridian Premier Adenovirus®; Stool samples were confirmed by EM and results compared to Orion Diarex® Rota-Adeno; eye swabs were confirmed by PCR
Serial two-fold dilutions of each virus suspension were assayed and the following results were obtained:

<table>
<thead>
<tr>
<th>Site IV Japan</th>
<th>Nasopharyngeal Swabs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenovirus Serotype</td>
<td>Protein Concentration (µg/ml)</td>
</tr>
<tr>
<td>Ad3</td>
<td>1.400</td>
</tr>
<tr>
<td>Ad4</td>
<td>0.600</td>
</tr>
<tr>
<td>Ad12</td>
<td>0.300</td>
</tr>
<tr>
<td>Ad17</td>
<td>0.150</td>
</tr>
<tr>
<td>Ad19</td>
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<tr>
<td>Ad27</td>
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</table>

**ASSAY PRECISION**

**Intra-Assay**

Two samples, one positive and one negative, were tested twenty times by three technicians. In each test, the positive sample produced a positive result, and the negative sample produced a negative result.

**Inter-Assay**

Positive and negative samples were run using test devices from different lots of SAS™ Adeno Test. In each test, the positive sample produced a positive result and the negative sample produced a negative result.

**BIBLIOGRAPHY**


**LIMITS OF DETECTION**

A study was performed at a University School of Medicine in Japan to measure the detection limits of the SAS™ Adeno Test.

**Note:** Please be advised that "relative" refers to the comparison of this assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgement can be made on the comparison assay's accuracy to predict disease.