



For the qualitative identification of human cytomegalovirus (CMV) immediate early antigen (IEA) in cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs).

FOR *IN VITRO* DIAGNOSTIC USE

Rx ONLY



INTENDED USE

The Diagnostic Hybrids, Inc. device, D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit, is intended for use in the qualitative identification of human cytomegalovirus (CMV) immediate early antigen (IEA) in cell cultures by immunofluorescence using fluoresceinated monoclonal antibodies (MAbs).

This product is not intended for use in testing blood or plasma donors and is not intended for use in direct identification of CMV in clinical specimens.

SUMMARY AND EXPLANATION OF THE TEST

CMV is a member of the herpesvirus group, which includes *Herpes simplex virus* types 1 and 2, *Varicella-zoster virus* (which causes chickenpox), and *Epstein-Barr virus* (which causes infectious mononucleosis). CMV is found universally throughout all geographic locations and socioeconomic groups, and infects between 50% and 85% of adults in the United States by 40 years of age.^{1,2,3} CMV is also the virus most frequently transmitted to a developing child before birth.^{1,4,5,6} CMV infection is more widespread in developing countries and in areas of lower socioeconomic conditions. For most healthy persons who acquire CMV after birth there are few symptoms and no long-term health consequences. Some persons with symptoms experience a mononucleosis-like syndrome with prolonged fever, and a mild hepatitis. Once a person becomes infected, the virus remains alive, but usually dormant within that person's body for life. Recurrent disease rarely occurs unless the person's immune system is suppressed due to therapeutic drugs or disease. Therefore, for the vast majority of people, CMV infection is not a serious problem. However, CMV infection is important to certain high-risk groups. Major areas of concern are (1) the risk of infection to the unborn baby during pregnancy (congenital Cytomegalic Inclusion Disease (CID) includes symptoms such as jaundice, transient petechial rash, hepatosplenomegaly, pneumonitis, microcephaly, and chorioretinitis; psychomotor retardation, blindness and hearing loss later in life; perinatal acquired at the time of delivery by contact with virus in the birth canal or by breast feeding; postnatal acquired by contact with individuals who are shedding virus),^{1,2,3} (2) the risk of infection to people who work with children (women of child-bearing age who previously have not been infected with CMV),¹ and (3) the risk of infection to the immunocompromised person, such as organ transplant recipients and persons infected with human immunodeficiency virus (HIV).^{6,7,8,9,10,11} Infectious CMV may be shed in the bodily fluids of any previously infected person, and thus may be found in urine, saliva, blood, tears,

semen, and breast milk.^{6,12} The shedding of virus may take place intermittently, without any detectable signs, and without causing symptoms.

PRINCIPLE OF THE PROCEDURE

The D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit uses a blend of CMV-IEA antigen-specific murine MAbs that are directly labeled with fluorescein for the rapid identification of CMV-IEA in cell culture.

Clinical specimens are inoculated into permissible cultured cell monolayers. These cells are fixed in acetone 1 to 4 days post-inoculation. The CMV-IEA DFA Reagent is added to the cells to identify the presence of any CMV-IEA antigens present. After incubating for 15 to 30 minutes at 35°C to 37°C, the stained cells are washed with the diluted Phosphate Buffered Saline (1X PBS) and, using the supplied Mounting Fluid, processed further for examination using a fluorescence microscope equipped with the correct filter combination for Fluorescein Isothiocyanate (FITC) at a magnification of 200-400X. Virus infected cells will contain bright apple-green fluorescent nuclei while non-infected cells will contain no apple-green fluorescence but will fluoresce red from the Evans Blue.

REAGENTS AND MATERIALS PROVIDED

The D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit contains the following:

CMV-IEA DFA Reagent

10 mL

One dropper bottle containing a mixture of two murine MAbs directed against CMV immediate early antigen (pp 72). The MAbs are both IgG1 (k) isotype. The buffered, stabilized, aqueous solution contains Evans Blue as a counter-stain and 0.1% sodium azide as preservative.

CMV Antigen Control Slides

5 slides

Five (5) individually packaged control slides containing wells with cell culture derived positive and negative control cells. Each slide contains one Negative well of non-infected cells and one Positive well of CMV infected cells. Each slide is intended to be stained only one time.

40X PBS Concentrate

25 mL

One bottle containing a 40X concentrate consisting of 4% sodium azide (0.1% sodium azide after dilution to 1X using demineralized water).

Mounting Fluid

7 mL

One dropper bottle of an aqueous, buffered, stabilized solution of glycerol and 0.1% sodium azide.

***Note:** A reagent drop will vary from 40 to 60 µL depending on the pressure and temperature of the solution, therefore, the number of examinations/tests per kit by procedure type is shown below.

Procedure Type	Number of Examinations per CMV-IEA DFA Reagent, 10 mL
Shell-vials, tests:	40 to 60
	<i>Assumptions: 4-drops, based on ~0.200 mL completely covering each shell-vial.</i>
Multi-well Plates, tests:	24-well plate: 40 to 60
	<i>Assumptions: 4-drops, based on ~0.200 mL completely covering each 24-well.</i>
	48-well plate: 50 to 80
	<i>Assumptions: 3-drops, based on ~0.150 mL completely covering each 48-well.</i>
Cell Spot, tests: <i>(from glass round bottom tube scraping)</i>	200
	<i>Assumptions: 1-drop, based on an average drop size of ~0.050 mL, covering 1 well (5 mm) of a 2-well slide.</i>

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microscope with the correct filter combination for FITC (excitation peak = 490 nm, emission peak = 520 nm); magnification 200X to 400X.
- Cell culture for CMV isolation according to the laboratory's method of choice. Suggested cell lines include H&V-Mix™ MixedCells™ human newborn foreskin, MRC-5.⁶ All are available from Quidel. Examples of CMV isolation methods include:
 - ▶ Shell-vials, with glass cover slips, containing monolayers of either a commercially prepared or user propagated cell line.
 - ▶ Multi-well plates (either 24-, or 48-well size), containing monolayers of either a commercially prepared or user propagated cell line.
- Live control viruses for positive culture controls: Known strains of CMV concentration for use in monitoring the cell culture and staining procedures. Such control virus strains can be obtained from Quidel.
- Cover slips (22 x 50 mm) for Antigen Control Slides and for specimen slides.
- Universal Transport Medium. Available from Quidel.
- Tissue Culture refeed medium (Eagle's Minimum Essential Medium with 2% fetal bovine serum, 25 mM HEPES and antibiotics). Available from Quidel.
- Reagent grade acetone (> 99% pure) chilled at 2°C to 8°C for fixation of Antigen Control Slides, shell-vials and cultured cell preparations.

NOTES:

- ▶ Keep the reagent-grade acetone container tightly sealed to avoid hygroscopic absorption of water, which may cause a hazy, non-specific, background fluorescence.
- ▶ A mixture of 80% acetone / 20% demineralized water is used for fixing cells in plastic multi-well plates. Store at ambient temperature (20°C to 25°C).
- Sterile graduated pipettes: 10 mL, 5 mL, and 1 mL.
- Sterile Pasteur pipettes or other transfer pipettes.
Caution: One should not use solvents such as acetone with polystyrene transfer pipettes.
- Fine-tipped forceps.
- Wash bottle, 200 mL.
- Bent-tip teasing needle (for removal of coverslip from the shell-vial); fashion the teasing needle by bending the tip of a syringe needle or similar object (i.e., mycology teasing needle) against a benchtop or with a pair of forceps taking care to avoid injury.
- Sodium hypochlorite solution (1:10 dilution of household bleach).
- Humidified chamber (e.g., covered Petri dish with a damp paper towel placed in the bottom).
- Glass microscope slides.
- Acetone-cleaned multi-well glass microscope slides.
- Blotters for multi-well glass microscope slides used to blot excess liquid from the mask to prevent spread of liquid or stained cells from one well to the other.
- Sterile nylon flock swabs or polyester swabs, which is non-inhibitory to viruses and cell culture.
- Incubator, 35°C to 37°C (5% CO₂ or non-CO₂, depending on the cell culture format used).
- Centrifuge with free-swinging bucket rotor.^{16,17,18}
- Demineralized water for dilution of 40X PBS Concentrate and for dilution of the reagent-grade acetone for use in polystyrene multi-well plates.
- Aspirator Set-up: Vacuum aspirator with disinfectant trap containing sufficient household bleach (5%) such that the concentration is not decreased by more than 10-fold as it is diluted with discarded fluids.
- Wash Container: Beaker, wash bottle or Coplin jar for washing slides.
- Fixing Container: Coplin jar, slide dish or polyethylene holder for slides for use in fixing the cells on the slides.
- Inverted Light Microscope: Used for examining the monolayers of cells prior to inoculation and examination for toxicity, confluency and for cytopathic effect (CPE). It should have between 40X to 100X magnification capability.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- No known test method can offer complete assurance that infectious agents are absent; therefore, all human blood derivatives, reagents and human specimens should be handled as if capable of transmitting infectious disease. It is recommended that reagents and human specimens should be handled in accordance with the OSHA Standard on Bloodborne Pathogens.¹³
 - ▶ Cell culture isolation may have some potential to be hazardous. Personnel working with these cultures must be properly trained in safe handling techniques¹⁴ and have experience with tissue culture before attempting this procedure.
 - ▶ All procedures must be conducted in accordance with the CDC 5th Edition Biosafety in Microbiological and Biomedical Laboratories, 2007, and CLSI Approved Guideline M29-A, Protection of Laboratory Workers from Instrument Biohazards and Infectious Disease Transmitted by Blood, Body Fluids, and Tissue.
- All specimens and materials used to process them should be considered potentially infectious and handled in a manner which prevents infection of laboratory personnel.
 - ▶ Biosafety Level 2 or other appropriate biosafety practices should be used when handling these materials.
 - ▶ Decontamination of specimens and cultures is most effectively accomplished using a solution of sodium hypochlorite (1:10 dilution of household bleach).
 - ▶ Although Antigen Control Slides have been shown to be non-infectious, the same precautions taken in handling and disposing of other infectious materials should be employed in their use.
- Acetone, a reagent that is required for the test but not provided in the kit, is a flammable, volatile organic solvent. Use it in a well-ventilated area and keep away from flames and other sources of ignition.
- Sodium azide is included in the 40X PBS Concentrate at a concentration of 4% (w/v), and in the other solutions in this kit at 0.1% concentration.
 - ▶ Sodium azide is considered poisonous. If the 40X PBS Concentrate is swallowed, seek medical advice immediately.
 - ▶ Aqueous solutions of sodium azide, when mixed with acids, may liberate toxic gas.
 - ▶ Sodium azide may react with lead and copper plumbing to form highly explosive metal azides.
 - ▶ Avoid disposal of these solutions down sanitary or industrial plumbing systems.
 - ▶ Avoid release to the environment.
- Evans Blue counter-stain is a potential carcinogen. If skin contact occurs, flush with water immediately.
- The CMV-IEA DFA Reagent is supplied at working strength. Any dilution of the DFA Reagent will decrease sensitivity.
- Do not expose the CMV-IEA DFA Reagent to bright light during staining or storage.
- Microbial contamination of the CMV-IEA DFA Reagent may cause a decrease in sensitivity.
- Reagents should be used prior to their expiration date.
- CMV Antigen Control Slides are microscope slides onto which either cultured *non*-infected cells or *cytomegalovirus*-infected cells have been grown then fixed (killed) with acetone; a drying agent is included in the foil envelope to preserve antigen integrity; there is no residual acetone present.
- Each CMV Antigen Control Slide should be used only once. Do not re-use a Control Slide. Store 1X PBS in a clean container to prevent contamination.
- Never pipette reagents or clinical samples by mouth; avoid all contact of clinical samples with broken skin.
- Avoid splashing and the generation of aerosols with clinical samples.
- Use aseptic technique and sterile equipment and materials for all cell culture procedures.
- Reusable glassware must be cleaned and thoroughly rinsed free of all detergents.
- Use of other reagents than those specified with the components of this kit may lead to erroneous results.
- Testing should be performed in an area with adequate ventilation.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.

- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.
- For additional information on hazard symbols, safety, handling and disposal of the components within this kit, please refer to the Safety Data Sheet (SDS) located at quidel.com.

Preparation of 1X PBS Solution

- After storage at 2°C to 8°C, some salts in the 40X PBS Concentrate may have crystallized. Warm the solution to ambient temperature (20°C to 25°C) to re-dissolve the crystals and mix.
- Add contents of the fully dissolved 25 mL 40X PBS Concentrate to 975 mL of demineralized water.
- Label the 1X PBS with a sixty (60) day expiration date after reconstitution and store at ambient temperature.

Preparation of 80% Acetone Solution

- Add 20 mL of distilled or de-ionized water to a 100 mL container.
- Add 80 mL of acetone to the container slowly and mix by inversion.
- Label the container as to contents, the date diluted, technologist's initials and store.

Storage

Table 1. Reagent Storage Conditions

CMV-IEA DFA Reagent	Store at 2°C to 8°C in the dark.
Mounting Fluid	
CMV Antigen Control Slides	Store at 2°C to 8°C.
40X PBS Concentrate NOTE: The Concentrate may crystallize when stored at 2°C to 8°C. The crystals will dissolve when the Concentrate is warmed to room temperature.	Store liquid at 2°C to 8°C prior to dilution.
1X PBS Solution	Store at ambient temperature (20°C to 25°C).

Stability

Reagent and components will retain their full potency through the expiration date shown on the label of each bottle when stored at recommended temperatures. Light exposure of the CMV-IEA DFA Reagent should be kept to a minimum.

Discard 1X PBS Solution if it becomes cloudy.

SPECIMEN COLLECTION AND PREPARATION

Proper collection and handling of the patient specimen are the most important factors in successful CMV identification. Specimen collection, processing, and cell culture isolation of viruses should be attempted only by personnel trained in such procedures. Care should be taken during all specimen collection and handling to avoid generation of aerosols.

For additional specimen collection and processing recommendations please refer to CLSI Approved Viral Culture Guidelines.¹⁵

Specimen Transport and Storage

Rapid specimen transport to the laboratory is ideal for optimum virus recovery. Ideally, specimen transport time should not exceed four hours. Specimens should be transported to the laboratory at 2°C to 8°C. This temperature can be attained by using cold packs, wet ice, foam refrigerant, or other coolants. The specimen should be processed and tested as soon as possible and then stored at 2°C to 8°C.

Specimens can be stored at 2°C to 8°C for up to 72 hours before being tested. If longer storage is required, the specimens should be frozen at –70°C or lower.

Freezing and thawing of specimens should be avoided since this will result in a loss of viability of viruses, leading to decreased sensitivity of the test.

All potentially infectious agents should be transported according to International Air Transport Association (IATA), International Civil Aviation Organization, (ICAO), Titles 42 and 49 of the U.S. Code of Federal Regulations, or other regulatory requirements, as may be applicable.

PROCEDURE

Preliminary Comments and Precautions

- Adhere to the recommended volumes and times in the following procedure to ensure that accurate results are obtained.
- When staining with fluorescent reagents and examining cells microscopically for fluorescence, it is very important to include controls, both positive and negative, to monitor the procedure and performance of the reagents. It is recommended that such controls be run with each batch of patient specimens.
- Place the closed, humidified chamber for holding slides during staining into the incubator for equilibration to 35°C to 37°C prior to staining. By doing this, the test slides and reagents will come to temperature quickly, yielding more rapid, intense staining.
- Bring the CMV-IEA DFA Reagent to ambient temperature (20°C to 25°C) prior to use, and immediately return to refrigerator after use for storage at 2°C to 8°C.

Regarding Cell Culture Testing

- Good Laboratory Practice dictates that positive and negative virus controls be run with each new batch of cells to confirm their performance in culturing specific viruses.
- It is good practice to retain the medium removed from the positive monolayers until after staining results have been obtained. If there is any question concerning the specimen results, the medium can be passed to another monolayer and incubated for the appropriate time period for repeat testing.
- When using cell cultures in polystyrene multi-well plates, dilute the acetone fixative to 80% by adding 20 mL of demineralized water to 80 mL of acetone.
- Do not allow the monolayers to dry before fixing; this can lead to high background staining and decreased sensitivity.
- Do not allow the DFA Reagent to dry on the monolayers; this can lead to high background.

Regarding Immunofluorescence Microscopy

- Examine the positive and negative controls before examining the test specimens. If one of these fails to perform as expected, review the steps and conditions under which the test was performed to determine the root cause(s). Do not report results for patient samples until controls perform as expected.
- Three aspects of the fluorescence microscope that must be functioning properly and optimally in order to achieve maximum brightness of fluorescence:
 - ▶ The activation light source has a finite life and as it ages, its output decreases, resulting in lower fluorescence intensity from the DFA Reagent.

- ▶ The light source is focused by a number of lenses and mirror(s). For maximum intensity, these must be properly aligned.
- ▶ The filters used in the light path must be appropriate for the particular fluor, in this case, fluorescein.
- Fluorescent artifacts may be observed in the cell monolayers:
 - ▶ Cell debris, lint, etc. can non-specifically adsorb the DFA Reagent, resulting in highly intense fluorescence. These can be identified by their morphology, i.e., they don't have the appearance of a complete cell and typically are not seen on the same plane of the monolayer as the other cells would be.
 - ▶ A low grade, yellow-green fluorescence may sometimes be seen, particularly in areas that have piled cells or are near holes in the cell monolayer. In both cases, the diffusion of the entrapped DFA Reagent is retarded during the wash step, resulting in the non-specific fluorescence.
 - ▶ Intense fluorescence around the periphery of slide wells is indicative of drying of the DFA Reagent during incubation, suggesting that it was incubated too long or the humidity was not well controlled.
 - ▶ Inadequate washing can lead to general low-grade fluorescence due to residual DFA Reagent remaining on the monolayer of cells.
- Protect stained slides and monolayers from light as much as possible during testing.
 - ▶ Bleaching or fading of the fluorescence of stained cells may occur on exposure to light, particularly light of high intensity.
 - ▶ This bleaching can occur when a stained cell is viewed in a fluorescence microscope for an extended period of time.

Cell Culture Testing – Shell-Vial

1. It is generally recommended that specimens be inoculated into a minimum of two shell-vials containing the same or different cell types that are permissive for CMV.
2. One shell-vial should be stained at 1-day post inoculation; the other shell-vial should be stained at 2 to 3 days post inoculation if the 1-day vial is negative.
3. Examine the monolayers for proper morphology prior to inoculation.
4. Aspirate maintenance medium from the monolayers and add 1 mL of appropriate refeed medium to each shell-vial.
5. Add 0.2 to 0.4 mL of prepared specimen to each shell-vial.
6. Centrifuge the shell-vials at 700xg for 1 hour at 20°C to 25°C.
7. Place stoppered shell-vials in an incubator at 35°C to 37°C.
8. When a monolayer is ready to be stained using the CMV-IEA DFA Reagent, remove the medium and add 1 mL of 1X PBS.
9. Swirl to mix and then aspirate.
10. Repeat this rinse with another 1 mL of 1X PBS and then aspirate.
11. Add 1 mL of fresh, chilled 100% acetone and allow to stand for 5 to 10 minutes at 20°C to 25°C.
Caution: Acetone is volatile and flammable; keep away from open flames.
12. Discard the acetone into a biohazard container.
13. Add 0.5 mL of 1X PBS to wet the monolayer.
14. Swirl and then aspirate completely.
15. Add 4 drops of the CMV-IEA DFA Reagent to the fixed monolayers of patient and control samples, and rock to **ensure complete coverage** of the monolayer by the Reagent.
16. Place stoppered shell-vials in a 35°C to 37°C incubator for 15 to 30 minutes.
17. Aspirate the CMV-IEA DFA Reagent from the monolayers.
18. Add 1 mL of the 1X PBS.
19. Remove the 1X PBS by aspiration; repeat the wash step, and again remove by aspiration.
20. Add 0.5 to 1.0 mL of demineralized water.
21. Remove the demineralized water by aspiration.

22. Lift the coverslip from the bottom of the shell-vial, grasping it with the fine tipped forceps; then transfer it, monolayer-side down, to a small drop of Mounting Fluid on a labeled microscope slide.
23. Examine the stained monolayers using a fluorescence microscope with magnifications between 200X to 400X. (See *Regarding Immunofluorescence Microscopy*.)
24. Refer to Section *INTERPRETATION OF RESULTS*.

Cell Culture Testing – Multi-well Plate

1. It is generally recommended that specimens be inoculated into a minimum of two wells containing the same or different cell types that are permissive for CMV. It is recommended that each replicate well be on a different multi-well plate. This allows each plate to be processed on the appropriate day.
2. One well should be stained at 1-day post inoculation; the other well should be stained at 2 to 3 days post inoculation if the 1-day vial is negative.
3. Examine the monolayers for proper morphology prior to inoculation.
4. Aspirate maintenance medium from the monolayers and add 1 mL of appropriate refeed medium to each 24-well plate monolayer; add 0.8 mL to each 48-well plate monolayer.
5. Add 0.2 to 0.4 mL of prepared specimen to the appropriate well of a multi-well plate.
6. Centrifuge the multi-well plates at 700xg for 1 hour at 20°C to 25°C.
7. Place the covered multi-well plates in a 35°C to 37°C incubator with a humidified, 5% CO₂ atmosphere.
8. When a monolayer is ready to be stained, remove the medium by aspiration and add 1 mL of 1X PBS.
9. Swirl to mix and then aspirate.
10. Repeat this rinse with another 1 mL of 1X PBS and then aspirate.
11. Add 1 mL of 80% acetone and let stand 5 to 10 minutes at 20°C to 25°C.
NOTE: Do not allow the 80% acetone fixative to remain in the polystyrene wells longer than 10 minutes since it may craze and cloud the plastic, making it difficult to examine the monolayers.
Caution: Acetone is volatile and flammable; keep away from open flames.
12. Remove the fixative by aspiration.
13. Add 0.5 mL of the 1X PBS to wet the monolayer.
14. Swirl and then aspirate completely.
15. Add 4 drops of the CMV-IEA DFA Reagent to the fixed monolayers of patient and control sample in each 24-well plate monolayer; add 3 drops of the CMV-IEA DFA Reagent to the fixed monolayers of patient and control samples in each 48-well plate monolayer. Rock to **ensure complete coverage** of the monolayer by the Reagent.
16. Place the covered multi-well plate in a 35°C to 37°C, humidified incubator for 15 to 30 minutes.
17. Aspirate the CMV-IEA DFA Reagent from the monolayers.
18. Add 1 mL of the 1X PBS and mix to wash.
19. Remove the 1X PBS by aspiration, repeat the rinse step, and again remove by aspiration.
20. Add 0.5 to 1.0 mL of demineralized water.
21. Remove the demineralized water by aspiration.
22. Add 3 drops of Mounting Fluid to each monolayer, and cover the plate.
23. Examine the stained monolayers using a fluorescence microscope with magnifications between 200X to 400X. (See *Regarding Immunofluorescence Microscopy*.)
24. Refer to Section *INTERPRETATION OF RESULTS*.

Quality Control

Cell Culture (optional)¹⁹

- Positive and negative virus controls should be run with each new batch of cells to confirm their performance in culturing specific viruses.
- To ensure viral sensitivity, a CMV-inoculated control monolayer should be included each time a new lot of cell culture is used.
- Also, non-inoculated monolayer from each lot should be stained to serve as a negative control. Adverse storage conditions or handling procedures will also be reflected in the negative control.
- If control cultures fail to perform correctly, results are considered invalid.

Reagents

- A fresh CMV Antigen Control Slide should be stained each time the staining procedure is performed to ensure proper test performance.
- The positive well will show multiple infected cells with bright apple-green fluorescent nuclei, with negative cells fluorescing a dull red due to the included Evans Blue counter-stain.
- The negative well will show only negative cells staining a dull red.
- Positive and negative controls must demonstrate appropriate fluorescence for specimen results to be considered valid. Antigen Control Slides may also aid in the interpretation of patient specimens.

INTERPRETATION OF RESULTS

Examination of Samples and Controls

- Examine controls first to ensure proper test performance before examining patient specimens.
- A positive reaction for CMV is one in which bright apple-green fluorescence is observed in the infected cells.
- Non-infected cells will fluoresce dull red due to the Evans Blue counter-stain included in the DFA Reagent.
- Examine the entire cell spot or monolayer of cells before reporting final results.
- Do not report results for patient samples unless controls perform as expected.

Artifacts of Staining

- Dried edges of the monolayer or cell clumps may non-specifically fluoresce due to antibody trapping.
- Dead, rounded cells may non-specifically fluoresce dull olive-green due to specimen toxicity or improper cell storage.
- Properly controlled humidity during staining and adequate washing between steps helps to eliminate non-specific staining.

Results from Culture Isolation/Confirmation

- A positive reaction is one in which bright apple-green fluorescent nuclei is observed in the infected cells.
- Examine the entire cell spot or monolayer of cells for CMV-specific fluorescent cells. If no fluorescent cells are found, report: "No cytomegalovirus identified."
- If one or more fluorescent cells are found, it should be reported as: "Cytomegalovirus isolated by cell culture."

LIMITATIONS OF THE PROCEDURE

- Inappropriate specimen collection, storage and transport may lead to false negative culture results.²⁰
- Incubation times or temperatures other than those cited in the test instructions may give erroneous results.

- Identification of CMV will vary greatly depending upon the specimen quality and subsequent handling. A negative result does not exclude the possibility of CMV infection. Results of the test should be interpreted in conjunction with information available from epidemiological studies, clinical evaluation of the patient and other diagnostic procedures.
- The effects of antiviral therapy on the performance of this kit have not been established.
- Since the MAbs used in this kit have been prepared using defined virus strains, they may not identify all antigenic variants or new strains of the viruses, should they arise. MAbs may fail to identify strains of viruses which have undergone minor amino acid changes in the target epitope region.
- Performance of the kit can only be assured when components used in the assay are those supplied by Quidel.
- Prolonged storage of the CMV-IEA DFA Reagent under bright light will decrease the staining intensity.
- Light background staining may occur with specimens contaminated with *Staphylococcus aureus* strains containing large amounts of protein A. Protein A will bind the Fc portions of conjugated antibodies. Such binding can be distinguished from viral antigen binding on the basis of morphology, i.e., *S. aureus*-bound fluorescence appears as small (~1-micron diameter), bright dots.

EXPECTED VALUES

The clinical studies described in ‘Specific Performance Characteristics’ used only specimens that were collected and cultured for the presence of CMV.

The specimen sources and positivity with the comparison devices are described in Table 2 below.

Table 2. Summary of Overall Specimen Source by Site

Site	Total	Blood	Body Fluid	Genital	G.I. – Upper	G.I. – Lower	Resp. – Upper	Resp. – Lower	Skin/Lesions	Tissue	Urine	Unknown
1	314	45	16	1	4	12	10	131	1	21	73	0
2	293	0	0	0	0	0	271	15	0	6	1	0
3	128	11	8	0	4	4	13	62	2	0	23	1
4	291	72	16	0	7	0	30	95	1	12	58	0
Total	1026	128	40	1	15	16	324	303	4	39	155	1

Blood: blood, buffy coat

Body Fluids: fluid, amniotic fluid, central spinal fluid (CSF/CF), pericardial fluid, pleural

Genital: Genital

G.I. – upper: gastrointestinal (upper), mouth, esophageal, ranula basil

G.I. – lower: gastrointestinal (lower), stomach, colon, sigmoid, bowel, stool, pleura

Resp. – upper: respiratory (upper), nasal (NS), nasopharyngeal (NP) aspirates, throat, nares

Resp. – lower: respiratory (lower), lung, lobes, bronchial, bronchial alveolar lavage (BAL), bronchial washing (BW), brushings, tracheal aspirates (TA), sputum,

Skin/Lesions: abdomen, conjunctiva

Tissue: biopsy (BX), liver, heart, lymph nodes, brain, bone marrow

Urine: urine

Unknown: investigator unable to specify source

The specimen Age/Sex demographics for the specimens cultured are tabulated below in Table 3.

Table 3. Age and Gender Distribution

Age Range	Male	Female	Gender Unknown	Total
0 to 1 month	0 / 37	3 / 17	0 / 0	3 / 54
> 1 month to 2 years	31 / 176	33 / 151	0 / 0	64 / 327
> 2 to 12 years	6 / 34	2 / 31	0 / 0	8 / 65
> 12 to 21 years	0 / 12	0 / 9	0 / 0	0 / 21
22 to 30 years	0 / 14	0 / 21	0 / 0	0 / 35
31 to 40 years	3 / 48	1 / 49	0 / 0	4 / 97
41 to 50 years	5 / 43	4 / 38	0 / 0	9 / 81
51 to 60 years	5 / 63	1 / 31	0 / 0	6 / 94
> 60 years	6 / 110	4 / 105	0 / 0	10 / 215
Unknown age	0 / 14	0 / 4	0 / 19	0 / 37
Total	56 / 551	48 / 456	0 / 19	104 / 1026

NOTE: Values are the # Positive/Total

SPECIFIC PERFORMANCE CHARACTERISTICS

A total of one thousand and sixty (1060) specimens were cultured and stained with one of two comparative devices and the D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit. Culture methods were consistent with recommendations in CLSI, M41-A. A combination of fresh (532) and archival (528) specimens were cultured and stained. A total of 34 specimens were excluded from final analysis, resulting in a total of 1026 results reported. Reasons for exclusion were specimen toxicity to cell culture (29), bacterial contamination of cell culture (1), non-specific fluorescence seen prohibiting interpretation (2), and unacceptable specimens (2). These evaluations were conducted at three external laboratory sites located in the United States and the Diagnostic Hybrids, Inc. in-house virology laboratory.

Percent Agreement between the D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit (D³ CMV-IEA ID Kit) and Comparison Devices was calculated for all the above analyzed specimens. (See Table 4 below.)

Table 4. Overall Comparison of D³ CMV-IEA ID Kit with Comparator Kits

		Comparator Device	
		+	-
D ³ CMV-IEA ID Kit	+	100	4
	-	4	918
		Total: 1026	

Positive percent agreement = 96.1% (100/104)

Exact 95% Confidence Interval = 90.5, 98.5

Negative percent agreement = 99.6% (918/922)

Exact 95% Confidence Interval = 98.9, 99.8

Study Site 1

Study site 1 collected and cultured a total of 314 fresh specimens during August, 2006. There were no specimens excluded from final analysis.

The culture protocol used at this site was as follows: two hundred microliters (200 µL) of each specimen were inoculated into individual wells of four 48-fill MRC-5 multi-well plates. The inoculated plates were centrifuged at 700xg for 60 minutes, incubated at 35°C to 37°C. One set of plates was stained at 2 days post-inoculation in accordance with the respective product insert (1 plate comparator and 1 plate subject device). The remaining set of plates was stained at 4 days post-inoculation in accordance with the respective product insert (1 plate comparator and 1 plate subject device). The results of this testing site are summarized in Table 5.

Table 5. Site 1 – Comparison of D³ CMV-IEA ID Kit with Comparator Kits

		Comparator Device	
		+	-
D ³ CMV-IEA ID Kit	+	16	1
	-	1	296
		Total: 314	

Positive percent agreement = 94.1% (16/17)
 Exact 95% Confidence Interval = 73.0, 98.9
 Negative percent agreement = 99.7% (296/297)
 Exact 95% Confidence Interval = 98.1, 99.9

Study Site 2

Study site 2 cultured a total of 300 specimens (72 fresh and 228 archival specimens) from August 31 through November 8, 2006. The archival specimens were collected from June, 2005 through September, 2006. They were stored at -70°C until re-cultured for this study. The specimens were not selected for this study based on previous culture results. Of the 228 archival specimens seven were excluded from the final analysis. Table 6 shows the reason for specimen exclusion.

Table 6. Study Site 2 – Excluded Specimens/Samples

Exclusion criteria – Toxic to cell culture	7
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The culture protocol used at this site was as follows: two hundred microliters (200 µL) of each specimen were inoculated into four MRC-5 shell-vials. The inoculated shell-vials were centrifuged at 700xg for 60 minutes, incubated at 35°C to 37°C. One set of shell-vials was stained at 1-day post-inoculation in accordance with the respective product insert (1 vial comparator and 1 vial subject device). The remaining set of vials was stained at 2 days post-inoculation in accordance with the respective product insert (1 vial comparator and 1 vial subject device). The results of this testing are summarized in Tables 7 and 8.

Table 7. Site 2 – Comparison of D³ CMV-IEA ID Kit with Comparator Kits with Archival Specimens

<i>Archival</i>		Comparator Device	
		+	-
D ³ CMV-IEA ID Kit	+	65	3
	-	0	153
		Total: 221	

Positive percent agreement = 100% (65/65)
 Exact 95% Confidence Interval = 94.4, 100
 Negative percent agreement = 98.1% (153/156)
 Exact 95% Confidence Interval = 94.5, 99.3

Table 8. Site 2 – Comparison of D³ CMV-IEA ID Kit with Comparator Kits with Fresh Specimens

<i>Fresh</i>		Comparator Device	
		+	-
D ³ CMV-IEA ID Kit	+	4	0
	-	0	68
			Total: 72

Positive percent agreement = 100% (4/4)
 Exact 95% Confidence Interval = 51.0, 100
 Negative percent agreement = 100% (68/68)
 Exact 95% Confidence Interval = 94.7, 100

Study Site 3

Study site 3 tested specimens from February 2007 through May 2007. A total of 146 fresh specimens were cultured. Of these 146 specimens, 18 were excluded from the final analysis. Table 9 shows the reasons for specimen exclusion.

Table 9. Study Site 3 – Rejected Specimens/Samples

Exclusion criteria – Toxic to cell culture	15
Exclusion criteria – Contaminated cell culture	1
Exclusion criteria – Inappropriate specimen	1
Exclusion criteria – Repeat specimen	1
Total	18

The culture protocol used at this site was as follows: two hundred microliters (200 µL) of each specimen were inoculated into two MRC-5 shell-vials. The inoculated shell-vials were centrifuged at 700xg for 60 minutes, incubated at 35°C to 37°C. The shell-vials were stained at 2 days post-inoculation in accordance with the respective product insert (1 vial comparator and 1 vial subject device). The results of this testing site are summarized in Table 10.

Table 10. Site 3 – Comparison of D³ CMV-IEA ID Kit with Comparator Kit with Fresh Specimens

<i>Fresh</i>		Comparator Device	
		+	-
D ³ CMV-IEA ID Kit	+	5	0
	-	1	122
			Total: 128

Positive percent agreement = 83.3% (5/6)
 Exact 95% Confidence Interval = 43.6, 97.0
 Negative percent agreement = 100% (122/122)
 Exact 95% Confidence Interval = 96.9, 100

Study Site 4

Study site 4 (Diagnostic Hybrids, Inc. in-house virology lab) cultured 300 specimens that were collected at a clinical reference laboratory located in the Eastern U.S. in February, 2007. These frozen prospectively collected specimens were stored at -70°C until cultured for this study. The specimens were not selected for this study based on previous culture results. Of these 300 archival specimens, 9 were excluded from the final analysis. Table 11 shows the reasons for specimen exclusion.

Table 11. Study Site 4 – Rejected Specimens/Samples

Exclusion criteria – Toxic to cell culture	7
Exclusion criteria – Non-specific fluorescence seen, unable to interpret	2
Total	9

The culture protocol used at this site was as follows: two hundred microliters (200 μL) of each specimen were inoculated individual wells of four 48-fill MRC-5 multi-well plates. The inoculated plates were centrifuged at 700xg for 60 minutes, incubated at 35°C to 37°C . One set of plates was stained at 1-day post-inoculation in accordance with the respective product insert (1 plate comparator and 1 plate subject device). The remaining set of plates was stained at 2 days post-inoculation in accordance with the respective product insert (1 plate comparator and 1 plate subject device). The results of this testing site are summarized in Table 12.

Table 12. Site 4 – Comparison of D³ CMV-IEA ID Kit with Comparator Kits

<i>Archival</i>	Comparator Device	
	+	-
D ³ CMV-IEA ID Kit	+	10
	-	279
		Total: 291

Positive percent agreement = 83.3% (10/12)

Exact 95% Confidence Interval = 55.2, 95.3

Negative percent agreement = 100% (279/279)

Exact 95% Confidence Interval = 98.6, 100

Analytical Sensitivity

Analytical sensitivity was studied for purposes of demonstrating comparability of the effectiveness of the D³ CMV-IEA DFA Reagent with that of a Comparator Device. A low-level inoculum was used to address analytical sensitivity in a cell culture system. This was done by first inoculating two 96-well cell culture plates (Hs27) with CMV diluted to a value of 1 TCID₅₀ and incubating at 37°C for 48 hours; then, one plate was stained with the subject D³ CMV-IEA DFA Reagent and the other plate was stained using the CMV-IEA DFA Reagent from the comparator device. This assay was performed three times, with an average of 35.3 ± 2.3 positive wells out of a total 96 wells identify with the subject, and an average of 38.3 ± 2.1 positive wells out of a total 96 wells with the predicate. These results are not statistically different by a paired t-test.

Analytical Specificity (Cross-reactivity Testing)

The D³ DFA Cytomegalovirus Immediate Early Antigen Identification Kit DFA Reagent was tested for cross-reactivity against a wide variety of cells and microorganisms. Stringent conditions for cross-reactivity testing were achieved by using a high concentration of the CMV-IEA DFA Reagent and relatively high titers of microorganisms. The DFA Reagent was prepared at 2X the concentration that is provided in the kit. No cross-

reactivity was observed for 58 virus strains or for 20 host culture cell types. Twenty-five (25) bacterial cultures, one yeast culture and three bacterial (*Chlamydia sp.*) and one protozoan commercially available slides, were stained and examined for cross-reactivity, including *Staphylococcus aureus*, a protein-A producing bacterium. Staining of *S. aureus* appeared as small points of fluorescence (see *LIMITATIONS OF PROCEDURE*). [See Tables 13, 14 and 15 below for cross-reactivity study results.]

Fifty-eight (58) virus strains were tested for cross-reactivity. Depending on the particular virus, 140 to 1,400 TCID₅₀ were inoculated into shell-vial culture and incubated for 24 to 48 hours, to yield a 1+ to 3+ infection, processed and stained with the 2X DFA Reagent according to the procedure as detailed in this product insert. No cross-reactivity was observed for the viruses listed below (Table 13).

Table 13. Virus Strains Tested for Cross-Reactivity with D³ CMV-IEA DFA Reagent

Organism	Strain or Type	Inoculum (TCID ₅₀)	Organism	Strain or Type	Inoculum (TCID ₅₀)
Adenovirus	Type 1	1,400	RSV	Long	1,400
	Type 3	1,400		Wash	1,400
	Type 5	1,400		9320	1,400
	Type 6	1,400	Parainfluenza 1	C-35	1,400
	Type 7	1,400	Parainfluenza 2	Greer	1,400
	Type 8	1,400	Parainfluenza 3	C 243	1,400
	Type 10	1,400	Parainfluenza 4a	M-25	1,400
	Type 13	1,400	Parainfluenza 4b	CH19503	1,400
	Type 14	1,400	HSV-1	IF	140
	Type 31	1,400		MacIntyre	140
Influenza A	Aichi	1,400	HSV-2	MS	140
	Malaya	1,400		Strain G	140
	Hong Kong	1,400	VZV	Webster	140
	Denver	1,400		Ellen	140
	Port Chalmers	1,400		AV92-3	140
	Victoria	1,400	Epstein-Barr	Commercially available slides stained.	
	New Jersey	1,400	Rubeola		
	PR	1,400	Mumps		
	WS	1,400	Echovirus	Types 4, 6, 9, 11, 30, 34	Commercially available slides stained.
Hong Kong	1,400				
Maryland	1,400	Coxsackievirus	Types B1, B2, B3, B4, B5, B6		
Mass	1,400				
Taiwan	1,400	Poliovirus	Types 1, 2, 3		
GL	1,400				
Russia	1,400				

Twenty (20) host culture cell types were tested for cross-reactivity. Cell cultures were prepared in shell-vial format. Confluent monolayers or cell spots were stained with the 2X DFA Reagent according to the procedure as detailed in this product insert, then examined for cross-reactivity. No cross-reactivity was observed for the following (Table 14).

Table 14. Cell lines Tested for Cross-Reactivity with D³ CMV-IEA DFA Reagent

A549	monolayer	NCI-H292	monolayer
BGMK	monolayer	pCMK	cell spot
HEp-2	monolayer	pRhMK	cell spot
HFF / Hs27	monolayer	RD	monolayer
LLC-MK2	monolayer	RhMK II	cell spot
McCoy	monolayer	RK (passage 1)	monolayer
MDCK	monolayer	R-Mix	monolayer
MRC-5	monolayer	Vero	cell spot
MRHF	monolayer	Vero 76	cell spot
Mv1Lu	monolayer	WI-38	cell spot

Thirty (30) microorganisms, including 24 bacterial cultures, one mycoplasma, one yeast and three chlamydial species and one protozoan commercially available slides, were stained with the 2X DFA Reagent according to the procedure as detailed in this product insert, then examined for cross-reactivity. Except for *Staphylococcus aureus*, which was cross-reactive with the CMV-IEA DFA Reagent (see above), all microorganisms tested negative. Concentrations for each bacterial organism cultured by Diagnostic Hybrids, Inc. for cross-reactivity testing were determined from suspensions of the bacteria in PBS by spectrophotometer according to McFarland standards of levels 1.0 and 2.0 (equaling approximately 3.0×10^6 and 6.0×10^6 CFU per mL). Slides were prepared with spots of 0.01 mL of the suspensions to give either 3.0×10^4 or 6.0×10^4 per spot. At the same time, 1 mL of each suspension was plated on an appropriate agar dish for colony confirmation. According to the confirmation agar cultures, initial concentrations of the bacterial organisms in the study ranged from 6.4×10^4 to 2.9×10^7 CFU. Results of testing are listed below (Table 15).

Table 15. Microorganisms Tested for Cross-Reactivity with D³ CMV-IEA DFA Reagent

BACTERIA	CMV-IEA DFA Reagent	CFU TESTED
Acholeplasma laidlawii	Negative	$\sim 6 \times 10^7$
Acinetobacter calcoaceticus	Negative	9.7×10^5
Bordetella bronchiseptica	Negative	1.7×10^5
Bordetella pertussis	Negative	4.6×10^6
Corynebacterium diphtheriae	Negative	2.5×10^6
Escherichia coli	Negative	2.6×10^5
Gardnerella vaginalis	Negative	5.0×10^5
Haemophilus influenzae type A	Negative	9.3×10^5
Klebsiella pneumoniae	Negative	6.4×10^6
Legionella pneumophila	Negative	6.5×10^4
Moraxella cartarrhalis	Negative	6.4×10^4
Mycoplasma hominis	Negative	$\sim 6 \times 10^4$
Mycoplasma orale	Negative	$\sim 6 \times 10^4$
Mycoplasma pneumoniae	Negative	$\sim 6 \times 10^4$
Mycoplasma salivarium	Negative	$\sim 6 \times 10^7$
Neisseria gonorrhoeae	Negative	1.3×10^6
Proteus mirabilis	Negative	2.1×10^6
Pseudomonas aeruginosa	Negative	1.0×10^7
Salmonella enteritidis	Negative	2.5×10^6
Salmonella typhimurium	Negative	1.8×10^6
Staphylococcus aureus	Positive*	1.0×10^7

BACTERIA	CMV-IEA DFA Reagent	CFU TESTED
Streptococcus agalactiae	Negative	9.6 x 10 ⁶
Streptococcus pneumoniae	Negative	8.0 x 10 ⁵
Streptococcus pyogenes	Negative	2.9 x 10 ⁷
Ureaplasma urealyticum	Negative	~6 x 10 ⁴
Chlamydomphila pneumoniae	Negative	Commercially available slides stained.
Chlamydomphila psittaci	Negative	
Chlamydia trachomatis	Negative	
YEAST		
Candida glabrata	Negative	8.7 x 10 ⁶
PROTOZOAN		
Trichomonas vaginalis	Negative	Commercially available slides stained.

* Staining of *S. aureus* appeared as small points of fluorescence while all other cultures were negative. This will be noted in labeling in the section "Limitations of the Assay": The Protein A produced by the bacterium, *Staphylococcus aureus*, will bind the Fc portion of some of the fluorescein-labeled monoclonal antibodies used in this kit. Such binding can be distinguished from viral antigen binding on the basis of morphology, i.e., *S. aureus*-bound fluorescence appears as small (~1-micron diameter), bright dots. Results from cell cultures with bacterial contamination must, therefore, be interpreted with caution."

WARRANTY STATEMENT

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IVD



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GLOSSARY

REF

Catalogue number



CE mark of conformity

EC REP

Authorized Representative
in the European Community

LOT

Batch code



Use by



Manufacturer



Temperature limitation



Intended use

R_x ONLY

Prescription use only



Consult e-labeling
instructions for use



Do not reuse

IVD

For *In Vitro* diagnostic use



40 to 60

Contains sufficient for 40 to 60 determinations

CONT NaN₃

Contents/Contains

NaN₃ 4%

Contents/Contains
