Key Code TSMX7787A

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PathoDx

Herpes Typing Kit

REF R62250 **EN**

1. INTENDED USE

PathoDx™ Herpes Typing Kit is an immunofluorescence test designed for the detection and typing of herpes simplex virus types I and II (HSV-I and HSV-II) in direct clinical specimens and following growth in tissue culture. Culture confirmation slides and shell vial assays may undergo detection and typing with a bivalent staining procedure. All direct clinical specimens which are negative or have inadequate numbers of cells must be re-evaluated by cell culture.

2. SUMMARY AND EXPLANATION OF THE TEST

Herpes simplex virus (HSV) is an ancient and ubiquitous virus, known to cause acute and recurrent infections in humans. The virus enters the mucous membranes (ocular, genital, or oral) and replicates locally.[12,16] Infection of neonates during passage through the birth canal may result in neurological damage and death.[2,15] In a small proportion of infected individuals, the virus may become latent, entering the sensory root ganglion and resulting in recurrent infections.[1]

In the 1960s, it was recognized that HSV consisted of two distinct types, HSV-I and HSV-II.[16] HSV-I was considered to be primarily associated with ocular and oral infection (above the waist) while HSV-II was considered to be a genital infection (below the waist). However, these distinctions have blurred and either strain of HSV may be isolated from herpetic lesions.

Typing of the virus is necessary for proper treatment of infected neonates and immunocompromised individuals,[3] adequate management of pregnant women,[14,15] and epidemiological studies.[14] In addition, individuals infected with HSV-II are prone to greater numbers of recurrent episodes than are those infected with HSV-I.[4] Moreover, HSV-II has been implicated as a cofactor with papilloma virus as a cause of cancer of the cervix.[16] Identification of the infecting strain may also aid in effective treatment of the infection.

The standard method for diagnosis of HSV has been growth in tissue culture cells.[11,14] Susceptible cells demonstrate typical cytopathic effect (CPE) usually within 1 to 4 days, but occasionally up to 7 days in culture.[5,13] Cytological changes such as ground glass nuclei, intranuclear inclusions, and multinucleate cells are considered diagnostic of HSV infections of tissue culture.[17] Immunologic assays such as ELISA,[14] immunoperoxidase staining,[11] and fluorescent antibody staining[5] have frequently been used to identify and type HSV isolates following growth in culture. Diagnosis may also be made directly from the sample, without cell culture amplification, if adequate numbers of infected cells are available.

Definitive identification of HSV-I or HSV-II can be made on the basis of pock size in chick embryo, sensitivity to antiviral drugs,[18] and restriction endonuclease mapping.[7,18] But only with the availability of monoclonal antibodies against HSV-I and HSV-II specific epitopes has the accurate identification and typing of HSV been made convenient.[2,7,8,16]

3. PRINCIPLE OF THE PROCEDURE

PathoDx Herpes Typing Kit uses four fluorescein-labeled monoclonal antibodies specific for HSV-I and HSV-II (two for HSV-I; two for HSV-II) to detect and identify herpes simplex virus. The HSV-I Typing Reagent contains two monoclonal antibodies which react with polypeptides having molecular weights of 85,000 and 142,000 daltons. The HSV-II Typing Reagent contains two monoclonal antibodies specific for HSV-II. One antibody recognizes a polypeptide of approximately 79,000 daltons, while the second antibody recognizes a 41,000 dalton molecule. When the typing reagents are added to wells containing herpes-infected cells, the fluorescein-labeled monoclonal antibodies react specifically with the viral antigens in the infected cells. After the unbound antibody is washed off with buffered saline, the viral antigens show a characteristic apple-green fluorescence while the cells counterstain red by Evans Blue under fluorescent microscopy. The reagents are specific for each HSV type and will not show any cross-reactive staining.

An Optional Bivalent Staining Procedure using combined HSV-I and HSV-II Typing Reagents is included for use in culture confirmation slides and shell vial assays.

4. REAGENTS

KIT CONTENTS

Herpes Typing Kit	100 Tests (R62250)
1. HSV-I Typing Reagent	1 dropper bottle (purple cap)
2. HSV-II Typing Reagent	1 dropper bottle (pink cap)
3. Mounting Fluid (R62230)	1 dropper bottle (black cap)
4. Herpes Typing Control Slides (R62251)	1 pack of 5
5. Instructions for Use	1

DESCRIPTION, PREPARATION FOR USE AND RECOMMENDED STORAGE CONDITIONS

See also **Warnings and Precautions**.

2°C–8°C

Store at 2 to 8°C, protected from light. Use on or before the expiration date marked on the label. All components must be at room temperature (15 to 28°C) before use; mix thoroughly by inversion.

Components of this kit are interchangeable with components of other lots of the same catalog number.

HSV I

HSV-I Typing Reagent

One plastic dropper bottle containing 5.0 ml of fluorescein-labeled purified murine monoclonal antibodies specific for HSV-I, Evans Blue counterstain, an inhibitor of non-specific staining, and 0.098% sodium azide in a protein-stabilized buffer solution.

HSV II

HSV-II Typing Reagent

One plastic dropper bottle containing 5.0 ml of fluorescein-labeled purified murine monoclonal antibodies specific for HSV-II, Evans Blue counterstain, an inhibitor of non-specific staining, and 0.098% sodium azide in a protein-stabilized buffer solution.

MOUNTING FLUID

Mounting Fluid

One plastic dropper bottle containing 5.0 ml of mounting fluid, consisting of buffered glycerol, with 0.01% sodium azide as preservative.

CONTROL SLIDE

Herpes Typing Control Slides

Five individually foil-sealed slides packaged in a zip lock bag with desiccant. Each control slide has four wells. The two upper wells contain RK-13 cells infected with HSV-I (strain MacIntyre), and the two lower wells contain RK-13 cells infected with HSV-II (strain MS). Before use, allow the slide to warm to room temperature (15 to 28°C) in the foil pack. Remove the slide by handling the edges only.

5. WARNINGS AND PRECAUTIONS

IVD

The reagents are for *in vitro* diagnostic use only.

For professional use only.

Please refer to the Safety Data Sheet and the product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

1. Sodium azide, at a concentration less than 0.1%, has been added to certain components as an antibacterial agent. To prevent buildup of explosive metal azides in lead and copper plumbing, reagents should be discarded into sewerage only if diluted and flushed with large volumes of water. Use copper-free and lead-free drain systems where possible.
2. **Clinical Specimens:** Follow routine biosafety procedures when collecting and processing HSV specimens. Consider specimens and all materials they contact as potentially infectious, and dispose of them in an appropriate manner. Do not pipette by mouth. Avoid generation of aerosols.
3. **Waste Material:** Sterilize all waste materials before discarding according to standard laboratory procedures and local regulations.
4. **Control Slides:** The control slides contain mammalian cells (RK-13) infected with HSV-I (MacIntyre) and HSV-II (MS). These have been treated and fixed to render the virus noninfectious. However, users should treat the controls with safety precautions appropriate for clinical specimens.
5. Evans Blue dye is present in the Reagent. Although present below the concentration for the product to be classified as carcinogenic, contact with the skin should be avoided.
6. Acetone (not provided) is a flammable organic solvent. Use in a well-ventilated area away from any potential source of ignition.

6. SPECIMEN COLLECTION, STORAGE AND TRANSPORT

For best results, specimens should be collected using polyester or cotton swabs (not calcium alginate)[9,10] within 3 to 7 days following the onset of symptoms.[1,12,13] Lesions which have started to heal will contain fewer viral particles and are not ideal as sampling sites. In addition, prior treatment of the infected area with disinfectants, soap, etc., may inactivate the virus. During collection of the specimens, care must be taken to avoid contaminating the swab with the surrounding area or blood.[9,10] Specimens intended for direct antigen detection should have an additional swab taken for culture confirmation of a negative direct test.

Place specimens for culture confirmation in viral transport medium (VTM) and transport immediately on ice to the laboratory. If specimens cannot be processed within 24 hours, they should be frozen at –20°C.

Vesicle

Aspirate the fluid with a 25–27 gauge needle and syringe and rinse into the viral transport medium (VTM). Firmly swab the base of the lesion to obtain adequate numbers of infected cells. Although the vesicle fluid is a good source of virus for culture,[12] infected cells are necessary for direct detection by fluorescent staining.

Ulcerated Lesion

Remove the crust with a sterile needle and discard. Remove any pus with sterile swab. Using a fresh swab, swab the base of the lesion vigorously to collect infected cells. Avoid drawing blood as this may interfere with the sensitivity of the assay.

7. PROCEDURE

MATERIALS SUPPLIED

Herpes Typing Kit (R62250) contains sufficient material for 100 tests, see **Kit Contents**.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Sterile 25–27 gauge needle (for removing fluid from vesicle and crust from dried lesion)
2. Sterile swabs (cotton or polyester) for collecting specimen and removing mucus or pus from ulcerated lesion
3. Viral Transport Medium (VTM), which is known not to inhibit the growth of HSV or cells in culture[9,10]
4. Microscope slides with wells 7–10 mm in diameter with acetone-resistant, hydrophobic coating. Wells must be sufficiently spaced to avoid specimen or reagent crossover (approximately 5 mm)
5. Acetone (reagent grade or better) stored in glass
6. Microscope slide box for storing and transporting slides
7. Culture tubes containing cells suitable for the isolation of HSV[13]
8. Known HSV-I and HSV-II strains as controls, such as ATCC® strains, MacIntyre (HSV-I), MS (HSV-II) or previously identified laboratory isolates
9. Sterile pipettes
10. Micropipette capable of dispensing up to 30 µl, with disposable tips.
11. Phosphate buffered saline (PBS: 0.01 M sodium phosphate, 0.15 M sodium chloride, pH 7.2–7.4, free of calcium and magnesium ions)
12. Cover slips, 22×50 mm
13. Beaker, dispensing bottle, or Coplin jar for washing slides
14. Absorbent paper for blotting slides
15. Fluorescence microscope with filters for fluorescein isothiocyanate (FITC) (maximum excitation – 490 nm; maximum emission – 520 nm) with objectives capable of 160–250X and 400–630X magnification
16. Moist, darkened chamber suitable for incubating slides

TEST PROCEDURE

Direct Specimen Slide Preparation

Step 1 Samples to be stained for direct antigen detection by fluorescent antibody must contain adequate numbers of infected cells. Collect samples as described above, ensuring that the bases of the lesions are vigorously swabbed.

Step 2 Firmly roll the swab onto 2 wells of a glass slide. Take care not to smear the swab on the slide to avoid distortion of the cells. Check the wells to ensure adequate cell coverage. If necessary, roll the swab across the wells again.

Note: Discard the sample into a biohazard bag.

Step 3 Allow the slide to air dry completely. Inadequate drying will result in loss of cells from the well during the staining process.

Step 4 Fix the slide with 0.5 ml of acetone for 10 minutes.

Step 5 Direct specimen slides may be transported to the laboratory at room temperature (15 to 28°C). If they are not stained within 4 hours, they can be stored at 2 to 8°C for 24 hours.

Inoculation Of Cell Cultures

Inoculate susceptible tissue culture cells according to standard procedures.[13] Briefly, the sample containing the swab is vortexed to release virus and infected cells, and the liquid expressed from the swab. The sample may be sonicated or vortexed with glass beads to disrupt cells. The sample is then centrifuged at 1000×g to remove cell debris, and the supernatant is used as the viral inoculum.

Positive and negative control cultures consisting of known HSV-I and HSV-II strains should be inoculated into cell culture along with each group of patient specimens. These may be ATCC® strains, MacIntyre (HSV-I), MS (HSV-II), or previously identified laboratory isolates.

Culture Confirmation Slide Preparation

Step 1 Examine the cell monolayer for CPE. When approximately 50 percent (2+ CPE) of the monolayer is involved, usually in 1 to 4 days, process the cells for HSV Culture Confirmation. CPE that has progressed beyond 2+ may cause hazing due to release of specific viral antigens (see **Notes on Technique** section).

Step 2 Remove the growth medium from the culture and place in a sterile tube in the event further passage is necessary.

Step 3 Add a small quantity of PBS (approximately 0.2 ml) to the tube containing the monolayer.

Step 4 Scrape and/or dislodge the cells from the surface of the tube using a Pasteur pipette. (Use a fresh pipette for each isolate.)

Step 5 Using the same pipette, aspirate the cell suspension gently to obtain a single-cell suspension. There should be sufficient cells for the suspension to appear visibly cloudy.

Step 6 Place 20 to 30 µl of the cell suspension (depending on well size) on each of two wells of a clean slide. Change pipette tips for each sample. Allow the slide to air dry completely.

Note: For the Optional Bivalent Staining procedure, place 20 to 30 µl of the cell suspension (depending on the well size) on one well of a clean slide.

Step 7 Place the slide in a Coplin jar or beaker and fix with acetone at room temperature (15 to 28°C) for 10 minutes. Discard the acetone after every 10–12 slides.

Step 8 Remove the slide from the acetone and allow to air dry completely.

If the fixed slides cannot be stained immediately, they may be refrigerated at 2 to 8°C for up to 24 hours. If a fixed slide cannot be stained within 24 hours, it can be stored at –20°C with desiccant in a closed container.[13] Allow slides to warm to room temperature (15 to 28°C) before staining.

Basic Typing Staining Procedure

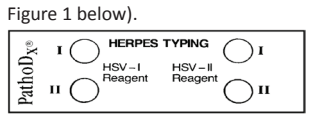
All components should be at room temperature (15 to 28°C) before staining. Mix the antibody reagents thoroughly by gently swirling the bottles.

Caution: Observe aseptic technique throughout the test procedure.

Direct Specimen and Culture Confirmation

Step 1 Place one to two drops of HSV-I Typing Reagent on the first well of the paired sample. Make sure the reagent covers the entire well. On the Herpes Typing Control Slide, place the HSV-I reagent on the left pair of wells labeled I and II, which contain HSV-I and HSV-II infected cells, respectively (see Figure 1 below).

Step 2 Place one to two drops of HSV-II Typing Reagent on the second well of the paired sample and control wells. Make sure that the reagent covers the entire well. On the Herpes Typing Control Slide, place the HSV-II reagent on the right pair of wells labeled I and II, which contain HSV-I and HSV-II infected cells, respectively (see Figure 1 below).



Step 3 Place the slides in a moist, darkened container and incubate for 30 minutes at room temperature (15 to 28°C). Do not allow the slides and the reagent to be exposed to light for extended periods. Do not allow the stain to dry on the slide, as nonspecific staining may occur at the edge of the well.

Step 4 Remove the slides from the box and rinse each well with a stream of PBS from a pipette directed above and to one side of each well. Ensure that there is no carryover of stain between wells.

Step 5 Place the slides in a Coplin jar with PBS. Wash the slides with agitation for 2 to 4 minutes, including one change of PBS. (Alternatively, use a dispensing bottle to wash slides with PBS or distilled water. If distilled water is used, allow slides to air dry before adding Mounting Fluid.)

Step 6 Tap or blot any excess PBS from the slide. Do not allow to dry.

Step 7 Immediately add a drop of Mounting Fluid and apply cover slip. Remove any air bubbles and excess mounting fluid with absorbent paper.

Step 8 The slide should be read immediately using a fluorescence microscope at 160–250X magnification. The morphology of infected cells may be confirmed at 400–630X magnification. If necessary, the slide may be stored in the dark at 2 to 8°C and read within 24 hours. Allow the slides to come to room temperature (15 to 28°C) before reading. After reading, the slides may be stored under desiccation at –20°C or –70°C.

Optional Bivalent Staining Procedure

This optional procedure may be considered for the detection and typing of HSV for culture confirmation slides and shell vial assays.

Step 1 Follow the test procedure for staining control slides as outlined above in the Basic Staining Procedure.

Step 2 Place one drop of HSV-I and HSV-II Typing Reagents in each sample well or shell vial.

Step 3 For slides follow steps 3 through 8 as outlined above in the appropriate sections.

Step 4 For shell vials see steps 11 through 15 in procedure below.

Shell Vial Culture Procedure

Using aseptic technique throughout the procedure, aspirate and discard the maintenance medium from the shell vials to be inoculated. Do not allow the cell monolayer to dry.

Step 1 Inoculate two shell vials with **0.2 ml** sample material and recap the vials.

Step 2 Centrifuge the vials at 600–700×g for 45 minutes at 18 to 35°C.

Step 3 After centrifugation, aspirate the inoculum and wash the monolayer with **2 ml** of sterile PBS or maintenance medium (pre-warmed to 35 to 37°C). Aspirate any remaining liquid. Add **2 ml** of fresh tissue culture maintenance medium before incubation.

Step 4 Incubate the duplicate shell vials at 33 to 36°C for 24 to 26 hours.

Step 5 Aspirate the maintenance medium so as not to disrupt the cell monolayer on the cover slip inside the vial.

Step 6 Rinse the monolayer twice with **1 ml** of PBS, added down the inside of the vial to avoid disrupting the cell monolayer.

Step 7 After aspirating the final rinse, immediately add **1 ml** of acetone.

Step 8 Aspirate the acetone and add **1 ml** fresh acetone. Allow the vials to fix for 10 minutes at room temperature (15 to 28°C).

Step 9 Remove the acetone and allow the cell monolayer to dry. (If the coverslip is not to be stained immediately, recap the vial and store at –20°C or below. When the cover slip is ready to be stained, allow the vial to reach room temperature before removing the cap.)

Step 10 Rinse the vial with **1 ml** PBS to increase surface cohesion. Aspirate the PBS from the vial before adding typing reagents.

NOTE: For the Optional Bivalent Staining Procedure, add 2 to 3 drops of each Typing Reagent to one vial.

Step 11 Add 2 to 3 drops (approximately 150 µl) of the HSV-I Typing Reagent to the vial, making sure that the reagent covers the entire cover slip. Repeat with the HSV-II Typing Reagent with the second shell vial. Recap the vials and incubate at room temperature (15 to 28°C) for 30 minutes in the dark.

Step 12 Aspirate the HSV-I and HSV-II Typing Reagents from the respective shell vials and wash each two times with **1 ml** of PBS.

Step 13 Remove the cover slips with a bent needle or forceps and gently tap or touch the edge of the coverslips on blotting paper to remove any excess PBS.

Step 14 Mount both cover slips on a glass microscope slide by placing them cell-side down onto a drop of Mounting Fluid.

Step 15 Remove any air bubbles and excess Mounting Fluid from the cover slips with absorbent paper. Examine by fluorescence microscopy using 100X to 400X magnification.

Notes on Technique

1. Fluorescence around the infected cells and in the background may occur if the CPE has progressed further than 2+ (50%) due to release of specific antigens from the cells. To avoid this problem, ensure that the infection of the monolayer has not progressed beyond this point before staining.
2. Artifacts may occur if the wells are allowed to dry after washing with PBS. Should the wells dry, rinse the slide briefly in PBS and mount immediately. Conversely, poor fluorescence may be seen if slides washed in distilled water are not allowed to dry before mounting.
3. Acetone will absorb moisture if it is not properly stored. This moisture will cause a nonspecific hazing following staining. Should this occur, use a fresh bottle of acetone and repeat the assay with a freshly prepared slide.

4. Nonspecific background fluorescence or greening may occur with some manufacturers slides due to the manufacturing process. To avoid the problem, wash the slides in acetone for 5 to 10 minutes before use.
5. Nonspecific greening will occur at the periphery of the well if the stain is allowed to dry on the slide. Ensure that there is sufficient stain to cover the well, and that the incubation chamber is well humidified.

8. QUALITY CONTROL

Testing Direct Clinical Specimens: To verify the specificity and staining pattern of each reagent, a Herpes Typing Control Slide should be included with each batch of direct clinical specimens. One well each of HSV-I and HSV-II infected cells (the two left wells) must be stained with HSV-I Typing Reagent and the remaining wells (the two right wells) stained with HSV-II Typing Reagent.

Positive Result: HSV-I infected cells (upper left well) in combination with HSV-I Typing Reagent. HSV-II infected cells (lower right well) in combination with HSV-II Typing Reagent.

Negative Result: HSV-I infected cells (upper right well) in combination with HSV-II Typing Reagent. HSV-II infected cells (lower left well) in combination with HSV-I Typing Reagent.

Testing Isolates from Cell Culture: Known strains or isolates of HSV-I and HSV-II must be cultured along with each group of patient specimens. These isolates act as tissue culture controls for viral growth. In addition, HSV-I and HSV-II Typing Reagents should be tested on slides made from cells infected with the HSV-I and HSV-II controls. These controls aid in the interpretation of patient results and establish reagent specificity, intensity of staining and presence of non-specific staining that may occur with the cell line used.

NOTE: If the positive and negative controls do not react as described in the **Interpretations** section, the reagents and assay performance should be re-evaluated.

9. INTERPRETATIONS

Direct Specimen Slide: Read the Herpes Typing Control Slide first to determine intensity of reaction and pattern of staining. In the sample slide, at least 20 non-superficial epithelial cells must be seen in each well for the specimen to be acceptable. A result is considered to be positive if at least one cell shows typical fluorescent staining with either of the typing reagents (or both reagents in the Optional Bivalent Procedure) as described below. All negative direct specimens or those with inadequate numbers of cells must be re-evaluated by cell culture.

Culture Confirmation: Read the control slide containing the known HSV-I and HSV-II isolates first to determine staining intensity and nonspecific staining. In the event that both HSV-I and HSV-II Typing Reagents stain positive on the culture isolate, retest the reagents on freshly prepared separate slides to confirm a dual infection.

Shell Vial Assay: Read the Herpes Typing Control Slide first to determine the intensity of reactions and patterns of staining, as well as for the presence of nonspecific staining.

Staining Pattern

HSV-I Infected Cells: HSV-I Typing Reagent, will show a diffuse bright apple-green fluorescence throughout. HSV-I infected cells should not show any specific fluorescence in the presence of the HSV-II Typing Reagent.

HSV-II Infected Cells: HSV-II Typing Reagent, will show a speckled distribution (granular pattern) of intense fluorescent staining in the cytoplasm and nucleus. HSV-II infected cells should not show any specific fluorescence in the presence of the HSV-I Typing Reagent.

Uninfected Cells: Uninfected cells counterstain red due to the presence of Evans Blue.

Dual Infection: A dual infection with HSV-I and HSV-II will reflect the staining patterns described above with the individual reagents.

Bivalent Staining: In single-type infections, the staining pattern will reflect that described for HSV-I Typing Reagent or HSV-II Typing Reagent. A dual infection will have a staining pattern that reflects both HSV-I and HSV-II infected cells.

10. LIMITATIONS

1. The intensity of the fluorescence is dependent on the intensity of the fluorescent light source, and may vary depending on bulb, filter size, etc. It is therefore essential that the fluorescence microscope be properly aligned and functioning. Prolonged exposure to light will also result in a decrease in fluorescence intensity.
2. The reagents are supplied at the optimal working strength. Dilution of the reagents will result in suboptimal results.
3. Mixing of reagents for use in the Optional Bivalent Staining Procedure is acceptable. The stability of the mix, however, has not been examined.
4. A negative result upon staining direct patient specimens does not exclude the possibility of an infection with HSV. All specimens negative on staining, or which have insufficient numbers of cells for evaluation, must be tested in cell culture for the presence of the virus. Interpretation of all results must include clinical evaluation of the patient and other diagnostic procedures.
5. Do not use direct specimen results as the sole means of diagnosing HSV when considering a Caesarean section. Prior culture results, when available, and clinical observation should be taken into account.
6. Testing specimens with only one of the two antibody reagents may result in incorrect results. Specimens must be evaluated with both antibody reagents.
7. The effect of concurrent or previous antiviral therapy on test performance is not known.
8. Positive HSV results must be interpreted in light of the patient's clinical circumstances. For example, a positive herpes result from a throat specimen may result from reactivation due to a febrile episode.
9. The direct test is for use only with those lesions symptomatic for HSV infection. It should not be used for diagnosis of asymptomatic individuals such as pregnant women.
10. The occasional transfer of cells between wells may occur during manufacture of the control slide. This may result in a low number of positive cells observed in upper right and lower left wells. The control slide will still serve as a suitable control to indicate reagent performance.

11. PERFORMANCE CHARACTERISTICS

Specificity: To verify the specificity of the PathoDx Herpes Typing Kit, various bacteria, fungi and viruses were stained with the reagents. The organisms (see chart) were grown in broth culture and 10 µl of a suspension containing 1×10^9 colony forming units per millilitre was fixed to a slide containing uninfected tissue culture cells. Viruses were grown in monolayers and tested when 2+ CPE was evident or after 7 days in culture. None of the organisms tested showed any reactivity with the reagents.

Organism	Strain
Acinetobacter calcoaceticus	ATCC® 17904
Adenovirus type 3	ATCC® VR3
Adenovirus type 5	ATCC® VR5
Adenovirus type 7	ATCC® VR7
Adenovirus type 10	ATCC® VR11
Adenovirus type 14	ATCC® VR15
Candida albicans	166 Nelsen/CA
Candida tropicalis	PN 1164
Chlamydia trachomatis Type E	ATCC® VR348B
Coxsackievirus A9	ATCC® VR186
Coxsackievirus B4	ATCC® VR184
Cytomegalovirus AD169	ATCC® VR538
Echovirus 4	ATCC® VR34
Echovirus 20	ATCC® VR50
Echovirus 22	ATCC® VR52
Enterococcus faecalis	PN 1120
Escherichia coli	ATCC® 29194
Haemophilus influenzae Type A	AANEN 1217
Influenza A	ATCC® VR100
Influenza B	ATCC® VR523
Klebsiella pneumoniae	ATCC® 33495
Lactobacillus acidophilus	PN 1126
Lactobacillus casei	PN 1078
Moraxella osloensis	PN 1339
Mycoplasma arginini	ATCC® 23838
Mycoplasma hominis	ATCC® 23114
Mycoplasma pneumoniae	ATCC® 15531
Mycoplasma genitalium	ATCC® 33530
Mycoplasma salivarius	ATCC® 23064
Neisseria gonorrhoeae	PN 1138
Neisseria gonorrhoeae	PN 1177
Neisseria gonorrhoeae	PN 1282
Neisseria meningitidis Group A	ATCC® 13077
Neisseria meningitidis Group B	ATCC® 13090
Neisseria meningitidis Group C	ATCC® 13102
Neisseria meningitidis Group D	ATCC® 13113
Neisseria sicca	PN 1101
Parainfluenza 1 strain C-35	ATCC® VR94
Parainfluenza 2 strain Green	ATCC® VR92
Parainfluenza 3 strain C-243	ATCC® VR93
Proteus mirabilis	ATCC® 25933
Proteus vulgaris	PN 1062
Pseudomonas aeruginosa	PN 1280
Respiratory syncytial virus	Strain 9320
Rhinovirus 32	ATCC® VR329
Rhinovirus 39	ATCC® VR340
Salmonella minnesota	R 595
Staphylococcus aureus Cowan I	ATCC® 12598
Staphylococcus epidermidis	PN 5204
Streptococcus agalactiae	090R
Streptococcus pyogenes	ATCC® 10389
Varicella-zoster virus strain Ellen	ATCC® VR586

In addition, the reagents were tested against common tissue culture cell lines to determine any possible cross-reactivity or nonspecific staining. All cell lines tested were found to be negative.

Cell Line	Strain
A549	ATCC® CCL 185
Baby Hamster Kidney (Clone 13)	ATCC® CCL 10
Flow 5000	Flow 59368
Hep 2	ATCC® CCL 23
L929	ATCC® CCL 1
LLC-MK2	ATCC® CCL 7
McCoy	Bartels
MRC-5	ATCC® CCL 171
RK-13	ATCC® CCL 37
Vero	ATCC® CCL 81
WI-38	ATCC® CCL 75

12. EXPECTED VALUES

Specimens were taken at two investigational sites from herpes-like lesions in male and female subjects according to instructions provided in the package insert. Direct specimens and cultured specimens were tested using the Basic Typing Procedure. Cultures were tested using the PathoDx Herpes Typing reagents and a reference culture identification/typing method. Direct testing was performed with the same PathoDx Herpes Typing reagents utilized for culture testing.

Specimen smears were made, air-dried, fixed with acetone for 10 minutes, and tested according to the manufacturer's package insert. Specimens were also cultured according to standard procedures. Cultures were incubated until 2+ CPE developed (50% of monolayer). Cells were then scraped, spotted onto two wells (approx. 6–8 mm diameter) of a glass slide, air-dried, fixed with acetone, and tested according to standard procedures.

In total, 313 patient specimens, comprising 109 males and 204 females, were tested for herpes at two investigational sites. The overall prevalence, defined by the reference cell culture fluorescent antibody staining method, was approximately 76%. Fifty-one percent of these herpes infections were identified as HSV-I and 49% were identified as HSV-II. PathoDx Herpes Typing correctly identified 169/170 cultures identified as positive by the reference method and 49/49 cultures identified as negative by the reference method (sensitivity 99.4%; specificity 100%, see **Table 1**). Of the 166 non-dual type positive cultures, PathoDx Herpes Typing yielded identical results in all 166 specimens (**Table 2**).

Table 1: PathoDx Culture Identification vs. Reference Culture Identification

PathoDx® / Reference	+ / +	– / –	– / +	+ / –	*n*	Sensitivity	Specificity
Site 1	81	21	0	0	102	100%	100%
Site 2	88	28	1*	0	117	98.9%	100%
Combined	169	49	1	0	219	99.4%	100%

*Identified by PathoDx on direct staining.

Table 2: PathoDx Culture Typing vs. Reference Culture Typing

Site 1 (*n* = 81)		Reference Culture Typing	
		HSV-I	HSV-II
PathoDx Culture Typing	HSV-I	69	0
	HSV-II	0	12

Site 2 (*n = 85)**		Reference Culture Typing	
		HSV-I	HSV-II
PathoDx Culture Typing	HSV-I	24	0
	HSV-II	0	61

*Five specimens not included in the above analysis were identified as dual type I and type II infections by the reference culture method. Of these, three were tested by PathoDx Herpes Typing kit and two were identified as dual type I and type II infections. The third was identified as a dual infection by the reference culture typing, but only as a type I by the PathoDx Herpes Typing kit.

Direct testing with PathoDx Herpes Typing correctly identified 37/40 culture-positive specimens and 27/27 culture-negative specimens when optimal specimen preparation technique was utilized (sensitivity 92.5%; specificity 100%, **Table 3**). In addition, direct typing yielded identical typing results to fluorescent staining of cell culture isolates by the reference method (**Table 3**). When an alternate specimen collection technique was employed involving the spotting of specimen supernatant obtained from vortexing specimen swabs after transport in viral transport medium, direct testing with PathoDx Herpes correctly identified 26/64 culture-positive specimens and 26/26 culture-negative specimens (sensitivity 41%; specificity 100%). Based upon the sensitivity and specificity estimates for the PathoDx Herpes Typing direct testing when optimal specimen preparation technique was utilized (sensitivity 92.5%; specificity 100%), the predictive value for a positive and negative direct result were calculated to be 100% and 81.6%, respectively, in a hypothetical 75% prevalence population.

Table 3: PathoDx Direct vs. Reference Culture Identification

PathoDx / Reference	+ / +	– / –	– / +	+ / –	*n*	Sensitivity	Specificity
	37	27	3	0	67	92.5%	100%

Typing (*n* = 37)		Reference Culture	
		HSV-I	HSV-II
PathoDx Direct	HSV-I	32	0
	HSV-II	0	5

In a separate study at a third site, 139 HSV clinical isolates (85 HSV-1 and 54 HSV-2), all of which were typed by the reference method, were inoculated into cell cultures according to standard procedures and incubated until a 3+ to 4+ CPE developed. The cells were scraped, spotted onto wells, and tested with the PathoDx Optional Bivalent Stain procedure. All 139 specimens were detected and correctly typed using this method (see **Table 4**).

Table 4: PathoDx Optional Bivalent Stain vs. Reference Method

HSV-1 Positive Samples		HSV-2 Positive Samples		% Correlation Between Kits	
Ref	PDx	Ref	PDx	HSV-1	HSV-2
85	85	54	54	100%	100%

In a similar study a total of 86 clinical isolates, known to be positive for HSV, were each inoculated into sets of three shell vials containing coverslips with Vero cell monolayers. The vials were centrifuged at 600×g for 45 min at room temperature, and incubated for 24–26 hours at 37°C. Two of the shell vials for each culture were stained and typed using the reference method. The third was stained and typed using the PathoDx Optional Bivalent Stain procedure. The correlation between the reference and PathoDx methods was 100% (see **Table 5**).

Table 5: PathoDx Bivalent Stain vs. Reference Method for Shell Vial Assay

HSV-1 Positive Samples		HSV-2 Positive Samples		% Correlation Between Kits	
Ref	PDx	Ref	PDx	HSV-1	HSV-2
48	48	38	38	100%	100%

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14. PACKAGING

REF R62250.....100 Tests/Kit

Symbol Legend

REF	Catalogue Number
IVD	*In Vitro* Diagnostic Medical Device
LAB	For Laboratory Use
	Consult Instructions for Use
	Temperature Limitation
LOT	Batch Code
	Use By
	Manufacturer

CE

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IFU X7787A, Revised December 2013 Printed in the UK

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