LIGHT DIAGNOSTICS™

PAN-ENTEROVIRUS DETECTION KIT

For the Preliminary Identification of Enteroviruses in Cell Culture

3460

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Intended Use

Light Diagnostics™ Pan-Enterovirus Detection Kit is intended as an indirect immunofluorescence screening reagent for the preliminary identification of enteroviruses from cell culture and not intended for testing directly on human specimens.

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Summary and Explanation

Enteroviruses, classified as picornaviruses, pico [small] + RNA [ribonucleic acid] + virus, are among the smallest and simplest ribonucleic acid (RNA) containing viruses known[1]. The RNA from all sequenced enteroviruses are similar in length, about 7400 nucleotides, and have identical organization[1].

The human alimentary tract is the predominant site of enterovirus replication where they were first isolated from enteric specimens. There are 67 numbered types of enteroviruses in the enterovirus family[1]: three polioviruses, twenty-three coxsackieviruses A, six coxsackieviruses B, thirty-one echoviruses, and four other enteroviruses. These viruses cause a wide variety of disease ranging from paralytic poliomyelitis, aseptic meningitis-encephalitis, myocarditis, pleurodynia, hand-foot-and-mouth disease, conjunctivitis, and numerous other syndromes associated with extra-intestinal target organs.

Enteroviruses, including echoviruses and coxsackieviruses, have been reported as major etiologic agents of aseptic meningitis[2]. Clinical syndromes associated with each type of enterovirus have also been reported[3]. Coxsackievirus A9 can cause aseptic meningitis, paralysis, exanthema, infant pneumonitis, and hepatitis; coxsackivirus A16 is a frequent cause of hand-foot-mouth disease. Coxsackieviruses B1-B6 can cause pleurodynia, aseptic meningitis, severe systemic infection in infants, meningoencephalitis, pericarditis, myocarditis, upper respiratory illness (cox B5) and undifferentiated febrile illness.

Echoviruses can cause aseptic meningitis, paralysis, encephalitis, ataxia, Guillain-Barre' syndrome, exanthema and respiratory disease. They have also been associated with diarrhea, epidemic myalgia, pericarditis, myocarditis and hepatic disorders. Enteroviruses 70 and 71 can cause paralysis, meningoencephalitis, acute hemorrhagic conjunctivitis and hand-foot-and-mouth disease.

Establishing an association between an enterovirus and a particular disease in a patient requires laboratory confirmation of infection by isolation of the virus or

documentation of specific serologic response in a properly timed specimen. Detailed descriptions of principles and procedures for diagnosis of enterovirus infections have been published[5-7]. Cell culture techniques have made the accurate detection of enteroviruses possible[8-12]. The identification of the enterovirus isolates help in the prevention, treatment and understanding of the enterovirus illnesses, and even discovery of new virus isolates. The 'gold standard' for typing enterovirus isolates is neutralization with type-specific pools of immune sera[12]. This method is time consuming (7 days or more) and expensive. As an alternative, a presumptive identification of an enterovirus may be done by screening culture isolates with an immunofluorescent screening reagent. The isolate can then be further identified by the use of type-specific monoclonal antibodies and/or group-specific monoclonal antibody pool(s) by indirect immunofluorescence[13-18].

Test Principle

Light Diagnostics™ Pan-Enterovirus Detection Kit can be used as a screening reagent for the preliminary identification of enteroviruses in cell culture using an indirect immunofluorescence assay (IFA). The monoclonal antibodies in the reagent will bind to enterovirus-infected cells and unbound monoclonal antibody is removed by rinsing with phosphate-buffered saline (PBS). A secondary FITC (fluorescein isothiocyanate)-labeled antibody is then added which will bind to the antigen-antibody complex. Unbound secondary antibody is removed by rinsing with PBS and the resultant complex can be visualized by fluorescence microscopy. Uninfected cells stain a dull red due to the presence of Evans Blue counterstain in the FITC-labeled secondary antibody reagent.

Kit Components

1. Pan-Enterovirus Blend Reagent - REF 3360: One 5 mL dropper vial containing a blend of two mouse monoclonal antibodies which recognize all enteroviruses including echoviruses, coxsackie A and B viruses, polio 1,2, and 3, and enteroviruses 70 and 71, protein stabilizer and 0.1% sodium azide (preservative). The Pan-Enterovirus Blend may cross-react with hepatitis A, reovirus 3, astrovirus and rhinovirus.

   Amount provided is sufficient for 125 tests. Estimate is based on test drop of 40µL; actual number of tests may vary.

2. Pan-Enterovirus Control Slides - REF 5069: Two control slides containing five infected (positive) wells and one non-infected (negative) wells. Each infected well represents a different enterovirus: Coxsackievirus A9, coxsackievirus B2, echovirus 30, enterovirus 71 and poliovirus 2. There is one uninfected (negative) well.

3. Normal Mouse Antibody – REF 5014: One 10 mL dropper vial containing normal mouse antibody to be used as a negative control in PBS with protein stabilizer and 0.1% sodium azide (preservative).

4. Anti-Mouse IgG:FITC Conjugate – REF 5008: One 10 mL dropper vials containing FITC labeled goat anti-mouse IgG in PBS, with 0.02% Evans Blue counterstain, 0.2% BSA, and 0.1% sodium azide (preservative).

5. Phosphate Buffered Saline - REF 5087: One packet of Phosphate Buffered Saline Salts yields 1 liter when dissolved in distilled water. Store in a clean closed container at room temperature.

6. Tween® 20 / Sodium Azide Solution (100X) - REF 5037: One 10 mL vial containing polyoxyethylene sorbitan monolaurate (Tween 20) and sodium azide (NaN_3) concentrate to be diluted 1:100 in PBS.

7. Mounting Fluid - REF 5013: One 10 mL dropper vial containing Tris-buffered glycerin, a fluorescence enhancer, and 0.1% sodium azide (preservative). Store at 2° - 25ºC.

Materials Required But Not Provided

- Acetone, reagent grade; stored in glass
- Distilled water or deionized water
- Sodium hypochlorite solution, 0.05% (1:100 dilution of household bleach)
- Sterile shell vials with 12 mm coverslips or culture tubes containing monolayer of cell line appropriate for growth of enteroviruses (RMK, MRC-5, etc)
- Tissue culture media such as RPMI or Eagle's Minimum Essential Medium (EMEM) with fetal bovine serum (FBS) and antibiotics, or equivalent
- Viral transport medium which is non-inhibitory to enteroviruses

- 0.1N NaOH
- 0.1N HCl
- Microscope slides, non-fluorescing
- No. 1 cover slips
- Blocking solution, such as 1% casein or Difco Bacto® Skim Milk with, 0.05% Tween 20, 0.02% sodium azide.
- Centrifuge capable of 700-950 x g with biohazard buckets and adapters for shell vials
- Fluorescence microscope with appropriate filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm) with 100x, 200x, 400x magnification (dry objective)
- Forceps
- Humid chamber
- Incubator, 37 ± 1°C
- Syringe filter, 0.45 micron
- Ultrasonic water bath
- Vortex mixer or sonicator

Warnings and Precautions

- Incubation times or temperatures other than those specified may give erroneous results. Any change must be validated by the user.
- The performance of the **Light Diagnostics™ Pan-Enterovirus Detection Kit** has not been determined on direct specimens.
- The monoclonal antibodies present in the **Pan-Enterovirus Blend Reagent** REF 3360 may show some background in certain cell lines, such as HEp-2 cells or some monkey cell lines.
- Slides prepared too early (<25% CPE) or too late (>95% CPE) can be difficult to read and can lead to false negative results.
- Sodium azide, present in the reagent, may react with lead and copper plumbing to form potentially explosive metal azides. Upon disposing of

solutions that contain sodium azide, flush plumbing with a large volume of water to prevent build-up.

- Handle all specimens and materials coming in contact with them as potentially infectious materials. Decontaminate with 0.05% sodium hypochlorite prior to disposal.
- Avoid contact with Evans Blue, present in the Anti-Mouse IgG:FITC Conjugate REF 5008, as it is a potential carcinogen. If skin contact occurs, flush with large volumes of water.
- Do not mouth pipette reagents.
- Do not allow shell vials or slides to dry at any time during the staining procedure.
- Pooling or alteration of any reagent may cause erroneous results.
- Acetone is extremely flammable and harmful if swallowed or inhaled. Keep away from heat, sparks or flame. Avoid breathing vapor. Use adequate ventilation.
- Mounting Fluid REF 5013 contains a fluorescence enhancer that may be destructive to mucous membranes. Avoid direct skin or mucous membrane contact. If contact occurs, flush with large volumes of water.

Stability and Storage

When stored at 2° - 8°C, the **Light Diagnostics™ Pan-Enterovirus Detection Kit** is stable up to the expiration date printed on the label. Do not freeze or expose to elevated temperatures. Discard any remaining reagent after the expiration date.

Specimen Collection

While procedures vary from laboratory to laboratory, some considerations on how to process specimen for enterovirus analysis are offered. The specimen type and its processing will depend on the clinical status of the patient and the laboratory request of the consulting physician. Specimens for enterovirus isolation should be transported on wet ice or cold packs and cultured as soon as possible. If storage is necessary, store at 2° - 8°C for up to 48 hours. If longer storage is necessary, store frozen at -70°C in appropriate media[19].

Specimen Processing

Body Fluids - For fluid specimens such as cerebrospinal fluid (CSF) inoculate 0.2 to 0.5 mL undiluted sample in each culture vessel.

Swabs - Specimens such as nasopharyngeal, throat or eye swabs in transport media should be agitated or vortexed to dislodge cells from the swab. Inoculate 0.2 to 0.3 mL in each culture vessel.

Fecal Material - If solid fecal matter is present, vortex in 2 to 5 mL of viral transport media or PBS. Discard the swab into sodium hypochlorite solution. Centrifuge at 950 x g for 10 minutes. If necessary, filter the supernatant through a 0.45 micron syringe filter to clarify. Inoculate 0.1 to 0.3 mL in each culture vessel.

Solid Tissues - Take small pieces of tissue specimen and place in 2 to 3 mL viral transport media. Grind tissue with tissue grinder. Centrifuge gently to sediment debris. Inoculate 0.1 to 0.2 mL supernatant in each culture vessel.

Culture/Isolation and Preparation for Staining:

Inoculation of Standard Tubes:

1. Immediately prior to inoculation with specimens, examine cell cultures for proper morphology.
2. Aspirate growth medium from tubes.
3. Add 0.2 to 0.5 mL of the inoculum to each tube.

   Note*: The use of multiple cell lines or replicate cultures may improve the isolation rate of the virus.*
4. Incubate tube cultures on a slant rack at 37°C for 1 hour.
5. After adsorption or centrifugation, aspirate the inoculum and add sufficient maintenance medium to completely cover the cell monolayer.
6. Incubate at 35° to 37°C in roller drums.
7. Examine the monolayer daily. The cells can be gently rinsed 2 to 3 times with pre-warmed maintenance media if it appears that the specimen is toxic to the monolayer.
8. Renew the cell culture every 3 to 5 days.

Note: A sample of each lot of the cell lines used for cell culture should be inoculated with representative enterovirus strains to establish susceptibility to enterovirus infection and subsequent development of CPE. Uninoculated cell cultures should also be grown and examined daily for contaminating viruses or mycoplasma. This will act as a control for normal cell morphology and could be useful in detecting early CPE. Unless these control cell cultures show appropriate growth, the results of the cell culture isolation should be considered invalid.

9. Examine tubes daily for cytopathic effect (CPE).

Shell Vial Inoculation Cultures:

1. Immediately prior to inoculation with specimens, examine cell cultures for proper morphology.
2. Aspirate growth medium from shell vials.
3. Add 0.2 to 0.5 mL of the inoculum to each shell vial.
4. Centrifuge at room temperature for 30 minutes at 500 to 700 x g.
5. After centrifugation, aspirate the inoculum and add sufficient maintenance medium to completely cover the cell monolayer.
6. Incubate at 35° to 37°C.
7. Examine shell vials daily for cytopathic effect and/or test for hemad-sorption. Shell vials can be stained between 24 and 96 hours post-infection or at optimal time points as determined by the individual laboratory.

Slide Preparation:

1. If CPE is observed, aspirate the culture medium from the tube and store at 2° - 8°C until testing has been completed. If additional testing is required, viral isolation can be attempted from this medium.

   ***Note**: An uninfected cell line control should also be processed in the same manner to act as a negative control.*

2. Gently rinse the cell monolayer three times with 1 to 2 mL of HBSS or PBS. Discard all rinses into sodium hypochlorite solution.

   ***Note**: PBS with Tween 20/sodium azide should not be used in viral isolation procedures.*

3. Add one tenth of the original culture volume of trypsin and let stand for 30 seconds. Gently tap the culture vessel to loosen the cells. Resuspend the cells in 2 mL HBSS or PBS. Centrifuge the cell suspension at 300 to 500 x

g for 10 minutes. Resuspend the cell pellet in 0.3 mL of sterile PBS to give a slightly cloudy suspension.

***Note**: Alternately, the cell monolayer may be scraped from the tube into a small volume PBS using a glass rod or sterile pipette.*

4. Centrifuge the cell suspension at 250 x g for 10 minutes at room temperature.
5. Resuspend cell pellet in 0.1 - 0.2 mL PBS and use to make cell spots in 6 - 8 mm slide wells and allow the slide to air dry completely.
6. Fix the slide in chilled (2° - 8°C) acetone for 10 minutes.
7. Remove the slide from the acetone and allow to air dry completely.
8. The slide should be stained as soon as possible. If storage is necessary, the slides should be kept at ≤ -20°C, with desiccant.

Test Procedure

Reagent Preparation:

PBS/Tween 20 Solution

1. Dissolve the contents of the PBS packet in 950 mL of deionized or distilled water.
2. Add the Tween 20/Sodium Azide 100X concentrate (10 mL) to the PBS and mix thoroughly.
3. Bring the mixture to 1 liter with deionized or distilled water. Transfer it to a clean, labeled storage container and cap tightly. Store at room temperature.

All other reagents are provided ready to use.

Suggested Immunofluorescence Staining Procedure:

1. Allow the acetone fixed control slide and/or test slide and reagents to equilibrate to room temperature.

   ***Note**: Do not allow slides to dry at any time during the staining procedure.*

2. *(Optional)* Preincubate the sample slide/well with PBS, 0.05% Tween 20, 0.02% sodium azide with 1% casein or 1% Difco Bacto Skim Milk for 15-30 minutes at 37°C. This eliminates most non-specific staining, but will not affect the specific enterovirus activity. Then rinse thoroughly with PBS/Tween 20.*

3. Add sufficient Pan-Enterovirus Blend Reagent REF 3360 or Normal Mouse Antibody REF 5014 (Neg control reagent) to cover the cells; 1 drop for cell spots and 4-6 drops for shell vials.
4. Incubate the slide at 37°C for 30 minutes in a humid chamber.
5. Rinse the slide gently with a squirt bottle of PBS/Tween 20 solution for 10-15 seconds to remove excess monoclonal antibody solution, taking care to direct the stream away from the well. For shell vials: aspirate reagent from vial and gently wash each shell vial 3 times with 1mL PBS/Tween 20 solution.
6. Place slide in a staining dish or Coplin jar (with slide holder or equivalent) and cover with PBS. Rinse 5 to 10 minutes. Gently agitate with magnetic stirring bar or by hand.
7. Shake off excess reagent from the slide and carefully dry the area surrounding the cell spot.
8. Add sufficient Anti-Mouse IgG:FITC Conjugate REF 5008 to cover the cells; 1 drop for cell spots and 4-6 drops for shell vials.
9. Repeat steps 4 to 6.
10. Mount under a coverslip using an aqueous Mounting Medium pH 8.5, REF 5013. For shell vials: Aspirate PBS/Tween 20 solution from shell vials. Raise each coverslip using a bent needle affixed to a small syringe and carefully remove with forceps. Mount each coverslip CELL SIDE DOWN on a glass slide with Mounting Fluid.
11. Wipe excess fluid from the edges of the slide.

    ***Note**: For best results, read slides immediately after preparation. If slides are to be stored after staining, store at 2° - 8°C, in a secure container in the dark.*

12. Examine with a fluorescence microscope at 100-200x for cells exhibiting the apple-green fluorescence of FITC. Detailed examination may be carried out at 400x.

    *Optional skim milk/casein pre-incubation step recommended for Pan-Enterovirus and Echovirus Blends.

    ***Note**: Performance of the fluorescence microscope is of critical importance in achieving satisfactory test results. While objectives, bulb intensity and*

wattage, and filters may affect results, use of a positive control will verify functioning of reagents, culture methodology, and microscope.

Interpretation of Results

Quality Control

Negative (uninfected cell controls) as well as positive controls should always be included with each test to assure proper performance of the procedure for the preliminary identification of enterovirus isolate(s), and to help identify non-specific staining. The positive control well should show the typical fine speckled 'starry night' staining pattern of apple-green fluorescence in the cytoplasm of infected cells and throughout the well. If Evans Blue counterstain is used, the negative control well should show cells staining a dull red color. Without Evans Blue, uninfected cells should not be visible.

***Note**: Scan the entire coverslip or slide well for the presence of infected cells.*

Positive controls may be prepared with appropriate enterovirus isolates or with positive clinical isolates. Enteroviruses for positive controls can be obtained from the American Type Culture Collection (ATCC®), Manassas, VA. Negative control can utilize known laboratory negative samples.

Culture Confirmation

A positive reaction is indicated by a bright apple-green fluorescence in the nucleus and/or cytoplasm of the infected cells which is greater and more intense than that seen in the uninfected cell control. A negative reaction is indicated by the absence of fluorescence and presence of a dull red color due to the Evans Blue counterstain, if used. All positive results should be further tested with enterovirus group blends and specific monoclonal antibodies. Pan-entero positive isolates which are negative with enterovirus group blends and/or specific monoclonal antibodies should be identified and confirmed by neutralization with type specific pools of immune sera. Pan-Enterovirus Blend-positive isolates should be reported as "isolate is a possible enterovirus, confirmation to follow".

A negative result may be due to a variety of factors such as: inadequate sample, improper specimen collection and handling, improper culture technique, or other factors mentioned in the "Troubleshooting" section. All negative results should be reported as "No virus observed". It is useful to examine the negative cells prior to the positive cells to determine if there is non-specific staining.

Limitations

- The **Pan-Enterovirus Blend Reagent** REF 3360 is intended as a tool to rule in the possibility of enterovirus. Cell cultures positive with the Blend should be further tested with more specific reagents and/or by neutralization in order to make a more definitive diagnosis of enterovirus.
- The **Pan-Enterovirus Blend Reagent** REF 3360 may cross-react with reovirus 3, hepatitis A, some strains of rhinovirus and astrovirus.
- Certain continuous cell lines may show speckled staining in the cytoplasm and/or nucleus. It is important that an uninfected control of the cell line be used as control.
- The monoclonal antibodies used in this reagent have not been characterized as to the particular antigens or epitopes recognized.
- The fine-speckled fluorescence of the staining pattern may be difficult to discern under low magnification with some isolates. It is recommended that slides be read using a 20X objective.
- If CPE has progressed beyond 2+, it may be difficult to identify intact cells and the resultant fluorescence may be mistaken for non-specific staining.
- Performance of the fluorescence microscope is of critical importance in achieving satisfactory test results. While objectives bulb, intensity and wattage, and filters may affect results, use of appropriate controls will verify functioning of reagents, culture methodology and microscope.
- Since the monoclonal antibodies in the reagent have been prepared using prototype strains, they may not detect all antigenic variants or new strains of enterovirus.

Expected Values

Samples were submitted to a laboratory in the south-western United States for enterovirus testing during 1995. Enteroviruses were isolated from 126 of the samples. Echovirus 9 (30.1%), coxsackievirus B2 (19.8%) and coxsackievirus A9 (10.3%) accounted for 60.2% of all isolates. A further 16.7% were identified as enteroviruses by neutralization[20] and the Pan-Enterovirus Blend Reagent, but were not further typed.

The samples which were submitted included rectal swabs (RS), cerebral spinal fluid (CSF), nasopharyngeal swab (NP), throat swab (TS), stool (S) and unknown (U). 61.9% of the enteroviruses were isolated from CSF, and another 15.9% from rectal swabs.

	RS	CSF	NP	TS	S	U
Number	20	78	10	10	7	1
Percent	15.9	61.9	7.9	7.9	5.6	>1

Specific Performance Characteristics

A total of 756 clinical specimens and known enterovirus isolates were tested with the Pan-Enterovirus Blend Reagent at two sites, an enterovirus reference laboratory in south-western United States and a public health laboratory on the west coast.

Three hundred and seventy-nine specimens were tested from 1994 through 1996 for enterovirus identification at the clinical virology laboratory. Of the 379 specimens, 80 were negative for enteroviruses. Those isolates exhibiting typical enterovirus CPE were tested with the **Light Diagnostics™ Coxsackievirus B**, **Echovirus, Enterovirus** and **Poliovirus Blends** and the **Pan-Enterovirus Blend Reagent**. Those isolates which were negative by the Group Blends, but positive with the **Pan-Enterovirus Blend Reagent**, were further identified by neutralization[20].

At the public health laboratory, enterovirus isolates which had been previously frozen and had been typed by neutralization, were tested for reactivity with the Pan-Enterovirus Blend Reagent and another commercial antibody for pan-enterovirus identification. One isolate each of echovirus 11 and 23, and 2 each of echovirus 9 and 22 did not grow well after being thawed and were negative on staining with the Pan-Enterovirus Blend Reagent.

Table 1. Number of Enterovirus Isolates Positive with Pan-Enterovirus Blend Reagent (Number of isolates which were negative)

Enterovirus Type	# of Isolates	Enterovirus Type	# of Isolates
Coxsackievirus A2	2	Echovirus 1	5
Coxsackievirus A3	1	Echovirus 2	1
Coxsackievirus A4	2	Echovirus 3	4
Coxsackievirus A5	1	Echovirus 4	18
Coxsackievirus A6	1	Echovirus 5	5
Coxsackievirus A7	1	Echovirus 6	17
Coxsackievirus A8	1	Echovirus 7	28

Table 1. (Continued)

Enterovirus Type	# of Isolates	Enterovirus Type	# of Isolates
Coxsackievirus A9	39	Echovirus 8 (2)	3
Coxsackievirus A10	2	Echovirus 9	68
Coxsackievirus A11	1	Echovirus 11 (1)	36
Coxsackievirus A12	2	Echovirus 12	3
Coxsackievirus A13	1	Echovirus 13	2
Coxsackievirus A14	1	Echovirus 14	6
Coxsackievirus A15	1	Echovirus 15	4
Coxsackievirus A16	2	Echovirus 16	4
Coxsackievirus A18	1	Echovirus 17	4
Coxsackievirus A21	2	Echovirus 18	15
Coxsackievirus A24	5	Echovirus 19	1
Coxsackievirus B1	26	Echovirus 20	6
Coxsackievirus B2	47	Echovirus 21	11
Coxsackievirus B3	34	Echovirus 22 (2)	3
Coxsackievirus B4	21	Echovirus 23 (1)	2
Coxsackievirus B5	33	Echovirus 24	4
Coxsackievirus B6	3	Echovirus 25	6
Poliovirus (1-3)	5	Echovirus 26	4
Poliovirus 1	16	Echovirus 27	5
Poliovirus 2	10	Echovirus 29	5
Poliovirus 3	6	Echovirus 30	60
Enterovirus 68	1	Echovirus 31	4
Enterovirus 69	2	Echovirus 32	4
Enterovirus 70	1	Echovirus 33	4
Enterovirus 71	10	Echovirus 34	2

Total Number of Samples: 756
Number positive with **Pan-Enterovirus Blend Reagent**: 670
Relative Sensitivity: 99.1%

Specificity and Cross-Reactivity

Prototype strains of each enterovirus type were obtained from The Centers for Disease Control, Atlanta, GA, California Department of Health Services, Berkeley, CA, University of New Mexico, Albuquerque, NM and the Texas Department of Health, Austin, TX. The Pan-Enterovirus Blend reacted with the prototype strain of each enterovirus.

Coxsackievirus	A1 – A22, A24 including A24v
Coxsackievirus	B1-6
Echovirus	1- 9, including 6’ and 6”
	11 – 34, including 11’
Enterovirus	68 – 71
Poliovirus	1 – 3

Cross-Reactivity

The **Pan-Enterovirus Blend Reagent** gave a positive staining result with the following viruses and cell lines:

Virus Strain	Cell Line	
Hepatitis A virus	A549	Slight
Astrovirus	HEp-2	Moderate
Reovirus – 3	LLC-MK$_2$	Negative
Rhinovirus-2	MRC-5	Slight
Rhinovirus-30	Vero	Slight
Adenovirus 5	PMK	Negative
Adenovirus 31	RD	Slight
Adenovirus 48	BGMK	slight

Troubleshooting

Specimen preparation is technique dependent and may affect the results obtained. In order to resolve any performance questions, all steps in the process must be analyzed.

A marked decrease in fluorescence may indicate:

1) Reagent deterioration,
2) Microscopy problems or
3) Other equipment or technique effects.

- Verify expiry date for all reagents used.
- If reagents are in-dating, verify microscope performance; re-read positive control.
- If problem is still not determined, verify all equipment operation as per package insert and repeat test.

Contact **EMD Millipore Corporation** - Technical Service at (800) 437-7500 for United States and Canada or (951) 676-8080. For additional assistance, visit www.millipore.com/offices.

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20. Data regarding the neutralization reagents used in these studies are available upon request from EMD Millipore Corporation.

Glossary of Symbols

Symbol	Used for	Symbol	Used for
REF	Catalog number		Use by YYYY-MM-DD or YYYY-MM
	Manufacturer	EC REP	Authorized representative in the European Community
	Caution, consult accompanying documents	Σ	Contains sufficient for <n> tests
IVD	*In vitro* diagnostic medical device		Temperature limitation
	Consult instructions for use		Biological risks
CONTROL	Control	CONTROL −	Negative control
CONTROL +	Positive control		

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