1. INTENDED USE

The MicroTrak® HSV 1/HSV 2 Direct Specimen Identification/Typing Test is intended for use in the identification and typing of herpes simplex virus from external lesions. All negative and inadequate specimens must be verified by tissue culture.

2. SUMMARY

Herpes simplex viruses (HSV) are ubiquitous among humans. Once acquired, HSV can remain latent in the regional sensory ganglia and, when reactivated, move back along the sensory nerves to produce recurrent infections (1,2). HSV infections include genital lesions, cold sores, pharyngitis, ocular keratitis, and encephalitis (3).

The viruses are classified as type 1 or 2 according to their genetic and antigenic composition. Although each type has been associated with a characteristic pattern of infection (oral HSV 1 and genital HSV 2), the site of infection is not an accurate predictor of the virus type. For example, HSV 1 is now suspected to cause a significant proportion of primary genital herpes (4,5).

Specific diagnosis of HSV infection is required in many situations, including infections of neonates, immunocompromised patients, or individuals suspected of having herpes encephalitis (3,6). In addition, specific diagnosis is useful in the counseling of sexually active individuals. Typing may have utility in (a) diagnosis, since it has been reported that the recurrence rate of genital HSV 1 infection is less than that of genital HSV 2 (4,5), (b) treatment, since it has been reported that the antiviral activity of chemotherapeutic agents can differ between the two HSV types (7), and (c) epidemiological research, where an association of HSV infection with other disease processes such as cervical carcinoma is being studied (8).

Virus isolation in tissue culture is routinely used for diagnosing HSV infections. While tissue culture amplifies small numbers of infectious organisms for detection, it requires special facilities and 1-7 days before a result can be reported (9,10). While culture has stood as a generally recognized reference method, recovery is recognized to be less than 100%.

Direct examination of viral antigen obtained from lesions provides results more rapidly (11-14). A poorly prepared slide can be rapidly identified (see Section 8, Evaluation and Interpretation of Test Results) so that another specimen can be obtained promptly. It must be recognized, however, that stained specimens identify viral antigen and no association with infectivity can be made.

Several laboratory methods of typing HSV have been reported (7,9), including plaque size, pock size on chorioic membrane, neutralization, ELISA, restriction endonuclease analysis, BDVU [E-5-(2-bromovinyl)-2-deoxyuridine] sensitivity, and immunofluorescence.

If typing is desired, the MicroTrak® HSV 1/HSV 2 Direct Specimen Identification/Typing Test can provide typing results within 30 minutes of specimen receipt. The test allows a simple, rapid procedure for the identification and typing of HSV in clinical specimens taken directly from external lesions (5,11,15).

Because direct antigen tests are not 100% sensitive and because of the potential for inadequate direct smears, specimens for tissue culture must be collected concurrently with specimens for the direct test. Direct specimens that are negative or inadequate for analysis should be verified by tissue culture.

3. PRINCIPLE

Monoclonal antibodies that react specifically with HSV 1 or HSV 2 have been prepared and labeled with fluorescein isothiocyanate. Three monoclonal antibodies used in the reagents have been described previously (15). One of these reagents contains a glycoprotein C complex that is specific for HSV 1 while the other reagents react with either a 140,000 dalton protein or a 38,000 dalton protein, both of which are specific for HSV 2. A fourth monoclonal antibody recognizes an HSV 2-specific epitope of a glycoprotein B doublet of 120-130,000 daltons.

After specimen is applied directly to paired slide wells, one well is stained with HSV 1 Reagent and the other with HSV 2 Reagent. The antibody conjugates bind specifically to their respective viral antigens and a rinse step removes unbound antibody. When slides are viewed under a fluorescence microscope, cells that are positive for the particular viral type show apple-green fluorescent staining that is characteristic of infection with HSV 1 or HSV 2, as demonstrated in the positive control wells; negative cells show only reddish-brown counterstaining, as demonstrated in the negative control wells (11,15).

Absence of positive cells in the specimen slide wells should be interpreted cautiously. Cell culture must be initiated at the same time the direct specimen is taken. This allows recourse to the culture result if a direct specimen is negative or inadequate for analysis.

4. PRODUCT DESCRIPTION, PREPARATION AND STORAGE

<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Product Description</th>
<th>Quantity/Volume</th>
</tr>
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<tbody>
<tr>
<td>8H349UL</td>
<td>MicroTrak® HSV 1/HSV 2 Direct Specimen Identification/Typing Test, consisting of: HSV 1 Reagent*</td>
<td>60 tests</td>
</tr>
<tr>
<td></td>
<td>HSV 2 Reagent*</td>
<td>2.0 ml</td>
</tr>
<tr>
<td></td>
<td>Mounting Fluid</td>
<td>6.0 ml</td>
</tr>
<tr>
<td></td>
<td>Reconstitution Diluent</td>
<td>2 x 5.0 ml</td>
</tr>
<tr>
<td>8H329UL</td>
<td>MicroTrak® HSV 1/HSV 2 Specimen Collection Kits**, each kit consisting of: Dual-well glass slide</td>
<td>20 kits</td>
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<tr>
<td></td>
<td>Dacron swabs (one large, one small)</td>
<td>1 slide</td>
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<tr>
<td></td>
<td>Acetone fixative</td>
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<td></td>
<td>0.5 ml</td>
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<td>8H399UL</td>
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<td>5 slides</td>
</tr>
<tr>
<td></td>
<td>HSV 2 Antigen Control Slides</td>
<td>5 slides</td>
</tr>
</tbody>
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* The HSV 1 and HSV 2 Reagents are supplied lyophilized. The indicated volume is that required for reconstitution. ** Sold separately.

Reagents and Reconstitution Diluent

The reagents contain fluorescein-labeled, purified murine monoclonal antibodies specific to HSV 1 or HSV 2, Evans blue counterstain, and a suppressor of non-specific staining in a protein-stabilized buffer solution.

The reconstitution diluent contains 0.1% sodium azide in deionized water.

To reconstitute a lyophilized reagent, remove the metal seal and mark the stopper to identify it with the original vial. Remove the rubber stopper; add 2.0 ml reconstitution diluent from one of the provided vials and then discard the remaining 3 ml diluent. Replace the stopper and gently swirl the vial to dissolve the powder. Record the reconstitution date on the reagent vial label. Repeat this procedure for the other reagent, using the second vial of reconstitution diluent. After reconstitution, allow the reagents to remain at a room temperature of 20-25°C for 30 minutes before use.

Store the reagents at 2-8°C when not in use. Do not freeze or expose to temperatures above 32°C. Do not expose to strong light. When handled as directed, the reconstituted reagents can be used for 12 weeks.

Precautions:

The MicroTrak® HSV 1/HSV 2 Direct Specimen Identification/Typing Test is designed for in vitro diagnostic use.

The direct specimen reagents and reconstitution diluent contain sodium azide. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.

Never pipette by mouth.

The following instruction should be adhered to when opening the silver flip seal cap:

- A tweezers, needle nose pliers, forceps, de-cappers, spatula or similar type of object should be used to open and peel off the flip-seal from the vial. When doing this action, ensure it is done outwards, away from the body.
- Latex gloves should also be worn to provide further protection to the user.

Mounting Fluid

Mounting fluid at pH 9.4 contains phosphate buffer, glycerol, and an agent to retard photobleaching. Store the mounting fluid at 2-8°C.

Control Slides

Each of the two control slides has two wells. The HSV 1 Antigen Control Slide contains fixed HSV 1-infected cells (ATCC strain MacIntyre). The HSV 2 Antigen Control Slide contains fixed HSV 2-infected cells (ATCC strain MS). In the staining procedure, HSV 1 Reagent is added to the left-hand well of each slide. HSV 2 Reagent is added to the right-hand well of each slide. The left well on the HSV 1 Antigen Control Slide and the right well on the HSV 2 Antigen Control Slide then serve as positive controls. The right well on the HSV 1 Antigen Control Slide and the left well on the HSV 2 Antigen Control Slide serve as negative controls. Because all four wells on the two slides actually contain HSV-infected cells, the slides provide an intertype specificity check in addition to a positive/negative reagent check.

Slides are provided in individual, sealed foil packs containing desiccant. Store the sealed slides at 2-8°C. Before removing a slide from its foil pack, leave it at a room temperature of 20-25°C for at least five minutes. Place the control slide immediately after removing it from the pack.
Precautions:
The viruses on MicroTrak control slides have been shown to be noninfectious in culture; however, users are advised to observe the same safety precautions as employed when handling and disposing of other potentially infectious biological materials. Note: Do not use the reagents, reconstitution diluents, control slides, or mounting fluid after the expiration dates printed on the container labels.

5. MATERIALS

Materials Provided
MicroTrak HSV 1/HSV 2 Direct Specimen Identification/Typing Test (contains reagents, reconstitution diluents, and mounting fluid)

Note: Use only the materials provided.

Materials Required but Not Provided
– For Direct Specimen Test Procedure
  • MicroTrak HSV 1/HSV 2 Direct Specimen Identification/Typing Test Control Slide Pack (contains HSV 1 Antigen Control Slides and HSV 2 Antigen Control Slides)
  • Recommended: MicroTrak HSV 1/HSV 2 Specimen Collection Kit (contains acetone in glass ampule with disposable dispenser, dual-well slide that allows specimen and reagent concentration for optimal sensitivity; large and small swabs; and a transport container with instructions for collection).
  • If the MicroTrak HSV 1/HSV 2 Specimen Collection Kit is not used, care should be taken to obtain specimen collection materials with the specifications described below.
  • Microscope slides with 8 mm wells – acetone-resistant paint required; wells should be sufficiently spaced to prevent reagent crossover. Sterile Dacron swab with rigid shaft to withstand forceful scraping of lesion. Acetone fixative – fresh, HPLC grade or equivalent; stored in glass. Container for slide transport to lab.
  • Sterile swab for removing unwanted pus from lesion
  • Sterile needle
  • Sterile water
  • Micropropels – 30 μl
  • Blotting paper
  • Moist chamber
  • Rinse – deionized or distilled water
  • Coverslips – 22 x 40 mm, #1 recommended
  • Fluorescence microscope with filter system for fluoroscence isothiocyanate (FITC); i.e., maximum excitation wavelength = 490 nm, mean emission wavelength = 520 nm; 200-250x and 400x magnification (oil not required).

Note: A well-functioning fluorescence microscope is crucial. Variations in bulb wattage, intensity and alignment, type of illumination, and filters may affect test performance. Use a positive control to verify adequate functioning of the staining procedure and microscope.

For Culture Isolation Procedure

Note: HSV tissue culture should only be attempted by laboratories experienced in procedures for viral isolation.

Syringe and sterile 26 or 27 gauge needle
Sterile swab (note that it has been reported that calcium alginate swabs are inhibitory to HSV tissue culture [10]).

Transport medium – commonly contains 1-2 ml sterile 10% nonhuman serum with either a balanced buffer solution or a tissue culture medium, and antibiotics. Appropriate media are noninhibitory to both HSV and tissue cells. May also contain charcoal or bentonite.

Cell cultures – use cell cultures determined to be susceptible to HSV infection; appropriate monolayers may include human embryonic kidney cells, human embryonic lung fibroblasts, human foreskin fibroblasts, and primary rabbit kidney cells.

Prepare a sufficient number of culture controls to ensure that each series of specimens can be read in parallel with positive and negative controls. The suspension medium and monolayers used to prepare positive and negative controls should be identical to those used for clinical specimens. For positive controls, inoculate a cell monolayer with a suspension of known HSV-infected cells (e.g., ATCC strains MS VR-540, or Mabthrype VR-539, or previously identified laboratory isolates). If an intertype specificity check is desired, HSV 1 and HSV 2 positive control strains can be used in parallel with positive and negative controls. The suspension medium and monolayers used are noninhibitory to both HSV and tissue cells. May also contain charcoal or bentonite.

Other media, supplies, equipment: depends on culture and typing methods employed.

(For further information on cell culture, see references 9 [pp. 329-336] and 16 [pp. 784-786].)

6. SPECIMEN COLLECTION

Collection of an adequate specimen is critical to the performance of any direct microbiological procedure. Samples of poor quality may be inadequate for analysis or result in false negative determinations. A sample for isolation in cell culture must be taken at the same time as the direct specimen. Culture is then available for analysis if a direct specimen is negative, contains too few cells, or contains inappropriate cell types.

External herpes lesions usually begin as one or more fluid-filled vesicles on a rash-like base of skin. After a few days, these vesicles break open and form shallow ulcers. As the outbreak subsides, the ulcers gradually crust over and the skin heals itself with minimal scarring. For best results, sample from early stage vesicular lesions rather than ulcerative or crusted lesions. Use of creams, lotions, ice, alcohol, vaginal sprays, sitz baths, etc., may reduce virus yield significantly. If possible, patients should avoid these topical treatments prior to specimen collection.

Precaution: During the collection and processing of samples, users are advised to observe the same safety precautions as employed when handling and disposing of other potentially infectious materials.

Note: To facilitate specimen collection for smears, use the MicroTrak HSV 1/HSV 2 Specimen Collection Kit. Also, see Section 12, Critical Parameters, and Section 13, Problem-Solving.

1. To expose the base of lesion:
   • Vescicles: Use a sterile needle to lift up the cap of the vesicle. Note that while vesicular fluid is not an adequate sample for the direct test, it is ideal for virus isolation. If vesicular fluid is present, aspirate with a sterile needle and syringe and inject it into an appropriate transport medium (TM).
   • Ulcers: Use a sterile swab to remove any unwanted pus without disturbing the base of the lesion; dispose of swab as appropriate for biologically contaminated material.
   • Crusts: Use a sterile needle to expose the base of the lesion.

2. Moisten the small Dacron swab in sterile water and vigorously swab the entire base of the lesion.
   • Vigorous scraping of lesion base is essential and can be expected to cause patient momentary pain.
   • Avoid drawing blood if possible.
   • Most virus-infected cells are at the base of lesion; laboratories require these nonsuperficial epithelial cells for diagnosis.

3. Immediately after sampling, firmly smear smear on each slide well, beginning in center and working in circles to edge of each well. Distribute equal amounts of material to each well. Use portion of swab that holds most material (e.g., material may be concentrated at swab tip).
   • Cover wells evenly and entirely.
   • Stay within well perimeters.

4. Immediately check each slide well for coverage. If necessary, hold the slide up to the light to examine each well. If no cloudy film is seen, reapply the swab over the well.

5. Dispose of swab as appropriate for biologically contaminated material.

Precaution: Observe the same safety precautions as employed when disposing of other potentially infectious materials.

6. Allow the specimen to air dry completely. Insufficient drying can result in cell loss during fixation.

7. As the direct specimen is drying, collect a sample for virus isolation if vesicular fluid was not collected. Press a sterile swab firmly against the edges and base of the lesion. Alternatively, samples for culture isolation can be taken simultaneously with the direct specimen.

Note: Swabs provided in the MicroTrak HSV 1/HSV 2 Specimen Collection Kit have not been evaluated in sample collection for culture.

8. Immense the swab in TM, swirl vigorously, and then express fluid by pressing the swab tip on the side of the tube. To avoid possible toxicity, do not leave swab in TM. Dispose of swab.

Precaution: Observe the same safety precautions as employed when disposing of other potentially infectious material.

9. When the direct smear is completely dry, lay the slide flat, flood it with 0.5 ml acetone fixative, and let the entire quantity evaporate.

10. Storage/Transport
   • Direct Slide: Store/transport either at a room temperature of 20-30°C or refrigerated at 2-8°C. For best use of results, stain slide immediately upon receipt in the laboratory so that inadequate specimens may be identified and immediately reported to the physician so that a second sample may be obtained (see Section 8, Evaluation and Interpretation of Test Results). If not stained within 3 days, the fixed specimen should be stored desiccated at ~70°C where its longevity will depend on its original quality and antigen titer. Never store the HSV specimen at ~20°C.
   • Culture TM: The culture specimen can be stored either refrigerated at 2.4°C or frozen at ~70°C until direct specimen test results are available (see Section 8, Evaluation and Interpretation of Test Results). Never store the HSV specimen at ~20°C. Avoid repeatedly freezing and thawing the specimen.

(For further information on specimen collection, see references 9 [pp. 325-326], 10 [pp. 1-7], and 16 [p. 784].)

7. PROCEDURE

Instructions

Note: Use of aseptic technique throughout this procedure is recommended.

A. Setup

1. Prepare the reagents according to the directions in Section 4.

2. Allow the reagents, control slides, and patient specimens to reach room temperature before use. To prevent condensation, leave the control slides in the foil packs with desiccant while bringing them to room temperature.

3. Swirl each reagent vial to mix thoroughly before use.
Note: To verify the performance of the staining procedure and microscope, stain and read an HSV 1 Antigen and an HSV 2 Antigen control slide in parallel with each series of patient specimens. Treat control slides exactly as specimens throughout the procedure described in this section. Use the appearance of control slides as a reference in evaluating patient specimens.

Precaution: The viruses on MicroTrak® control slides have been shown to be noninfectious in culture; however, users are advised to observe the same safety precautions as employed when handling and disposing of other potentially infectious biological materials.

B. Staining and Mounting
1. To stain a control slide, place 30 μl of HSV 1 Reagent in the left-hand well. Change the pipette tip, then place 30 μl of HSV 2 Reagent in the right-hand well.

2. To stain a patient specimen, place 30 μl of HSV 1 Reagent in the left-hand well. Change the pipette tip, then place 30 μl of HSV 2 Reagent in the right-hand well.

Ensure that the entire area of each well is covered. Return the reagents to refrigeration immediately after staining. (Also see Section 12, Critical Parameters.)

Precaution: Observe the same safety precautions as employed when handling and disposing of other potentially infectious biological material.

Note: If necessary, spread the reagent over the smear with the pipette tip to avoid taking care not to disturb the fixed specimen. After spreading, always change pipette tips. Cross-contamination of reagents and samples could cause false results.

2. Incubate the slides in a well-humidified chamber for either 15 minutes at 37°C or 30 minutes at room temperature. Do not allow the antibodies to dry on the specimen; drying will cause dense staining around the perimeter of the well. If slides have this staining pattern, repeat the test. To prevent drying, periodically inspect the chamber for adequate humidification and remove the slides from incubation promptly at either 15 or 30 minutes. Do not expose slides to light stronger than normal U.V. room light during incubation. (Also see, Problem-Solving.)

3. Aspirate the excess reagent.

4. Immediately rinse the slides for 5-10 seconds with a gentle stream of deionized or distilled water from a wash bottle. Aim the stream of water at the slide surface above the wells. Hold the slide at a 45° angle as indicated in the drawing. Direct a gentle but continuous stream of water back and forth above the wells so that the water flows down over the well. Gently shake off excess water and wick the remaining moisture from the edge of each slide with blotting paper. Allow them to air dry.

Note: Do not direct the stream of water directly at the wells.

5. Add a drop of mounting fluid to each slide well. Place a coverslip on top of the slide and remove all air bubbles.

Note: Use only the mounting fluid that comes in the same kit as the reagent.

C. Reading

Read the slides using a suitable fluorescence microscope (see Section 5, Materials). For optimum clarity, use 200-250x magnification for screening and 400x magnification for confirmation of morphology.

Note: If it is not possible to read the slides immediately after staining, store them in the dark at 2-8°C and read them within 24 hours for best results. Allow the slides to reach room temperature before reading, or condensation will occur, obscuring the specimens.

8. EVALUATION AND INTERPRETATION OF TEST RESULTS

Scan each well for intact cells displaying fluorescent staining that is characteristic of infection with HSV 1 or HSV 2, as described here:

Cell type
Nonsuppurative epithelial: typically basal/parabasal or multinucleated giant cell

Distribution of staining
Entire cell, greater concentration around edge

Quality of staining
Fluorescent, apple-green, granular (clustered points of light)

Disgard staining of cell debris and staining of a flat, nonfluorescing green quality.

Control Slides

The positive controls should show cells with characteristic fluorescent staining; the negative controls should not display characteristic fluorescence although the reddish-brown counterstained cells should be visible. The appearance of the positive control should be used as a reference in evaluating patient specimens.

If the positive controls cannot be distinguished from negative controls, steps should be taken to differentiate among four possible sources of error: (1) the microscope; (2) the staining procedure; (3) the control slides; and (4) the reagents. First, check the expiration dates of the reagents and control slides. If either is expired, discard it and stain new control slides using unexpired components. If the reagents and control slides are not expired, check the microscope alignment and redate the control slides. If differentiation still cannot be made, stain a second set of control slides, observing staining procedures carefully. A satisfactory result at this stage indicates that the original staining procedure may have been faulty. An unsatisfactory result suggests that the reagents may not be functioning. Review the history of the reagents for conformity with instructions in the package insert.

Patient Specimens

Positive diagnosis is made when at least one intact cell displaying characteristic fluorescent staining is identified in a fixed, stained smear.

The differentiation between HSV 1 and HSV 2 is based on the reagent well in which characteristic staining was found. Dual infections with both HSV 1 and HSV 2, though rare, may be diagnosed by identifying characteristic fluorescent staining in both reagent wells.

If the direct specimen is positive in either well, HSV infection is indicated, and the type is determined by the well that is positive. The possibility of dual infections with both HSV 1 and HSV 2 may be checked by typing of isolates.

Negative results are reported when both fixed, stained smears are free of characteristic fluorescent staining. At least 20 intact counterstained nonsuppurative epithelial cells must be visible in each well before negative results can be reported (see below).

No diagnosis can be made with specimens which fail to meet criteria for distinguishing positive or negative results.

Inadequate specimens are reported when (1) neither fixed, stained smear is positive and (2) either or both of the fixed, stained smears contain fewer than 20 intact counterstained nonsuppurative epithelial cells. Polyomaviruses, leucocytes or red blood cells should not be counted. Inadequate specimens cannot be reported as negative. If a specimen is inadequate, obtain a fresh specimen for staining or refer to culture for diagnosis.

If neither direct specimen well is positive (i.e., they are negative, inadequate for analysis, or a combination of these), then the isolation sample must be cultured. For details on cell culture technique, see references 9, 10, and 16.

9. LIMITATIONS

1. Performance of the MicroTrak® HSV 1/HSV 2 Direct Specimen Identification/Typing Test has been established only for the identification and typing of herpes simplex virus in direct specimens from external lesions.

2. The test sensitivity has not been evaluated in certain sampling situations. Do not use the test to detect asymptomatic viral shedding, to evaluate cervical specimens, or to test newborns without lesions. Specifically, do not use the test as routine surveillance of asymptomatic, pregnant women near term or at time of delivery.

3. Optimal performance of this test depends on the collection of an adequate patient specimen and proper slide preparation technique.

4. Performance of this test in the evaluation of patients treated with therapeutic agents has not been established.

5. Poor sample quality may cause direct specimens to be falsely negative or inadequate for analysis. Therefore, all specimens that are negative or inadequate for analysis must be tested by culture.

6. Test results should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

10. EXPECTED VALUES

Of 439 specimens that were adequate for analysis by the direct specimen test, 213 (49%) were positive by a reference culture confirmation test, and 220 (50%) were positive by the direct specimen test (see Section 11, Performance). For the direct specimen test, the percentages of positive HSV results were 48% in female populations, and 52% in male populations; the percentages were 50% in genital specimens, 23% in oral specimens, and 46% in specimens of other anatomical origins. Of the 177 direct positive genital specimens, 13 (7%) were HSV 1 positive and 164 (93%) were HSV 2 positive. All 6 of the direct positive oral specimens were HSV 1 positive.

Expected values may vary depending upon the patient population tested.

11. PERFORMANCE

A. Identification of HSV

Comparison to Tissue Culture

The MicroTrak® HSV 1/HSV 2 Substrate Preparation/Typing Test was compared to cell culture/CPE by four independent laboratories located in the Southern, Northeastern, Western, and Northern United States. At all study sites, cultures were cross validated with the MicroTrak® HSV 1/HSV 2 Culture Confirmation/Typing Test (17). Three study sites were herpes clinics within large metropolitan hospitals; the fourth site was a university associated STD clinic. MicroTrak® positive patients reported to these clinics because of suspected primary or recurrent HSV lesions at various body sites.

Lesions were sampled by scraping the base with two swabs simultaneously. One swab was used to apply specimen directly to a dual-well microscope slide, which was then tested with the MicroTrak® Direct Specimen Test. The second swab was placed in transport medium and sent to the laboratory where two tissue culture tubes were inoculated. Transport media were used were Hanks balanced salt solution and orbital, closed, veal infusion/welgenal stabilizer, minimum essential medium, and welgenal calf serum, or heart infusion, all containing antibiotics; cell lines were used human embryonic lung (diploid) cells, human embryonic tonsil, human embryonic lung, monkey kidney cells, or human foreskin cells (see reference 9 [pp. 322, 325]). Cultures were examined daily for the first 3 to 5 days and every other day for up to 2 weeks. When a monolayer exhibited 2+ CPE, or after 2 weeks if no CPE developed, it was further evaluated with the MicroTrak® HSV 1/HSV 2 Culture Confirmation/Typing Test (17).

A total of 439 samples were processed through the complete diagnostic protocol and data analysis. An additional 157 slides met neither the criteria for positive nor negative determination; the significance of this observation is discussed later in this section. Of the 439 diagnostic samples, 248 were from males, 187 were from females, and 4 were not differentiated by patient sex. By lesion site, 352 of the adequate specimens were genital, 22 were oral, and 65 were of other anatomical origins. The patient populations were not differentiated by age.

Obtaining adequate specimens is a key factor in any attempt, cytological or immunological, to demonstrate HSV in cells (14-16). In evaluating the expected performance of this test, it is essential to recognize the importance of making the diagnosis from slides that meet the established criteria (see Section 8, Evaluation and Interpretation of Test Results). In the clinical
1. Collection of an adequate specimen is critical to the performance of the test. The following instructions will aid in the collection of the cells required for diagnosis:
- Virus-infected cells decrease in number as the herpes lesions heal. For best results, obtain specimens from early vesicular lesions (9,12,13).
- When collecting multiple specimens from the same patient, it is preferable to sample separate lesions. If this is not possible, take the MicroTrak® specimen first from the base of the lesion to avoid collection of serous exudate.
- Before fixing, ensure that material smeared on the slide is visible in the slide wells. When properly covered, the slide well will have an opaque appearance.

2. To verify the performance of the staining procedure and microscope, test an HSV 1 Antigen and an HSV 2 Antigen control slide in parallel with each series of patient specimens. Use the appearance of control slides as a reference in evaluating patient specimens.

3. Swabs provided in the MicroTrak® HSV 1/HSV 2 Specimen Collection Kit are not tested for use in HSV culture testing.

4. Each working reagent (i.e., reagent reconstituted with exactly 2.0 ml reconstitution diluent) is optimized to identify herpes simplex virus. Dilution, adularization, or pooling from different lots of the working reagent may result in loss of sensitivity.

5. Keep each smear within the perimeter of its well; this ensures that reagent will not be drawn out of the well when stained.

6. In the given test procedure, studies have shown that 30 µl of reagent is sufficient to cover a cell smear contained in an 8 mm slide well. Modifications of the procedure may lead to difficulty in covering the entire smear.

7. If only one of the reagents is used, a negative result will not rule out the possibility of infection with the other HSV type; similarly, a positive result will not rule out the possibility of a dual infection with both HSV types. Use both reagents before reporting results.

8. Pooling the type 1 and 2 reagents dilutes the antibodies and may reduce sensitivity. Do not mix type 1 and 2 reagents or use them together on one well.

9. The fluorescence microscope must be equipped with a filter system for observing fluorescein isothiocyanate (FITC) and with a correctly aligned bulb of high intensity.

13. PROBLEM SOLVING

1. If too few cells are repeatedly observed, modify the collection or smearing procedure appropriately (e.g., by pressing harder with the swab during collection or during placement on the slide; by rotating the swab to remove sample from all portions when smearing; by holding slide to light to check coverage).

2. If the counterstained cells or the positive controls are not visible through the fluorescence microscope, replace or realign the bulb and check the filters.

3. Use clean slides only. Foreign matter may cause irregular fluorescent patterns. Take care to avoid contaminating specimen handling. For quality and consistency in specimen collection material, use the MicroTrak® HSV 1HSV2 2 Specimen Collection Kit.

4. Dense staining around the perimeter of the well may indicate that the reagent dried before rinsing or during the incubation. Reagent drying may cause misinterpretation of test results; if wells exhibit this staining pattern, the test should be repeated.

5. The following conditions may cause nonspecific background haze; note the recommendations for eliminating these problems:
   a. Slides other than those provided by Trinity Biotech plc. may have been chemically treated by the manufacturer. Acetone soaking (for 5-10 minutes) of slides before use may alleviate this problem.
   b. Acetone fixative may have hygroscopically absorbed water. Keep acetone bottle tightly capped. Always store acetone in glass.
   c. Drying of the slide after the rinse step may have been inadequate. Ensure that the slide is dry before mounting.
   d. Areas of the slide containing no patient specimen material may be hazy. Smear the entire surface of the well with the swab.
   e. Yellow staining indicates autolysis of infected virus and should be disregarded.
   f. If negative control wells appear to contain positive cells, cells from the positive well may have been transferred by improper rinsing. Rinse the slides for 5-10 seconds with a gentle stream of deionized or distilled water from a water bottle. Aim the stream of water at the slide surface above the wells. Hold the slide at a 45° angle as indicated in the drawing in Section 7, Procedure. Direct a gentle but continuous stream of water back and forth above the wells so that the water flows down over the well. Gently shake off excess water and wick the remaining moisture from the edge of the slide with blotting paper. Allow them to air dry.

Note: Do not direct the stream of water directly at the wells.

Another possible cause of negative control wells appearing positive is reagent cross-contamination. To prevent this, always change pipette tips between use of reagents and always return the reagent rubber stoppers to their original vials.

8. A higher than expected rate of dual infections may indicate that cell material has been smeared between the wells, creating a bridge for reagent crossover. Stay within well perimeters when smearing.

14. RISK AND SAFETY

Sodium Azide

Glycerol
R22: Harmful if Swallowed.
R32: Contact with acids liberates toxic gas.
R36/38: Irritating to eyes and skin.
S36: Wear suitable protective clothing.

Prepared in accordance with requirements foe EEC label
ENEC 247-852-1
ENEC 200-299-5

Notation: Adulteration of reagents, use of instruments without specified capabilities, or other failure to follow instructions as set forth in this labeling can affect performance characteristics and stated or implied label claims.

This product is protected under U.S. Patent No. 4,582,791.

Monoclonal antibodies developed for Trinity Biotech plc., by Genetic Systems Corporation, Woodenville, Washington, U.S.A.
REFERENCES