Procedure	SAS™ Adeno Test

Prepared by	Date Adopted	Supercedes Procedure #

Review Date	Revision Date	Signature

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PRINCIPLE OF THE TEST

The SAS™ Adeno 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.

REAGENTS

Test device containing a monoclonal a monoclonal anti-adenovirus, colored conjugate, and polyclonal coated on a membrane.
Extraction buffer that contains 0.1% sodium azide

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.
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 AND STABILITY

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

SPECIMEN COLLECTION & PREPARATION

Specimens for virus isolation should be collected as soon as possible after the onset of symptoms, preferably within 7-10 days. 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.

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.

Follow the package insert for specific instructions of specimen collection depending on the type of specimen to be collected.

Specimen Storage: 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 -20ºC or colder until ready for testing.

PROCEDURE

Material Provided

1. Test devices containing monoclonal anti-adenovirus colored conjugate, and polyclonal coated on a membrane.
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 collection swabs
2. Precision micropipette and micropipette tips
3. Vortex or centrifuge
4. Timer
5. Adenovirus positive controls
6. Adenovirus negative control

DIRECTION FOR USE

Allow the pouch (test device), specimen and /or controls to reach room temperature (15º-30ºC) 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 150 µl) 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 concentration of antigen.

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. A colored line of any intensity in the S (specimen) 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 are 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 tested is positive or negative.
If the line should fail to appear in the C area of the device, the test should be repeated. 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. 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.

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 procedure.
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.

PERFORMANCE CHARACTERISTICS

The SAS™ Adeno Test was evaluated in laboratories, clinics, and hospitals in the United States, Japan and France for detecting adenovirus and/or adenovirus antigens in tissue culture supernatant, stool specimens, eye swabs and nasopharyngeal swabs.

SENSITIVITY & SPECIFICITY

Test results of studies completed in the United States to Meridian Adenoclone® for tissue culture demonstrated a 100% sensitivity and 100% specificity as well as a 100% overall agreement. Tests results completed in France and Japan to Orion Adenolex™ Dry for stool suspension demonstrated a 100% sensitivity and 93.8% specificity as well as a 96.4% overall relative agreement.

Test results of studies completed in France and Japan to Diarlex™ Rota-Adeno for stool suspension demonstrated a 86.3%-100% relative sensitivity and a 73.9%-96.9% relative specificity as well as a 84.15-98.2% relative agreement. Test results if studies completed in Japan to Meridian Adenoclone® for eye

swabs demonstrated a 100% sensitivity a 100% specificity as well as a 100% relative agreement. Test results of studies completed in Japan to PCR for nasopharyngeal demonstrated a 69.8%-97.6% sensitivity a 90.5%-100% specificity and a 86.7%-99.05 agreement.
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 adenovirus antigen were assayed and tested with the SAS™ Adeno Test. Refer to the package insert for results.

CROSS REACTIVITY

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

Microorganisms
Streptococcus pneumoniae
Hemophillus influenzae
Neisseria gonorrhea
Staphylococcus epidermidis
Pseudomonas 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 11
Coxsackievirus A-14
Coxsackievirus B-4
Enterovirus
Rhinovirus
Coronavirus
Influenza A
Influenza B
Parainfluenza-1
Small Round virus
Parainfluenza-2
Parainfluenza-3
Parainfluenza-4
Mumps
Measles

Coxsackievirus A-24 Simian
Rotavirus Astrovirus

BIBLIOGRAPHY

1. Hierholzer, J. C. 1996. Adenoviruses, p. 947-955. P. Murray et al. Editors, Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington DC.
2. Schmitz, H., R. Wigand, and W. Heinrich. 1983. Worldwide epidemiology in human adenovirus infections. J. Epidemiol. 117:455-466.
3. Uchio, E., H. Matsushima, H. Komaura, T. Maki and T. Nakazawa. 1998. Clinical Evaluation of a simple one-step diagnostic test kit for the detection of Adenovirus in nasopharyngeal specimens. The 38th Interscience conference on antimicrobial agents and chemotheraphy (ICAAC). Abstract, H66. P. 334.
4. Saitoh-Inagawa, W., Oshima, A., Aoki, K., et al. 1996. Rapid diagnosis of adenoviral conjunctivitis by PCR restriction fragment length polymorphism analysis J. Clin. Microbiol. 34:2113-2116.
5. Lennette, D.A. 1985. Collection and Preparation of Specimens for Virological Examination. P. 687-693. In E. H. Lennette. A. Balows, W.J. Hausler, Jr., and H.J. Shadomy (eds.), Manual of clinical microbiology, 4th ed. American Society for Microbiolgy. Washington DC.
6. Chernesky, M.A., C.G. Ray, and T.F. Smith, Laboratory diagnosis of viral infections. Coordinating ed., W.L. Drew. Amerian Society for Microbiology, Washington DC.
7. Kowalski, R., R., and Y. J. Gordon. 1989. Comparison of direct rapid tests for the detection of adenovirus antigen in routine Conjunctival specimens. Ophthalmology 96:1106-1109.
8. Uchio, E., Aoki, K., Saitoh, W., Itoh, N., Ohno, S. 1997. Rapid diagnosis of adenviral conjunctivitis on Conjunctival swabs by 10-minute immunochromatography. Ophthalmology 104:129 4-1299.
9. Brandt, C.D., Kin, H. W., Vargosdo, A. J., Jeffries, B.C., Arrobio, J.O., Ringe, B., Parrot, R.H. and Chanock, R.M. Infections in 18,000 infants and children in a controlled study of respiratory tract disease. I. Adenovirus pathogenicity in relation to serological type and illness syndrome. Am. J. Epidemiol. 90: 484-500, 1969.
10. Uhnoo, I., Wadell, G., Svensson, L., Johansson, M.E. Importance of enteric adenoviruses 40 and 41 in acute gastroenteritis in infants and young children. J. Clinl. Microbiol. 20:365-372, 1984.
11. Laverty, C.R., Russell, P., Black, J., Kappagoda, N., Benn, R.A.V., and Booth, N. Adenovirus infection of the cervix. Acta Cytol. 21”114-117, 1977.
12. Mogabgab, W.J., Mycoplasma pneumonia and adenovirus respiratory illnesses in military and and university personnel, 1959-1966. Am. Rev. Respiratory Dis. 97:345-358, 1968.

13. Kemp, M.C., Hierholzer, J.C., Cabradilla, C.P., and Obijesti, J.F. The changing etiology of epidemic
Keratoconjunctivitis: Antigenic and restriction enzyme analysis of adenovirus types 19 and 37 isolated
Over a 10 year period. J. Infect. Dis. 148:29-33, 1983.
14. Mallet, R., Riberre, M., Bonnenfant, F., Labrune, B., and Reyrole, L. Les pneumopathies graves a
Adenovirus. Arch Fr. Pediatr. 23:1057-1073, 1966.
15. Ishii, K., Nakazone, N., Fujinaga, K., et al, 1987. Comparative studies on aetiology and epidemiology
Of viral conjunctivitis in three countries of East Asia-Japan, Taiwan, and South Korea. Int. J. Epidemiol 16:98-103.