Laboratory Procedure Manual

Analyte: **Varicella Antibody (gp)**

Matrix: **Serum**

Method: **Enzyme immunoassay**

as performed by: CDC/NCID
Division of Adolescent and School Health
Group 81 MS C22
Atlanta, GA 30329

Contact: William Bellini, MD

Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

File Name	Variable Name	SAS Label
MMRV_F	LBXVGP	Varicella (gp) antibody

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

The presence of IgG antibody to varicella-zoster virus (VZV) is measured using an enzyme immunoassay (EIA) developed by the staff of the National VZV Laboratory (NCID/DVRD/VEHB). This method has been validated against a number of other laboratory-based VZV serologic methods and performs comparably to all of them (manuscript in preparation). Lentil-lectin-purified glycoprotein antigens derived from VZV-infected human fibroblast cells (obtained through CRADA with Merck & Co.) is coated on the wells of a 96-well microtiter plate, which is subsequently incubated with a diluted test specimen. Control (normal tissue) antigen (also obtained from Merck & Co.) is also prepared from uninfected fibroblasts and is plated separately into different wells and incubated with test serum to account for any nonspecific antibody reactivity. After unbound serum components are removed by washing, an antibody-enzyme conjugate, consisting of anti-human IgG antibody coupled to alkaline phosphatase, is added to wells and incubated. The conjugate binds only to human IgG antibodies that are in turn bound to the antigen coated on the plates. A colorimetric substrate for the enzyme is added to the wells and incubated for a sufficient time to permit color development, at which point the reaction is stopped chemically. The enzyme-substrate reaction results in a yellow-colored product that can be measured using a spectrophotometer set to a wavelength of 405 nm. gpELISA has been demonstrated to have both higher sensitivity and specificity than whole cell ELISA; since the antigens are available in limited supply, however, it is used to test all specimens that test in the negative or equivocal range by whole cell ELISA and to detect seroconversion to vaccine (which requires the higher sensitivity).

Varicella-zoster virus (VZV) is the etiologic agent of chickenpox (varicella) and shingles (zoster). Routine VZV vaccination of children aged 18 months to 2 years, and of susceptible adolescents and adults, has been recommended by the ACIP since 1996. VZV serology is useful for determining whether an individual is susceptible to infection by the virus, particularly in high-risk groups such as immunocompromised patients, hospital employees, transplant recipients, and pregnant women.

2. SAFETY PRECAUTIONS

Consider all serum specimens for analysis potentially positive for infectious agents including HIV and the hepatitis B virus. Observe universal precautions; wear protective gloves, eye wear, and lab coat during all steps of this method because of infectious contamination hazards. Place all plastic and glassware contaminated with serum in a plastic autoclave bag for disposal. Keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach solution when work is finished. Biosafety level 2 containment and practice as described in CDC/NIH publication #88-8395 are recommended for handling test specimens and kit reagents(4).

Material Safety Data Sheets (MSDS) for phenylmethylsulfonyl fluoride, EDTA, EGTA, deoxycholate, aprotinin, NP-40, sodium fluoride, Tween-20, disodium nitrophenyl phosphate and sodium hydroxide are available through the National Center for Infectious Diseases (NCID) computer network. Risk is minimal because the chemicals are required in small quantities, are well-packaged, and require limited handling by the operators.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

1. Currently, raw data are transferred electronically from instrument readout files into a computerized spreadsheet. This spreadsheet is designed for the management of CDC National VZV Laboratory test results. It functions within the Excel (Microsoft Corporation, Redmund, WA) software program. For VZV IgG ELISA test results, adjusted mean test optical density readings are recorded (average optical density from two normal tissue control wells, is subtracted from

the average optical density from two test antigen wells). Positive test cut-offs were established empirically by analyzing operator and test variation of results from 16 serum specimens (8 positive, 8 negative) tested twice a day (two different operators) on three different days. Internal standards (strong positive, weak positive, negative) are run on each test plate. Reporting of test results includes both the adjusted mean test optical density reading and the practical outcome (positive/negative) is done directly from the spreadsheet file in printed form or by electronic transfer.

2. A physical, written log is generated for all specimens received. The log includes batch receipt dates and updated details concerning the handling, manipulation, and analysis of specimens, as well as any problems that occur.

3. Finished data sheets are reviewed by the supervisor. All data files are printed out and stored in hard copy form and are archived to CD-ROM format and on hard drive.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

1. No special instructions, such as fasting or special diets, are required.

2. Serum is the preferred sample, although specimens of plasma and blood collected and dried onto filter paper blots for reconstitution in the laboratory are also acceptable.

3. Minimum sample volume is 0.2 mL. For blood spots, two completely filled circles of blood (reconstituted to 0.5 mL) are required.

4. The appropriate amount of serum/plasma is dispensed into a Nunc cryovial or other plastic screw-capped freezing vial labeled with the participant's ID. Blood spots are collected through finger or heal prick onto the underside of prepared filter blot pads (provided by the VZV lab) as instructed.

5. Serum and plasma specimens collected in the field should be frozen, and then shipped on dry ice by overnight mail. Once received, specimens should be stored at –20^{o}C until analyzed. Residual specimens are refrozen at -20 ^{o}C. Blood spot specimens must be permitted to completely dry before placing them into individual zip-lock bags. They should be shipped at ambient temperature within one week of collection.

6. Specimens may be stored at 4-8 ^{o}C for up to 7 days, or stored frozen at -20 ^{o}C.

7. Subsequent freezing and thawing must be avoided as it may lead to loss of activity.

8. Slightly and moderately lipemic, hemolyzed, or icteric samples are acceptable. Samples that are grossly lipemic, icteric, or hemolyzed are to be brought to the attention of the laboratory manager or technical supervisor (reconstituted blood spots are invariably hemolyzed).

9. Specimens may be stored in glass or plastic vials, as long as the vials are tightly sealed to prevent desiccation of the sample.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

a. Instrumentation

(1) OPTImax tunable microplate reader, model 0112-0050 (Sunnyvale, CA)

(2) Automatic plate washer, model SKANwasher 400 (Sterling, VA)

b. Other Materials

(1) Brinkmann Eppendorf Repeater Pipette, # 22260201 (Brinkmann Instruments, Inc, Westbury, NY)

(2) Biohit Proline pipet, 0.5-50 µL, # 720-020 (Vangard International, Neptune, NJ)

(3) Biohit Proline pipet, 20-200 µL, #720-030 (Vangard International, Neptune, NJ)

(4) Pipetus-Akku pipetting aid, Daigger & Co. # HX2038A (Hirschmann Laborgerate)

(5) Pipettor, model P 1000, 2-1000 µL, (Gilson, Middleton, WI)

(6) Pipettor, P 200, 0.2- 200 µL, (Gilson, Middleton, WI)

(7) Pipettor, P 20, 0.02-20 µL, (Gilson, Middleton, WI)

(8) Immulon2 flat bottom microtiter plates, (Dynex Technologies, Inc, Chantilly, VA)

(9) 2.0 mL polypropylene screw-cap cryovials (Sarstedt, Newton, NC)

(10) Pipette tips, RT-200, 1000 µL capacity (Rainin Instruments, Woburn, MA)

(11) Pipette tips, RT-250, 250 µL capacity (Rainin Instruments, Woburn, MA)

(12) Pipette cartridges, 5 mL Combitip Plus, # 22 26 640-3 (Brinkmann Instruments, Inc, Westbury, NY)

(13) Glassware: 1000-, 500-, and 200-mL bottles for storing solutions (Wheaton); 400-, 250-, and 100-mL beakers; 250-, 100-, and 50-mL graduated cylinders (Pyrex or Kimax brand; clean, reusable glassware is provided by the Scientific Resources Program, NCID)

(14) Phenylmethylsulfonyl fluoride, >99% pure, # P7626, (Sigma, St. Louis, MO)

(15) Ethylenediaminetetratacetic acid (EDTA), 0.5M, pH 8.0, # 15575-012 (Gibco BRL, Grand Island, NY)

(16) Ethylene glycol-bis (β-aminoethyl ether)-N, N,N',N'-tetraacetic acid (EGTA), free acid, 97% pure, # E0396 (Sigma, St Louis, MO)

(17) Deoxycholic acid, sodium salt (deoxycholate), >99% pure, # D5670 (Sigma, St. Louis, MO)

(18) Aprotinin, 5-10 TIU/ml, #A6279 (Sigma, St. Louis, MO)

(19) Nonylphenoxy polyethoxy ethanol (NP-40, aka Igepal CA-630), # I3021 (Sigma, St. Louis, MO)

(20) Sodium Fluoride, 99% pure, # S7920 (Sigma, St. Louis, MO)

(21) Polyoxyethylenesorbitan, mixed fatty acid (Tween 20), # P1379 (Sigma, St. Louis, MO)

(22) Carbonate buffer, 0.5M, pH 9.6, # 996996 (SRP/NCID/CDC)

(23) Diethanolamine buffer, pH 9.8, # 993182 (SRP/NCID/CDC)

(24) Phosphatase substrate tablets (p-nitrophenyl phosphate, disodium), # 104-105 (Sigma, St. Louis, MO)

(25) Sodium hydroxide, 3.0 N (stopping solution), # LC24420 (LabChem, Inc., Pittsburgh, PA)

(26) Corning pH meter, model 430 (Corning, Inc., Acton, MA)

(27) Balance, model AE50 (Mettler Instrument Co., Highstown, NJ)

(28) Dehydrated skim milk, # 0032-17-3 (DIFCO Laboratories, Detroit, MI)

(29) VZV strain Webster (American Type Culture Collection (ATCC), Rockville, MD)

(30) HLF human fibroblast cell line (ATCC, Rockville, MD)

(31) Household bleach (any vendor)

(32) Fetal bovine serum (FBS), # A-1111 (Hyclone Labs)

(33) RPMI 1640 medium w/ sodium carbonate, w/ l-glutamine, # (Life Technologies, GibcoBRL, Grand Island, NY)

(34) L-glutamine 200 mM (100X), # 25030-024 (Life Technologies, GibcoBRL, Grand Island, NY)

(35) Conjugate (goat anti-human IgG, phosphatase-labeled, affinity-purified), # 075-1002, Kirkegaard and Perry Laboratories, Inc

(36) Vortex Genie 2 mixer (Fisher Scientific, Atlanta, GA)

c. Reagent Preparation

Unless otherwise specified, all chemicals are American Chemical Society “Reagent Grade.” Reagents are prepared with double-distilled water.

(1) <u>Preparation of cell culture media</u>

Growth medium is prepared in the laboratory using Eagles MEM, which is supplemented with 10% heat-inactivated FBS, 100 µg/ml of streptomycin, 100 µg/ml of penicillin, and 300 µg/ml of glutamine. Maintenance medium is prepared by substituting 2% FBS for 10% FBS. All media are filtration sterilized by passage through a 0.45-micron filter.

(2) Preparation of varicella EIA antigen

(uninfected control antigen is prepared using the same protocol without the virus inoculation step (step d)).

(a) HLF cells are suspended in growth medium at 1.5 X 10^5 cells per milliliter.

(b) Seed T-150 culture flasks with 50 ml of cell suspension each.

(c) incubated at 35^{o}C, 5% CO_2 for 48 hours.

(d) Inoculate each flask with approximately 5 X 10^5 pfu/ml of infectious VZV, strain Webster.

(e) Incubate cells for 4-7 days until they reach the 3+ CPE stage.

(f) Harvest cells with rubber policeman, transferring to 50 ml conical centrifuge tubes; pellet cells by centrifugation for 10 minutes at 2000 rpm. Discard supernatant fluids.

(g) Pool cell pellets into a single 15 ml conical centrifuge tube and pellet cells by centrifugation at 2000 rpm for 10 minutes

(h) Remove supernatant fluid and resuspend cell pellet in 5 ml of RIPA lysing buffer.

(i) Place tube on ice for 15 minutes.

(ij Pellet nuclei from lysed cells by centrifugation at 900 X g for 20 minutes.

(k) Titrate the VZV and control antigen in 96-well microplates to determine which dilution will be used in the test proper. For a typical antigen preparation, a dilution of 1:1000 of VZV and control antigen is appropriate.

(l) Antigen may be stored in frozen aliquots of 0.125 µl at –70 ^{o}C, although our observations suggest that the antigen is stable at 4 ^{o}C for up to six months. We prefer to store antigen in liquid form at refrigerator temperature (4 ^{o}C).

(3) Coating of viral antigen and control antigen to the 96-well microtiter plate.

(a) Add 100 µl of the working dilution of viral antigen and control antigen to each96-well plate (viral antigen in odd columns and control antigen in even columns).

(b) Plates are stacked (placing an empty 96-well plate on the top of the stack), and are placed in the refrigerator at 4 °C for at least 18 hours.

(c) Plates may be used up to one week after preparation without detectable change in performance.

(4) RIPA lysing buffer (5X stock and working solution)

5X stock solution: To 196 ml of double-distilled water, add 2.0g of deoxycholic acid, sodium salt; 0.04g phenylmethylsulfonyl fluoride, 4.0 mL EDTA (0.5M solution), 0.76g EGTA, 0.08g NaF, and 100µL aprotinin solution. Stir until all solids are completely in solution. Store at 4 °C and use within 6 months.

1X working solution: Add 20mL of RIPA stock to 80mL of double-distilled water. Add 0.5mL NP-40 detergent and stir until dissolved. Store at 4 °C, and use within 2 weeks.

(5) p-nitrophenyl phosphate substrate

Purchased as preweighed tablets and stored at ≤ 20 °C. Solution is prepared by dissolving one tablet in 5mL Diethanolamine buffer, pH 9.8. Use immediately.

(6) 10% Hypochlorite solution

Add 300mL bleach to 2700mL of double-distilled water. The solution is stable for 1 week when stored at 20-25 °C.

(7) 0.01 N NaOH solution

Dissolve 8g NaOH in 2.0L of double-distilled water. Place solution in an empty dispense bottle. Can be stored indefinitely at 20-25 °C; use as needed.

(8) 0.01 N HCl solution

Dilute 1.6666g of 12 mol/L HCL with 2.0L of double-distilled water. Can be stored indefinitely at 20-25 °C; use as needed.

d. Standards Preparation

Identify individuals who have been previously tested and determined to be high positive, low positive, or negative for varicella-zoster, and obtain serum from each. Use individual serum samples (not pooled). Add 10 µL of a 10 g/dL sodium azide solution per mL of serum (final dilution of sodium azide is 1:1000), aliquot sera into 5.0 mL lots and freeze at –20°C. These QC materials are stable indefinitely, unless contamination occurs. These sera are screened for HBs antigen and antibody to HIV before accepting for use.

Controls included on each plate: High positive, low positive, negative.

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. Calibration Curve

No calibration curve is generated by the user as part of these assay methods. The calibration is either automatic or performed periodically by contracted service personnel.

b. Verification Procedure

The instrument used to read assay results (Section 6.a.1.) is equipped to analyze the positive and negative controls for each test series. If, within a testing series, positive or negative controls do not conform to specifications as defined in the product literature, the entire series is invalidated by the instrument.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

a. Preliminaries

(1) Serum or plasma: Unpack samples immediately after arrival, and check for damage and leakage during transport. Check the vial specimen numbers against the packing list and box vials. If specimens arrive frozen, work rapidly to avoid thawing and store at $-20^{o}C$. Thawed specimens are stored at $4^{o}C$ if they are to be tested within one week.

b. Sample Preparation

(1) Serum/plasma specimens:

(i) Dilute 20.0 µl of specimen in 380 µl of blotto supplemented with 1% normal HLF cell lysate and 1% fetal calf serum (final dilution of 1:20).

(ii) Vortex all tubes.

c. Instrument Set-up

(1) Operation of the SkanWASHER 400

(i) Switch on the main power.

(ii) Verify that the waste inlet reservoir tubing is connected.

(iii) Verify that inlet reservoir is filled and pressurized.

(iv) Verify that plate carriage is in position for flat bottom plates.

(v) Run prime program before washing plates.

(2) Operation of the OPTImax tunable microplate reader

(i) Switch on main power to OPTImax reader.

(ii) Switch on Compac Elite 4/75 CXL laptop computer.

(iii) Run Softmax Pro application.

(iv) Verify communication between OPTImax reader and computer.

d. Operation of Assay Procedure

(1) Prepare 1:1000 dilutions of VZV test antigen and normal tissue control antigen in carbonate buffer, in sufficient quantity to provide 200 µl per antigen per specimen (including three control sera).

(2) Add 100 µl of VZV antigen (V) or 100 µl of normal tissue control antigen (NT) to two wells each for every test (T) and control serum in the pattern shown below:

(3) Plates are refrigerated overnight ($4^{o}C$).

(4) Select program on ScanWASHER to aspirate and wash 4 times (PBS/Tween. Vent the pressurized reservoir and insert the microtiter plate. Start program and remove plate when washes are complete. Repeat for the number of plates required.

(5) Block plates with blotto for 1 hour at ambient temperature.

(6) Repeat wash program (aspirate and 4 washes) using PBS/Tween.

(7) Add serum dilutions and control serum dilutions to appropriate wells in plate at 100 µl per well (2 test wells and 2 normal tissue wells per serum).

(8) Incubate plates for 30 minutes at ambient temperature.

(9) Aspirate and wash plates 4 times with PBS/Tween 20.

(10) Dilute goat anti-human IgG:alkaline phosphatase conjugate antibody 1:1000 in PBS.

(11) Add 100 µl of conjugate dilution to all wells.

(12) Incubate at ambient temperature for 30 minutes.

(13) Aspirate and wash plates 4 times in PBS/Tween.

(14) Prepare disodium nitrophenyl phosphate colorimetric substrate by dissolving two pre-weighed tablets in 10 ml of 0.9M diethanolamine buffer (this much needed for each 96-well plate set-up).

(15) Add substrate to wells at 100 µl per well.

(16) Allow the enzymatic reaction to proceed for 10 minutes at room temperature.

(17) Stop the colorimetric reaction using 100 µl of 2.5N NaOH solution.

(18) Plates are read in the OPTImax reader using a wavelength of 405nm.

e. Recording of Data

Templates have been created for the OPTImax reader that automatically correct the optical density readings against the blank, calculate mean test O.D. (T.O.D.) and mean normal tissue O.D. (N.O.D.) for each specimen, and adjust the final O.D. value by subtracting N.O.D. FROM T.O.D. All raw and calculated data are automatically saved to an Excel spreadsheet for each run.

(1) Quality Control Data

Stong positive, weak positive and negative sera with established performance data with this assay are used to determine whether or not individual test runs are valid. These data (both raw O.D. readings and adjusted O.D. readings are recorded together with specimen test data for each test run).

(2) Analytical Results

Both raw data and adjusted O.D. readings are automatically recorded after each test run.

(3) Data Maintenance

All test results are maintained both as electronic Excel spreadsheets and in hard copy.

f. Replacement and Periodic Maintenance of Key Components

(1) Instruments are on service contract and, except for basic daily routine maintenance are serviced by a representative from Molecular Devices Corporation.

(2) Incubator temperatures, ScanWASH fluids, and room temperature are monitored and documented weekly; Freezers and refrigerators are under continual remote monitoring, and are monitored manually and documented weekly.

(3) All micropipettors used in testing clinical specimens are checked for calibration every 6 months. Pipettors that do not conform to specifications are sent out for recalibration in accordance with the manufacturer's recommendations. Calibration records are kept for each pipettor by serial number.

g. Calculations

(1) Cut-off calculation has been established empirically by testing multiple (10 each) VZV seropositive and seronegative in duplicate daily test runs (performed by two different operators), performed on three different days. The resulting 6 data sets were statistically analyzed for variation due to different operators, variation day-to-day, test run to test run, etc. The mean negative test O.D. plus three standard deviations (based on a cumulative variation from all sources) was used to establish a cut-off value.

(2) The negative control serum must produce an optical density reading of less than 0.05, or the test run is invalidated.

(2) Results in the equivocal range are retested at a lower serum dilution.

9. REPORTABLE RANGE OF RESULTS

The following cut-off ranges have been established:

0.000 – 0.049	negative
0.050 – 0.200	equivocal
0.201 and above	positive

Results are recorded both as mean adjusted O.D. reading and by their objective rating as positive, negative or equivocal.

10. QUALITY CONTROL (QC) PROCEDURES

Prepare a log of control results for each run including the date tested; the run number; the mean absorbance for each control; and a notation when an individual control or run is unsatisfactory.

Calculate the mean and standard deviation for each control on the basis of the first 40 acceptable runs with a lot of antigen.

Select at random one serum specimen from every other run for repeat testing (2.5%).

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

a. Repeat the entire EIA run using new serum dilutions.

b. If controls continue to fail, consult the supervisor for other appropriate actions.

c. Do not report results from runs in which the controls did not meet acceptable limits.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

High non-specific reactivity of a serum specimen with the normal tissue control may obscure a weakly positive specimen in particular. Specimens with high nonspecific reactions should be reevaluated using VZV latex bead agglutination or FAMA.

13. REFERENCE RANGES (NORMAL VALUES)

There are no established normal ranges for this assay.

14. CRITICAL CALL RESULTS ("PANIC VALUES")

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens reach and maintain 20-25^{o}C during testing. After analysis, the specimens are stored at –20^{o}C or lower.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

An FDA licensed latex bead agglutination test (VZVScan) performed equivalently with this IgG EIA in a validation study conducted in this laboratory. This method, while not preferred for handling large numbers of specimens, can be substituted for the IgG EIA, or may be used as an adjunct to clarify equivocal results obtained by EIA. If the analytical system fails, the specimens should be refrigerated at 4-8^{o}C until the analytical system is restored. If long-term interruption is anticipated, specimens are refrozen at –20^{o}C or lower.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Standard record-keeping means (e.g., electronic, data files, laboratory notebook) are used to track specimens. Records are maintained indefinitely. Specimens are retained at the laboratory for at least one year, and then may be placed in archival storage at the CDC facility in Lawrenceville, GA. Only numerical identifiers are used. All personal identifiers are kept masked and available only to the project coordinator in order to safeguard confidentiality.

19. SUMMARY STATISTICS AND QC GRAPHS

Qualitative assays are assays with a positive, negative or indeterminate result. Since the controls do not generate quantitative values, plots are not generated for quality control purposes.

REFERENCES

Gershon AA, Forghani B. Varicella-zoster virus. In: Lennette, EH, Lennette DA, Lennette ET, editors. Diagnostic procedures for viral, Rickettsial and chlamydial infections, Washington (DC); American Public Health Association, Inc., 1995:601-613.

Forghani B. Varicella-zoster virus antibodies. In: Bergmeyer HU, editor. Methods of Enzymatic Analysis. Vol. X, 3rd ed. Weinheim, Germany: Verlag Chemie International, Inc., 1986:267-282.

Centers for Disease Control and Prevention. 1996. Prevention of varicella: recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR 45(RR-11):1-36.

U.S. Department of Health and Human Services. Biosafety in microbiological and biomedical laboratories. HHS publication (NIH) 88-8395. Washington: U.S. Government Printing Office, 1988.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Martha L. Thieme and Denise R. Brown, CDC, Division of Viral and Rickettsial Diseases, Viral Exanthems and Herpesvirus Branch, in the development of this methodology.

-