

SAS™ Adeno Test

A Rapid Test for the Qualitative Detection
of Various Adenovirus Serotypes
Present in Eye Swabs, Nasopharyngeal Secretions,
Fecal Samples and Cell Cultures.

For *In-Vitro* Diagnostic Use
Store at 15° to 30°C

For Technical Assistance Call 800-272-2710
Outside the USA Call 210-699-8800



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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, 8, 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 provided extraction buffer solution. The buffer containing the extracted specimen is then added to the devices sample 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 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.

10. Do not use kit or materials beyond expiration date.
11. Do not mix or interchange lots of SAS™ Adeno Test devices or reagents.
12. Extraction buffer contains sodium azide, which may react with lead and copper plumbing to form explosive metal azides. Azide build-up may be avoided by flushing drains with large volumes of water after disposal.
13. Avoid microbial contamination of reagents or incorrect results may occur. Contamination of samples could cause false results.
14. Use aseptic technique and sterile equipment for all tissue culture procedures.
15. Use separate pipettes or pipette tips for each sample, control and reagent.
16. Do not reuse test devices or kit materials.

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 loose 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 tube containing 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

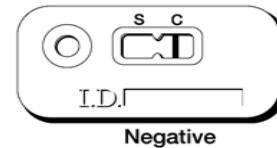
Allow the pouch (test device), specimen and/or controls to reach room temperature (15° - 30°C) prior to testing. Swabs or samples must be extracted using the provided SAS™ extraction buffer. Rub the swab carefully against the tube containing extraction buffer.

1. Remove the test device from the protective pouch and place it on a flat surface. Label the device with patient 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

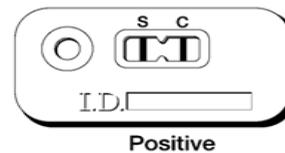
Negative Results

The test is negative if only one colored line appears in the C (control) area.



Positive results

The test is positive if two colored lines appear. One colored line will appear in the S (specimen) area and one in the C (control) area. Any colored line in the S area should be considered positive. Colored lines may be lighter or darker than each other.



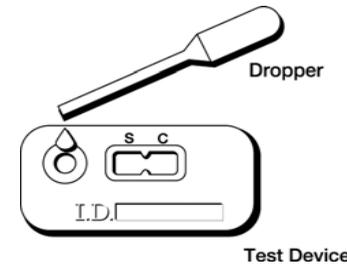
Invalid Results

The test is invalid if no colored line appears in the C (control) area even if a colored line appears in the S (specimen) area. If this occurs, add 1 to 2 additional drops of sample and wait for 5 minutes. If a colored line does not appear in the C area, the test is invalid and should be repeated. Colored lines that appear after 15 minutes are not diagnostic and should be ignored.

QUALITY CONTROL

Each test device includes a built in procedural control. Correct procedural technique and test device performance is confirmed when a colored line appears in the C (control) area of the device. The procedural control line should appear in the C area with all sample types (eye swabs,

nasopharyngeal secretions, fecal samples, and cell culture supernatant), whether the sample is tested is positive or negative. Warning: The procedural control does not test for the presence or absence of adenovirus.



An adenovirus positive tissue culture of a purified adenovirus antigen can be used as a control.

The control line contains an anti-mouse antibody that captures the colored conjugate antibody. If the line should fail to appear in the C area of the device, the test should be repeated (see the "Invalid Results" section). If the line in the C area still does not appear, contact our Technical Support Department at (210) 699-8800. It is recommended that when a new shipment of product is received, negative and positive controls for adenovirus should be tested and the appropriate results obtained. (See NCCLS C24-A for guidance on appropriate quality control practices.)

LIMITATIONS OF THE PROCEDURE

1. SAS™ Adeno Test is highly sensitive and specific for adenovirus antigen. The monoclonal antibody in this test reacts with the group specific hexon antigen. It will detect all known serotypes, but cannot be used to differentiate types.
2. The test is highly dependant on the collection and transportation of clinical specimen. Care should be taken to adhere to proper procedures.
3. A negative result does not exclude the possibility of adenovirus infection in the patient. False negative results may occur due to low concentration levels of the adenovirus antigen below the sensitivity level of the test, improper sampling or handling of the specimen, failure of the cell culture, etc.
4. Test results depend on the level of antigen in clinical specimens and may not correlate with cell cultures.
5. Adenovirus may be found in both solid and loose stools. Our data was obtained using both stool types.
6. All positive results should be interpreted with caution, since adenovirus is capable of latency and recrudescence. Asymptomatic shedding may occur up to 18 months after infection. Enteric adenovirus may be found in the stools of asymptomatic children.

Test results should be interpreted in conjunction with information available from epidemiological studies or clinical evaluations of patient or other diagnostic procedures.

7. Co-infection with bacterial pathogens is possible. Therefore, bacteriological tests should be performed in parallel with this test to rule out bacteriological etiology.
8. False positives could occur with high levels of *S. aureus* possessing Protein A.
9. Certain transport media containing gelatin may interfere with the test.

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 (10). 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. Ocular 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 samples were CPE formation in cultures and to Meridian Premier Adenoclone®; Stool samples were confirmed by EM and results compared to Orion Diarlex™ Rota-Adeno; eye swabs were confirmed by PCR

and the results compared to Meridian Premier Adenoclone®; and nasopharyngeal swabs were confirmed by PCR. Results are summarized in the following tables:

Site I U.S. Tissue Culture					
Comparison	CPE Formation		Meridian Adenoclone®		
SAS™ Adeno Test	+	-	+	-	
	91	0	13	0	
Performance Statistic	Value	95% C.I.	Value	95% C.I.	
	100%	75.3%-100%	100%	75.3%-100%	
Sensitivity	100%	88.1%-100%	100%	88.1%-100%	
	100%	91.6%-100%	100%	91.6%-100%	
Specificity	100%	88.1%-100%	100%	88.1%-100%	
	100%	91.6%-100%	100%	91.6%-100%	
Agreement	100%	91.6%-100%	100%	91.6%-100%	
	100%	91.6%-100%	100%	91.6%-100%	

Site II & III France & Japan Stool Suspension					
Comparison	EM		Orion Adenolex™ Dry		
SAS™ Adeno Test	+	-	+	-	
	52	2	23	2	
Performance Statistic	Value	95% C.I.	Value	95% C.I.	
	94.6%	84.9%-98.9%	100%	85.2%-100%	
Sensitivity	96.7%	88.7%-99.6%	93.8%	79.2%-99.2%	
	95.7%	90.2%-98.6%	96.4%	87.5%-99.6%	
Specificity	95.7%	90.2%-98.6%	96.4%	87.5%-99.6%	
	95.7%	90.2%-98.6%	96.4%	87.5%-99.6%	
Agreement	95.7%	90.2%-98.6%	96.4%	87.5%-99.6%	
	95.7%	90.2%-98.6%	96.4%	87.5%-99.6%	

Site II & III (cont'd) France & Japan Stool Suspension Diarlex™ Rota-Adeno		
SAS™ Adeno Test	+	-
	25	4
Performance Statistic	Value	95% C.I.
	100%	86.3%-100%
Relative Sensitivity	100%	86.3%-100%
	100%	86.3%-100%
Relative Specificity	88.9%	73.9%-96.9%
	88.9%	73.9%-96.9%
Relative Agreement	93.4%	84.1%-98.2%
	93.4%	84.1%-98.2%

Site IV Japan Eye Swabs					
Comparison	PCR		Meridian Adenoclone®		
SAS™ Adeno Test	+	-	+	-	
	93	1	48	5	
Performance Statistic	Value	95% C.I.	Value	95% C.I.	
	52.3%	44.6%-59.8%	100%	92.6%-100%	
Sensitivity	99.1%	95.2%-99.9%	100%	86.3%-98.0%	
	99.1%	95.2%-99.9%	100%	86.3%-98.0%	
Specificity	99.1%	95.2%-99.9%	100%	86.3%-98.0%	
	99.1%	95.2%-99.9%	100%	86.3%-98.0%	
Agreement	70.6%	65.0%-75.7%	100%	91.3%-98.7%	
	70.6%	65.0%-75.7%	100%	91.3%-98.7%	

Site V Japan Nasopharyngeal Swabs			
Comparison	PCR		
SAS™ Adeno Test	+	-	
	23	0	
Performance Statistic	Value	95% C.I.	
	88.5%	69.8%-97.6%	
Sensitivity	100%	90.5%-100%	
	100%	90.5%-100%	
Specificity	100%	90.5%-100%	
	100%	90.5%-100%	
Agreement	95.2%	86.7%-99.0%	
	95.2%	86.7%-99.0%	

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.

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.

Serial two-fold dilutions of each virus suspension were assayed and the following results were obtained:

Adenovirus Serotype	Protein Concentration (ug/ml)	SAS™ Adeno Test Results*
Ad3	1.200	+
	0.600	+
	0.300	+
	0.150	+
	0.075	±
	0.038	-
Ad4	0.872	+
	0.436	+
	0.218	+
	0.109	+
	0.055	+
	0.027	±
Ad7	0.740	+
	0.370	+
	0.185	+
	0.093	+
	0.046	+
	0.023	±
Ad19	0.850	+
	0.427	+
	0.213	+
	0.107	+
	0.053	+
	0.027	±
Ad37	0.850	+
	0.427	+
	0.213	+
	0.107	+
	0.053	+
	0.027	±

* + = Positive
± = Weak Positive
- = Negative

CROSS REACTIVITY

The following organisms and viruses have been tested and showed no cross-reactivity:

Microorganisms

S. pneumoniae
H. influenza
N. gonorrhoea
S. epidermidis
P. aeruginosa
Chlamydia pneumoniae

Viruses

Polyomavirus
Herpes simplex 1
Herpes simplex 2
Echovirus 1
Echovirus 4
Echovirus 6
Echovirus 9
Echovirus 11
Echovirus 27
Echovirus 30
Echovirus 33
Poliovirus II
Coxsackievirus A-14
Coxsackievirus A-24
Rotavirus
Small Round virus

Coxsackievirus B-4
Enterovirus
Rhinovirus
Coronavirus
Influenza A
Influenza B
Parainfluenza-1
Parainfluenza-2
Parainfluenza-3
Parainfluenza-4
Mumps
Measles
Simian
Astrovirus

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.

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