PathoDx HERPES TYPING KIT

INTENDED USE
PathoDx™ Herpes Typing Kit is an immunofluorescence test designed to identify and type isolates of the Herpes simplex virus (HSV) types I and II (HSV-I and HSV-II) in direct clinical specimens and following growth in tissue culture. The Herpes simplex virus (HSV) types I and II are two dimorphic, DNA viruses with two different capsids and shell vial assays may undergo detection and typing with a blank staining procedure. All direct clinical specimens which are processed with inadequate numbers of cells must be re-examined by cell culture.

EXPLANATION OF THE TEST

Herpes simplex virus (HSV) is an ancient and ubiquitous pathogen, known to cause acute and recurrent infections in humans. The virus enters the mucous membranes (ocular, genital, or oral) and replicates in the infected cells. Directly from infected cells, the virus can also be shed and transmitted to others through vesicle fluid or through the bloodstream. In 1906, it was recognized that HSV consisted of two distinct types, HSV-I and HSV-II, a recognition that was first confirmed by restriction endonuclease mapping. However, only with the availability of monoclonal antibodies against HSV-I and HSV-II have these types been made convenient. Immunologic assays such as ELISA, Enzyme Immunoassay and Immunoperoxidase staining, have frequently been used to identify and type HSV isolates following growth in cell culture along with each group of patient specimens. These may be ATCC strains, MacIntyre strains, some of which are identified laboratory isolates (approximately 5 mm), and shell vial assays may undergo detection and typing with a blank staining procedure. All direct clinical specimens which are processed with inadequate numbers of cells must be re-examined by cell culture.

DEFINITIONS

- **HSV-I**: Typing Reagents: A protein-stabilized buffer solution.
- **HSV-II**: Typing Reagents: A protein-stabilized buffer solution.
- **Cell Culture** Speaker of HSV-I and HSV-II Typing Reagents from the well size) on one well of a clean slide.
- **Control Slides**: The controls contain mammalian cells (RK-13) infected with HSV-I (MacIntyre) and HSV-II (MS). These controls are included in each kit; however, if additional controls are needed, reagents are commercially available. The morphology of infected cells may be confirmed at 160X-250X and 400X-630X magnification.

- **CPE** (cytopathic effect): The viral infection will cause an inability to detect the type of infection present. The specimen will be confirmed at 160X-250X and 400X-630X magnification.

- **Culture Confirmation Slide Preparation** Speaker of HSV-I and HSV-II Typing Reagents from the well size) on one well of a clean slide. Change pipette tips for each sample. Allow the slide to air dry before adding Mounting Fluid.

- **Direct Typing Procedure** Speaker of HSV-I and HSV-II Typing Reagents from the well size) on each of two wells of a clean slide. Change pipette tips for each sample. Allow the slide to air dry before adding Mounting Fluid.

- **Direct Typing** Speaker of HSV-I and HSV-II Typing Reagents from the well size) on a well of a clean slide.

- **Formalin-fixed paraffin-embedded tissue** (FFPE) material: A technique used to preserve tissue samples for long-term storage and subsequent analysis. The tissue samples are fixed in formalin, embedded in paraffin wax, cut into thin sections, and stained with various dyes for microscopic examination.

- **Indirect Typing** Speaker of HSV-I and HSV-II Typing Reagents from the well size) on one well of a clean slide. Change pipette tips for each sample. Allow the slide to air dry before adding Mounting Fluid.

- **Immunofluorescence** A technique used to detect and localize specific antigens in tissues or cells by using fluorescently labeled antibodies. The antibodies bind to the antigens, and the fluorescence is detected under a microscope.

- **Monoclonal Antibody** A type of antibody that is produced by a single clone of genetically identical B lymphocytes. These antibodies are specific for a single epitope on an antigen and are used in various diagnostic and research applications.

- **Negative Control** Speaker of HSV-I and HSV-II Typing Reagents from the well size) on one well of a clean slide.

- **Positive Control** Speaker of HSV-I and HSV-II Typing Reagents from the well size) on one well of a clean slide.

- **Sample** A portion of the material being tested for the presence or absence of a specific antigen or analyte.

- **Specimen** A material collected from a patient for diagnostic or research purposes. Specimens can include blood, tissue, fluids, or other biological materials.

- **Tissue culture** A technique used to grow cells in a controlled environment, typically in a petri dish or vial. This allows researchers to study the behavior of cells under various conditions.

- **Vesicle**: A fluid-filled structure found on the skin or mucous membranes. Vesicles can be open or closed and may contain fluid filled with viral particles.

- **Viral Transport Medium**: A solution used to transport clinical specimens to the laboratory for analysis. It contains buffers, nutrients, and preservatives to maintain the viability of the virus.

- **Well**: A small, circular depression in a glass slide where reagents are applied in a micro-dilution format.

- **Waste Material** Materials that are contaminated with infectious substances and require special handling and disposal.

- **Wipe**: A material used to clean surfaces or collect specimens. Wipes can be made of various materials, such as cotton, gauze, or nylon, and can be used with solvents or disinfectants.

- **Wipe Solutions** Solutions used to prepare surface wipes, such as those containing alcohol, disinfectants, or other antimicrobial agents.

- **Working Solution** A concentration of a reagent that is used in diagnostic procedures, typically prepared from the stock solution by dilution with a suitable solvent.

- **Xylene**: A volatile, odorless, flammable liquid used in histology to remove paraffin from tissue sections for microscopic examination.

- **Xylene Fixative**: A solution used to fix tissue specimens in xylene for subsequent histological examination.

- **Yeast Extract**: A nutrient source used in media to support the growth of various microorganisms, particularly fungi.

- **Zinc Chloride Solution**: A solution used to fix tissue specimens in preparation for histological examination.
10. The occasional transfer of cells between wells may occur due to manufacturing of the product. To avoid the problem, wash the slides after application of the reagents and use a transfer pipette to avoid cross-contamination.

11. Nonspecific greening will occur at the periphery of the well if the slide is allowed to dry on the slide. Ensure that there is sufficient stain to cover the well, and that the incubation is conducted in a humidified chamber.

II. CONTROL PROTOCOL

Testing Direct Clinical Specimens: To verify the specificity and sensitivity of the PathoDx Herpes Typing Kit, the slides should be included with each batch of direct clinical specimens. One well of HSV-I and HSV-II infected cells (the left two wells) was stained with HSV-I Typing Reagent, and the other well (the two right wells) was stained with HSV-II Typing Reagent.

Positive Reactions: To verify the specificity of the PathoDx Herpes Typing Kit against HSV-I and HSV-II infected cells, positive reactions were observed with HSV-I Typing Reagent. HSV-I infected cells (lower right well) in combination with HSV-II Typing Reagent.

Positive Result: HSV-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 clinical specimens to determine the specificity and sensitivity of the kits in vitro and in vivo. In addition, HSV-I and HSV-II Typing Reagents should be tested against common tissue culture isolates infected with the HSV-I and HSV-II controls. These controls aid in the interpretation of patient results and help to 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 this section, the reagents and assays should be re-evaluated.

III. 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 should be included with each batch of direct clinical specimens. These isolates act as tissue culture controls for the sample slides. The controls were included with each batch of direct clinical specimens. To verify the specificity and sensitivity of the 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 by a titer of 1/40 by the Herpes Typing kit.

Direct testing with PathoDx Herpes Typing correctly identified 37/40 culture-positive specimens and 27/27 culture-negative specimens when optimal preparation technique was utilized (sensitivity 92.5%; specificity 100%). Table 3 in Appendix 2. In addition, direct typing yielded identical typing results to fluorescent staining of cell culture isolates by the Reference Laboratory. When an alternate specimen collection technique was employed including the spotting of specimen supernatant obtained from a previous experiment of specimens after transport in transport medium, direct testing with the PathoDx Optimal Blotant Stain procedure was successfully completed (sensitivity 46%; specificity 100%). 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 result were calculated to be 100% and 86%, respectively, in a hypothetical prevalence population.

IV. SUMMARY

11. In a similar study at a third site, 139 HSV clinical isolates (85 HSV-I and 54 HSV-II), 100% were detected and correctly identified 108/139 cultures identified as positive by the reference method, were inoculated into cell cultures according to standard procedures. A third was stained and typed using the PathoDx Optimal Blotant Stain procedure. The correlation between the reference and PathoDx methods was 100% (see Table 4).

5. Positive HSV results must be interpreted in light of the patient's clinical circumstances. For example, a positive result may indicate a recent history of genital herpes or may occur in a patient with a history of recurrent infections. The presence of HSV in the genital area, for example, may indicate a recent history of genital herpes or may occur in a patient with a history of recurrent infections.

2. Positive HSV results must be interpreted in light of the patient's clinical circumstances. For example, a positive result may indicate a recent history of genital herpes or may occur in a patient with a history of recurrent infections. The presence of HSV in the genital area, for example, may indicate a recent history of genital herpes or may occur in a patient with a history of recurrent infections.

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 to ensure that there is sufficient stained material for examination.

12. EXPECTED VALUES

Specimens were taken from 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 in accordance with the 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 instructions. Specimens were also cultured according to standard procedures. Cultures were inoculated until 2+ CPE developed (approx. 6–8 mm diameter) of a glass slide, air-dried, fixed with acetone for 10 minutes, and tested according to the manufacturer's package instructions.

In total, 313 patient specimens, comprising 109 males and 204 females, were tested for herpes at two investigational sites. In the sample slide, at least 20 non-superficial epithelial cells should be included with each batch of direct clinical specimens.

10. In a similar study at a third site, 139 HSV clinical isolates (85 HSV-I and 54 HSV-II), 100% were detected and correctly identified 108/139 cultures identified as positive by the reference method, were inoculated into cell cultures according to standard procedures. A third was stained and typed using the PathoDx Optimal Blotant Stain procedure. The correlation between the reference and PathoDx methods was 100% (see Table 4).

In a separate study at a third site, 139 HSV clinical isolates (85 HSV-I and 54 HSV-II), 100% were detected and correctly identified 108/139 cultures identified as positive by the reference method, were inoculated into cell cultures according to standard procedures. A third was stained and typed using the PathoDx Optimal Blotant Stain procedure. The correlation between the reference and PathoDx methods was 100% (see Table 4).

For technical assistance please contact your local distributor.