8-1988

Comparison of Five Different Serological Assays for Determination of Antibody Levels Against Herpes Virus Type 1 and 2

Murat Sami Küçük

Abstract

To aid in the diagnosis of certain clinical types of Herpes virus infections, we evaluated and compared five serological methods for measuring Herpes virus antibody. The five tests we compared are complement fixation test (CF), indirect immunofluorescent antibody assay (IFA), anticomplementary immunofluorescent assay (ACIF), enzyme linked immunoassay test (ELISA), and the latex agglutination test (LA). The main objective of this study was to establish which of the currently available serologic tests was best suited for clinical laboratory use and which may then replace the complement fixation test. In the comparative testing, one hundred twenty serum samples were divided into four groups which were based on their complement fixation test results. The groups were negative, low positive, high positive and blind samples. A commercial indirect fluorescent antibody test was compared and was found to be easy to perform and cost effective. The problems with the IFA test is that it has low specificity and high false-positive predictability. A third test compared was a commercially available ELISA kit, which was shown to be rapid and sensitive. This test appeared to have a high false-positive rate based upon results obtained from cell culture control beads. The ACIF test was also included. It eliminated the false positive problems observed with the IFA. The final test method was a commercial latex agglutination kit, which performed similarly with the other tests. In our hands we have found the latex agglutination test to be most ideal.

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COMPARISON OF FIVE DIFFERENT SEROLOGICAL ASSAYS FOR DETERMINATION OF ANTIBODY LEVELS AGAINST HERPES VIRUS TYPE 1 AND 2

by

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A Thesis in Partial Fulfillment of

the Requirements for the Degree Master

of Science in Microbiology

August 1988

Each person whose signature appears below certificates that this manuscript in his opinion is adequate, in scope and quality, for a thesis for the degree Master of Science

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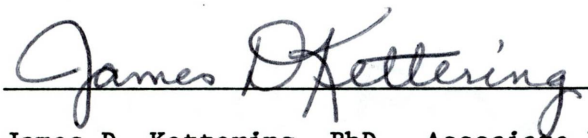

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INTRODUCTION

Herpes Simplex virus (HSV) is a common infectious agent of man and occurs in two forms that are biologically and serologically distinguishable. These are designated as types 1 and 2 (HSV 1 and HSV 2). HSV causes two types of infections - primary and recurrent. Acute disseminated primary infection (gingivostomatitis, herpes genitalis), recurrent infection with intermittent virus shedding (herpes labialis or genitalis), and virus latency in neural ganglia between clinical episodes are all typical clinical manifestations. Neonatal herpes is one of the important primary infections of Herpes simplex virus.

Neonatal herpes is a serious disease often acquired by passage through an infected birth canal. Estimates of the general risk of neonatal herpes, if the virus is present in the maternal genital tract at the time of delivery can vary from 40 to 80%. This risk can be prevented by cesarean section if maternal genital herpes is properly diagnosed. Mortality in disseminated neonatal infections approaches 67 percent, while another 18 percent have significant morbidity (Petersdorf et. al. 1988). Occasionally the herpes infection is acquired either transplacentally or postnatally (Vaughan et. al., 1988). If the primary infection occurs concurrently with early pregnancy, infection of the fetus can result in abortion, stillbirth, or congenital deformities. Most of the neonatal herpes infections are caused by HSV-2 (Vaughan et. al., 1986). Maternal primary herpes genitalis carries a greater risk of intrauterine infection of the baby than does the recurrent form of genital herpes. The risk of serious disease is greater in premature

infants (White and Fenner, 1986).

Laboratory diagnosis of Herpes virus infections includes cell culture isolation and serum antibody determinations. Isolation in cell culture is currently regarded as the most sensitive laboratory procedure available for the diagnosis of herpes virus. Distinctive foci of swollen cells appear within 1-5 days. However, the diagnosis can be made as early as 24 hours by immunofluorescent (IF) or immunoperoxidase staining of cultured cells (White et. al., 1986). The most commonly used serological tests are complement fixation, indirect immunofluorescent (IFA), anticomplementary immunofluorescent (ACIF), enzyme linked immunoassay (ELISA), latex agglutination (LA), neutralization, and the indirect and direct hemagglutination assay (Rose and Friedman,1986).

The advantages of the complement fixation test are:

1. reproducibility
2. specificity
3. ability to measure antibodies to a wide variety of antigens
4. stability and long lasting property of complement fixing antibodies in the adult.

The disadvantages the of complement fixation test are:

1. slow and cumbersome (labor intensive)
2. requires an overnight incubation
3. exact titration of its multiple components precludes its occasional use for the serodiagnosis of HSV alone.
4. after prolonged storage some sera exhibit anti-complementary

activity (White et. al., 1986; Rose et. al., 1986)

The first test to be compared with CF was the IFA test. The advantages of the IFA test include sensitivity, speed, serum samples can be run individually, and a well defined procedure. The disadvantages of the IFA test include (Rand et. al 1986; Rose et.al., 1986):

1. Strong nonspecific staining of cells which is the result of nonspecific conjugate attachment. This nonspecifity can be minimized by:
   (a) using cells in which less Fc receptors are induced by HSV infection
   (b) blocking the Fc receptors with nonhuman IgG
   (c) using the anticomplementary immunofluorescence (Rose et. al., 1986)
   (d) using fluorescein isothiocyanate conjugated (FITC) Staphylococcal protein A (SPA) instead of FITC antibody to human IgG
   (e) treatment of IFA slides with 10% glacial acetic acid for 10 minutes (Gallo, D., 1986) to decrease the nonspecific staining of the cells
2. An ultra-violet microscope is required.

The second method was the ELISA. A commercial kit called "HSV Bio-EnzaBead Titration Kit" was used. This is an immunoenzyme test system for the quantitation of antibodies to Herpes simplex virus. The test is based on the principle of a solid phase immunoassay for detecting antibody, but differs by using a magnetic force to transfer

the solid phase from one reaction mixture to another (Smith and Gehle, 1977). The advantages of ELISA are its objectivity (automated readings of results), sensitivity, potential for automation and the long shelf life of the enzyme-conjugate reagents. The major disadvantage is the cost of the commercial kits or, alternatively, the experience and expertise needed to construct an ELISA in an individual laboratory.

The third method was ACIF. In this test, complement is activated only by antibody bound to its specific antigen and not by IgG bound to Fc receptors (Rose et. al.,1986, Kettering et. al., 1977). Slides were prepared in our laboratory.

The fourth method was a commercial LA slide test, called "Virogen HSV Antibody Test". Latex agglutination slide tests can be used for qualitative and quantitative detection of herpes simplex virus antibody in serum. The "Virogen HSV Antibody Test" uses "Reagent Latex" particles which are coated with purified herpes simplex virus antigens. The antigens are common to both HSV-1 and HSV-2.

Serum antibody determinations are intended to be used for the detection and quantitative determination of antibody to HSV in human sera (White et. al., 1986; Petersdorf et. al., 1988). These assays are limited in their use, and hence are utilized as a laboratory aid in diagnosing primary HSV infection. In addition they can serve as a useful tool for epidemiological studies. For example, in infants, an increase in IgM is suggestive of intrauterine infection, whereas increased IgG levels in infants less than six months old may be of maternal origin. Elevation of IgG antibodies after six months is

clinically meaningful because it is suggestive of an early congenital or postnatal infection (White et. al., 1986).

The goals of this study were:

1. to find a more practical serological method to replace complement fixation.
2. to identify a cost effective serological method.
3. to eliminate the subjectivity presently associate with complement fixation.
4. to find a test with increased sensitivity.

To achieve the desired goal, this study compared the indirect immunofluorescent (IFA) test, anticomplementary immunofluorescent test (ACIF), enzyme linked immunoassay test (ELISA), latex agglutination test (LA) with complement fixation test (CF). The criteria for comparison include:

1. Time required to obtain laboratory results
2. Labor required to perform the test,
3. Total cost (labor plus cost of the materials which included the cost of the kit if commercially available)
4. Sensitivity
5. Specificity
6. False-positive and false-negative predictive values
7. Ease of performance (subjective, objective, automated).

MATERIALS AND METHODS

SERUM SPECIMENS:

The 160 serum samples used in the study were selected from sera sent to the Clinical Laboratory or Faculty Medical Laboratory at Loma Linda University Medical Center, Loma Linda, California. For HSV-CF testing, samples were selected on the basis of their complement fixation test value. Samples were divided into three groups, namely, high titer group (>1:64), low titer group (1:8 to 1:32), negative titer group (<1:8). A forth group, unknowns, were selected randomly by an independent party. All samples were stored in capped glass tubes at -20°C after being aliquoted.

COMPLEMENT FIXATION TEST (CF):

Serum samples were submitted to the microbiology laboratory of the Loma Linda Medical Center for HSV antibody testing by the standard CF microtiter method (Rose et. al., 1986). The CF test is based on the binding of complement to an antigen antibody complex, which will prevent the complement mediated lysis of the hemolysin - sensitized indicator sheep red blood cells (SRBC)(Lot # 6026, M.A. Bioproducts). The test is performed in 2 steps. First, a titrated concentration of antigen (Herpesvirus Hominis 1 CF antigen-Vero cells- Lot # 40607003, M.A Bioproducts), serum sample dilutions and a fixed amount of complement (Guinea pig complement: Catalog No: 44-010-43, Lot #. 44010027 Flow laboratories) are allowed to react at 2-8°C overnight. Second, a volume of hemolysin-sensitized sheep H-RBC (Hemolysin-rabbit anti-sheep, Cat #44.012-44, Flow Laboratories) is added to the mixture, which is

then incubated at 37°C for 15 minutes. An uninfected tissue control antigen (Herpesvirus Hominis 1 CF Tissue Control Antigen, Lot # V948035C, Flow Laboratories) is included. Negative and positive serum controls are used (Herpesvirus human immune serum for CF, Cat #42-605-41, Lot #:42605005, Flow Laboratories). Veronal Buffered Saline (VBS) is used for dilutions (see appendix I for preparation of VBS).

Performance of the CF Diagnostic Test:

Preparation of antigen, sheep red blood cells, hemolysin titration, sensitized cell suspension and complement titration are explained in detail in the appendix section.

1. Label serological tubes (12x75 mm) for the 1:8 dilutions of unknown sera and known positive serum.
2. Add 350 μl of VBS to each tube.
3. Add 50 μl of unknown serum to its labeled tube
4. Add 50 μl of known positive serum to its labeled tube and mix with vortex.
5. Inactive the serum dilutions for 30 minutes in a 56°C heat block. During this incubation period, label the plates
6. When the 1:8 dilutions have been in the heat block for 30 minutes, remove them and allow them to cool at room temperature.
7. Add 25 μl VBS to all wells, except first well in each row and NS wells.
8. Add 50 μl of the 1:8 dilution of known positive serum and unknown serum to the assay wells labeled 1:8. At

the same time, add 25 μl of known positive serum to the control wells (SC) and control antigen wells (NS).

9. Using a 25 μl microdiluting pipette, pick up 25 μl of the 1:8 dilutions and transfer to the wells labeled 1:16 dilution; mix it and transfer 25 μl to the next well which is marked as 1:32. Repeat the process through the 1:256 dilution of each serum.

10. Determine the volume of test antigen required by multiplying the number of patients plus positive control by 0.2 ml, add 0.4 ml excess for complement titration.

11. Prepare the test antigen at the optimal dilution determined during the antigen titration in tubes.

12. Add 25 μ of the optimal dilution of test antigen to the wells in the test rows and to the wells for complement-antigen control.

13. Add 25 μ of the tissue control antigen to the NS wells and the wells for complement-control antigen control.

14. Place the plates on the vibrator to mix. After mixing, remove the plates and place at 4°C for 30 minutes or until cold before adding the complement.

15. Prepare enough complement (see complement titration) for all the wells of the test (25 μ per well).

16. Dilute complement units to be 1.5, 1.0 U, and 0.5 U. (complete the tubes containing 300, 200, 100 ml of VBS with pretitrated complement which is accepted as 2U complement;

tubes will have 1.5U, 1.0U, and .5U of complement respectively)

17. Add 2 U complement to appropriate well.
18. Add 1.5 U, 1.0 U, 0.5 U complement to the appropriate complement-Antigen control + antigen control wells.
19. Place the plates on the vibrator to mix. Incubate at 2-4°C overnight.
20. Remove plates from 4°C, place at room temperature for 10 minutes.
21. Add 50 μl sensitized sheep RBC (sensitized at least 10 minutes in room temperature) to every well used in plate. Mix on the vibrator for 40 seconds.
22. Incubate for 15 minutes at 37°C. Read.
23. Record results in the log book as shown in Antigen Titration.

Treatment of Anticomplementary Activity:

1. Add 150 μl of unknown serum with 50 μl complement and then incubate for 30 minutes at 37°C.
2. Add 1.0 ml of VBS to the complement treated serum. Heat inactivate at 56°C for 30 minutes with rest of unknown serum. (Final dilution of complement treated serum is 1:8.)

Interpretation:

1. Complement control wells 2 U and 1.5 U should show complete hemolysis, 1 U partial to 2+, 0.5 U no hemolysis.

2. Serum control wells should show complete hemolysis.
3. NS serum control wells should show complete hemolysis.
4. Wells of test should be read as they are. Titer is the highest dilution that shows a 3+ fixation.
5. When controls are not correct, the test is not valid.
6. A single CF test is of little diagnostic value in establishing HSV infection. It can be used as a screening test in infants suspected of HSV. When possible, the serological diagnosis of HSV infection should be confirmed by virus isolation.
7. Caution should be exercised in that patients with significant rise in CF antibody titer against HSV may exhibit a four-fold or greater titer rise against Varicella-Zoster (VZ) antigen. This is indicative of the presence of minor common antigens between two viruses.

Limitations:

The CF test is useful for detecting the increase in specific IgG antibodies that occurs several weeks following an infection; however, the standard CF test does not reliably detect the IgM antibodies that develop earlier. The moderately low sensitivity of the test makes it unreliable when used to determine immunity or provide evidence of a past infection. The CF test is also inhibited by high levels of rheumatoid factor.

The antigen-antibody reaction is detected by the fixation of complement. If the serum contains antibodies to the HSV, the complement

is bound by the resulting antigen-antibody complex and is therefore unable to lyse the H-RBC indicator system. The unlysed cells then settle to the bottom of the well. If there are no antibodies in the patient's serum, the complement is free to lyse the H-RBC and this reaction will be visually manifested as a clear red fluid.

INDIRECT FLUORESCENT ANTIBODY ASSAY (IFA):

A commercial IFA test "HSV-CHECK", (Product No. IF1091 Lot # 5307X, Diagnostic Technology, Inc., Hauppauge, NY) was tested. The HSV-CHECK kit utilized an indirect immunofluorescent antibody (IFA) method for the detection of antibody to HSV. HSV-2 infected cells were immobilized on a glass slide and any addition of patient serum containing antibodies to HSV will bind to the antigen substrate and would not be rinsed off. Subsequently, when fluoresceinated anti-human globulin was added to the reaction site it would bind to the antibodies, if present, causing the HSV-infected cells to fluoresce when viewed through a UV microscope. The presence of antibody to HSV is demonstrated by a greenish-yellow fluorescence in the virally infected cells. Negative serum did not show this fluorescence.

Test Procedure:

(materials, interpretations of results and test limitations are included in appendix)

1. Prepare a 1:10 dilution of the patient specimen using the prepared PBS.
2. Remove slide from foil packet and label appropriately.

3. Using a Pasteur pipette, add a sufficient quantity (approx. 15-20 μl) of the appropriately diluted serum sample so as to cover each reaction site.
4. Place the slide in a humidity chamber and incubate at 37°C for 30 minutes.
5. Remove slide from chamber and briefly rinse with a gentle stream of PBS; avoid directing PBS at wells. Immerse slide in PBS for 5 minutes.
6. Very briefly (approx. 1-2 seconds) dip slide into distilled water so as to prevent the formation of any PBS crystal between wells when drying.
7. Allow the slide to air dry by standing on end on paper towels.
8. Add just enough of the appropriately reconstituted conjugate to cover each reaction site (approx. 15-20 μl).
9. Incubate slide at 37°C in a humidity chamber for 30 minutes.
10. Rinse slide as described in Steps 5,6, and 7.
11. Place a small drop of mounting fluid on each reaction site just before reading and carefully cover with a No. 1, 22 x 50 mm cover slip; avoid trapping air bubbles.
12. Read slide as soon as possible at 150X to 200X magnification.

One variation of this procedure was that fourteen of the CF negative serum samples were run with the aforementioned commercial IFA-treated with 10% glacial acetic acid for 5 minutes (Gallo, 1986).

ANTICOMPLEMENTARY IMMUNOFLUORESCENCE (ACIF):

We prepared the ACIF slides in our laboratory using Human fibroblast cells to culture HSV-1 and HSV-2. The results of HSV-1 were compared to HSV-2 and then compared with the other four methods. Infected and uninfected cells were mixed to provide a control background. After fixing the slides with acetone for 10 minutes at room temperature the slides were stored at -20°C. The ACIF test was performed as described by Kettering, et al., 1977.

1. Make a map of slide on the paper.
2. Mark the slide.
3. Add 25 μl of the positive control serum on positive control well.
4. Add 25 μl of the negative control serum on the corresponding well.
5. Add 25 μl of beforehand titrated patients serum to the corresponding wells.
7. Incubate the slide in a humidity chamber for 30 minutes at 37°C.
8. Remove slide from chamber and briefly rinse with a gentle stream of PBS; avoid directing PBS at wells. Immerse slide in PBS for 5 minutes.
9. Very briefly (approx. 1-2 seconds) dip slide into distilled water so as to prevent the formation of any PBS crystal between wells when drying.
10. Allow the slide to air dry by standing on end on paper

towels.

11. Add 25 μl of previously titrated C′ to each well except to the conjugate control well.
12. Repeat incubation process.
13. Repeat 8,9, and 10.
14. Add 25 μl of anticomplementary immunofluorescent reagent to cover each reaction site (approx. 15-20 μl).
12. Incubate slide at 37°C in a humidity chamber for 30 minutes.
13. Rinse slide as described in Steps 8,9,10.
14. Place a small drop of mounting fluid on each reaction site just before reading and carefully cover with a No. 1, 22x50 mm cover slip; avoid trapping air bubbles.
15. Read slide under the U.V. microscope as soon as possible at 150X to 200X magnification.

ENZYME LINKED IMMUNOASSAY (ELISA):

(Detailed steps of the test are included in the appendix section)

The commercial HSV Bio-EnzaBead Titration Kit from Organon Teknika (Catalog No. 37178/9142-20) was used. The principle of this test is based on the technique of solid phase immunoassay for detecting antibody and uses a magnetic to transfer the solid phase from one reaction mixture to another. The antigen was bound to the surface of beads made of ferrous metal. Negative control beads, similarly prepared from uninfected cell cultures, were included in the kit to detect potential nonspecific reactions. The beads were placed in the dilution of serum to

be assayed. If antibodies are present in the sample, they would attach to the antigens on the beads. The beads were washed to remove unbound human serum protein and then released into a reaction mixture containing peroxidase conjugated anti-human globulins. The conjugate would bind to the patient's antibody molecules if these were attached to antigen on the surface of the beads. The beads were then washed to remove unbound conjugate and placed in a substrate solution. The appearance of color indicated the presence of human antibody to the antigens on the bead. To determine the antibody titer, the sample was serially diluted. Each dilution was treated as described above and the endpoint was established as the highest dilution which yielded a 1 to 2+ color intensity. The 96 well plate was read by the "EIA reader" (Bio-Tek Instruments Inc., model no:EL308.) The EL308 reader is a microprocessor based bench top spectrophotometer designed to accurately measure and record the absorbance values of samples in 96 well microplates.

LATEX AGGLUTINATION TEST:

A commercial LA test " Virogen HSV Antibody Test", (Wampole Laboratories, Cranbury, New Jersey) was included. The Virogen HSV Antibody Test Reagent Latex particles are coated with purified herpes simplex virus antigens which are common to both HSV-1 and HSV-2. In the presence of HSV antibody, a specific and sensitive immunochemical reaction results in visible agglutination of the latex particles. There are two protocols, namely Qualitative and Quantitative. In our study we used the quantitative protocol. The quantitative protocol consists of

preparing serial dilutions of sera on the slide, adding "REAGENT LATEX", mixing, rotating the slide on a serologic rotator for 10 minutes, and observing for agglutination. The observation of an agglutination pattern with the naked eye was very difficult to read due to the small particle size. A dissecting microscope was used to accurately read the agglutination pattern. An antibody titer was obtained by determining the reciprocal of the dilution in the last well containing a positive reaction. The positive and negative controls included in the kit should be tested with each run, in accordance with the established quality control protocol.

CRITERIA FOR THE ANALYSIS OF RESULTS:

Percent sensitivity, specificity, false-positive and false-negative results were calculated as described by Grinner et. al., 1981.

A = True positive (sera positive with three or more of the five test)

B = True negative (sera negative with three or more of the five test)

C = Comparative assay positive result

D = Comparative assay negative result

Percent sensitivity = A/(A+C)*100

Percent specificity = D/(B+D)*100

False-positive rate = B/(B+D)*100

False-negative rate = C/(A+C)*100

The P value was calculated by the student T test (Level of significance accepted was $P \leq 0.05$).

RESULTS

The results are shown for four serum groups in Table I through VII. Each test was compared individually with all others to determine any significant differences. The tests were also compared for their sensitivity, specificity, false-positive and false-negative predictive values as described above. These results are summarized in Table VII.

CF vs IFA:

The sensitivity of the CF test was lower than that of the IFA test. The specificity of the CF yielded higher values than IFA. In addition, the false-positive rate was 0% with CF in contrast to IFA which gave 43%. The titers remained higher with IFA compare to CF ($P = 0.0002$). Nine out of 15 of the CF negative serum samples gave positive results with IFA. In addition three of the nine gave a 1:8 or greater titer with the IFA slides treated with 10% glacial acetic acid. These titers ranged between 1:8 and 1:64. Five of these CF-negative, IFA-positive serum samples produced a positive titer (1:100) with the ELISA method. All of the CF positive serum samples remained positive with IFA. Figure I shows the correlation between CF and IFA.

CF vs ACIF:

CF and ACIF showed excellent correlation ($P = 0.5912$). Figure II illustrates this. One of the fifteen CF-negative gave a positive titer with ACIF. This serum sample remained positive with ELISA. None of the CF-positive were negative with ACIF.

ACIF-HSV-1 vs ACIF-HSV-2:

In an antibody comparison of HSV-1 with HSV-2, the ACIF-HSV-1 results were in close agreement with the ACIF-HSV-2. The difference was not statistically significant (P = 0.6612). In 5 serum samples the test was capable of distinguishing specific antibodies to either HSV-1 or HSV-2, thus separating the two agents.

CF vs ELISA:

The sensitivity of the ELISA was 4% (Table 6) higher than CF. The specificity of the ELISA, however, was lower than CF (100% for CF vs 38% of ELISA). Nine of the fourteen CF negative samples gave 1:100 titers when measured by the ELISA technique. None of the CF-positive sera remained negative with ELISA. Some of the ELISA negative control beads gave positive results with high titer sera. Figure III shows the relationship between CF and ELISA.

CF vs LA:

The sensitivity of the LA test was higher than that of the CF test (100% for LA vs 94% for CF). The specificity of the CF test was higher than that of the LA test (100% for CF vs 90% for LA). The false-positive rate of the two tests was zero. The false-negative rate of the two tests was essentially the same. Seven of seventeen CF-negative sera gave a low positive titer with the LA. Twenty-one of the 41 sera gave the same titer (same titer = one titer below, higher, or the same). The difference between CF and LA was significant (P = 0.0039). Figure IV

illustrates this the relationship.

IFA vs IFA*:

(IFA* slides treated with 10% glacial acetic acid for 5 minutes to eliminate the non-specific Fc receptors.)

The acid treated slides stained less brightly with the patient sera, as compared to the untreated slides. Negative and positive controls reacted similarly. Fourteen of the CF-negative serum samples were compared in this evaluation. Three of the 14 gave 1:8 with the acid treated slides, while nine of the 14 gave titers ranging between 1:8 to 1:64 with the commercial IFA test slides (see Table V).

IFA vs ACIF:

The indirect fluorescent antibody test was more sensitive than the anticomplementary immunofluorescence assay ($P = 0.0014$). The IFA titers remained higher in general as can be seen in at Figure V. Eighteen of the ACIF-negative sera gave low positive titers with IFA.

IFA vs ELISA

Sensitivity, specificity, false-positive rate, and false negative rate of the IFA test was parallel to the ELISA results. The IFA correlated very well with the ELISA ($P = 0.6892$). Figure VI shows the correlation between the ELISA and the IFA.

IFA vs LA:

The sensitivity of the two tests was 100%. Specificity of the LA

test was higher than IFA (90% for LA vs 57% for IFA). In addition, the false-positive rate of IFA was significantly higher than LA (57% for IFA vs 0% for LA). The IFA correlated well with those measured by LA (P = 0.5848). Figure VII shows the relationship between these two tests.

ACIF vs ELISA:

Serum ELISA titers measured very high compared to the ACIF. No direct statistical evaluation could be performed due to the differences in dilution levels used in these two procedures. Figure VIII does show the relative titers obtained for the two tests. ACIF yielded higher specificity (100 of ACIF vs 38%). False-positive rate was higher with ELISA test (69% for ELISA vs 0% for ACIF).

ACIF vs LA:

The ACIF test yielded higher specificity than LA (100% vs 90%). False-positive rate was zero for both, while the false-negative rate was higher for LA. Serum titers remained higher with LA.

ELISA vs LA:

For both of these tests the sensitivity was 100%. The LA yielded higher specificity (90% vs 38%). A zero false-positive rate was obtained with LA, while the ELISA gave a 38% false-positive rate. The false-negative rate was zero with ELISA, while LA produced a 10% false-negative rate.

DISCUSSION

The complement fixation test has historically been considered the "gold standard" of HSV serology. It is a labor intensive procedure that requires two days before the results can be obtained. Several carefully pre-titrated controls are required to insure test performance. In addition to that fact, after prolonged storage, some sera exhibit an anticomplementary activity. A well trained technician must perform the test with careful attention to the titration of complement. Failure to do so will invalidate the entire test.

There are several reasons however that the CF test remains popular and is widely used. These include reproducibility, specificity, acceptable sensitivity, ability to measure antibody to a wide variety of antigens and the fact that it is easy to read the result.

For the ACIF test we prepared our own HSV-1 and HSV-2 slides and then compared the corresponding HSV-1 and HSV-2. The ACIF titers gave similar results with those of CF and the difference was not statistically significant. The two tests have similar sensitivity and specificity. The ACIF test, however, is less complicated to perform than the CF.

When ACIF is compared to IFA, two disadvantages of ACIF are noted. One is that ACIF requires the use of complement, which may be unstable and requires prior titration. Secondly, it requires an extra incubation period. A major advantage of the ACIF over IFA is that ACIF eliminates the non-specific binding of IgG to the Fc-receptors, which is induced by HSV infection. Complement binds only to true antigen-

antibody complexes, not to Fc-receptor-IgG binding (Rose et. al., 1986).

With the Indirect Fluorescent Antibody test, the procedure is simple, and there are several commercial kits available. The only technical disadvantage of IFA, which is also true for ACIF, is that it requires a UV microscope and a well-trained individual for interpreting IFA results. The major problem with commercial IFA kits in this study is the possibility of reading false-positive results. In our study we calculated 43% false-positivity with the IFA test. Some other studies calculated the false-positivity of IFA ranges between 16% and 62% (Rand, et. al,. 1986; Rose et. al., 1986). The presence of Fc-receptor binding sites of HSV infected cells increases false-positive readings (Westmoreland, et. al, 1976; Yasuda, et. al, 1968).

HSV is an enveloped virus, and one of the several viral glycoproteins projecting from the envelope possesses IgG Fc-receptor activity. It can bind to human as well as some other species of IgG (White et. al., 1986). These receptors result in nonspecific fluorescent staining of the infected cells, regardless of the presence of specific viral antibody in the serum specimens. At present, there are three practical solutions to circumvent this problem. One method utilizes anticomplementary immunofluorescence assay (Kettering, et. al., 1977). Complement is activated only by true antigen-antibody binding. A second is the use of fluorescein isothiocyanate conjugated (FITC)-SPA (Rose et. al.,1986). SPA is Staphylococcal protein A which is known to bind to the Fc portion of human IgG and, therefore, could be used to distinguish between the binding of an antibody Fab or its Fc portion. A

third solution is suggested by Gallo, D.,1986. She uses a 10% of glacial acetic acid for 5 minutes. We titrated 15 serum samples with acid treated, commercial IFA slides. Three of the fifteen of CF, ACIF-negative serum stayed positive with acid treated commercial IFA. The acid-treated HSV-2 slides did not stain as brightly as the untreated slides. This study would indicate that treatment with 10% glacial acetic acid decreases serum titers in general and may eliminate any low positive results.

The detection of antibody by the HSV Bio-EnzaBead Titration kit is based upon the principle of solid phase immunoassay for detecting antibody. The results of the ELISA test were recorded using two methods. We used an ELISA reading machine which is a microprocessor based bench top spectrophotometer designed to record absorbance values of samples in 96 well microplates. In addition, the optical density of the ELISA test was estimated using the naked eye. Both gave comparable results, but use of the ELISA readers is preferred because one can obtain an objective and quantitative end point. A major problem with the commercial ELISA kit was that the negative control beads produced positive results with 6 of 15 high titer sera. For this reason, it seems very difficult to rely on this type of ELISA kit due to the fact that some antibodies may not be measured in some sera.

Using the Latex agglutination test the agglutination particles were very small. It was therefore difficult to distinguish positive from negative results, since the agglutination pattern was indistinct. Clarity of background seen in a positive agglutination was also

difficult to detect. To enhance ease of interpretation, results were read under the dissecting microscope at 10X, which allowed for more distinct readings. This study shows that LA can eliminate the false positives and decrease false negative readings. The commercial LA test is the easiest to perform, and gives accurate results and has several advantages over the other tests. It is rapid, requires only a simple rotator and but a dissecting microscope is useful to facilitate readings. Results did not vary from those observed in the other tests. A final advantage is the relative simplicity of the test performance which can result in either a qualitative or quantitative titration.

TABLE I: SERUM SAMPLES WHOSE CF TITERS WERE UNKNOWN PRIOR TO TESTING WITH OTHER METHODS

No	CF	IFA	ACIF-1	ACIF-2	LA
1	<1:8	1:16	<1:8	1:32	<1:8
2	<1:8	1:32	<1:8	1:16	≥1:32
3	<1:8	<1:8	<1:8	<1:8	<1:8
4	<1:8	*	<1:8	<1:8	1:32
5	<1:8	1:64	<1:8	<1:8	*
6	<1:8	<1:8	<1:8	<1:8	*
7	<1:8	<1:8	*	*	*
8	<1:8	<1:8	<1:8	1:8	*
9	<1:8	<1:8	<1:8	<1:8	*
10	<1:8	1:32	<1:8	1:32	*
11	<1:8	<1:8	<1:8	<1:8	<1:8
12	1:16	1:128	1:8	<1:8	1:256
13	1:16	1:64	1:64	1:64	*
14	1:16	1:32	<1:8	<1:8	*
15	1:16	*	1:8	1:8	*
16	1:32	1:128	1:3	1:32	*
17	1:32	1:128	*	*	*
18	1:64	1:64	1:16	<1:8	*
19	1:64	1:256	*	*	*
20	1:64	1:256	*	*	*

CF — Complement Fixation Test

IFA — Indirect Fluorescent Antibody Test

ACIF-1 — Anticomplementary Immunofluorescence Test with HSV-1 antigen

ACIF-2 — Anticomplementary Immunofluorescence Test with HSV-2 antigen

ELISA — Enzyme-Linked Immunoassay Test

LA — Latex Agglutination Test

TABLE II: SERUM SAMPLES WHICH GAVE TITERS 1:64 OR HIGHER WITH COMPLEMENT FIXATION TEST AND THEIR CORRESPONDING TITERS WITH IFA, ACIF, ELISA, AND LA

No	CF	IFA	ACIF-1	ACIF-2	ELISA	LA
1	1:256	1:2048	1:128	1:256	1:6400	*
2	1:128	1:1024	1:16	1:32	1:6400	*
3	1:128	1:1024	1:32	1:32	*	*
4	1:128	1:1024	1:8	<1:8	1:6400	1:256
5	1:128	1:1024	1:32	1:64	1:6400	*
6	1:128	1:1024	1:64	1:64	1:6400	*
7	1:64	1:512	1:128	1:64	1:6400	1:64
8	1:64	1:128	*	*	*	*
9	1:64	1:256	1:32	1:32	1:6400	*
10	1:64	1:128	1:32	<1:8	1:3200	1:64
11	1:64	1:128	1:16	<1:8	1:800	*
12	1:64	1:256	1:32	1:64	*	*
13	1:64	1:256	1:32	1:8	*	*
14	1:64	1:256	1:16	1:32	*	*
15	1:64	*	*	*	1:800	1:16
16	1:64	*	*	*	1:800	1:32
17	1:64	*	*	*	1:6400	1:256
18	1:128	*	*	*	1:6400	1:256
19	1:64	*	*	*	1:3200	1:64
20	1:64	*	*	*	*	1:256
21	1:64	*	*	*	*	≥1:1024
22	1:128	*	*	*	*	1:512
23	1:256	*	*	*	*	1:128

TABLE III: SERUM SAMPLES WHICH GAVE TITERS WITH COMPLEMENT FIXATION TEST BETWEEN 1:8 AND 1:32 AND THEIR CORRESPONDING TITERS WITH INDIRECT FLUORESCENT ANTIBODY TEST, ANTI-COMPLEMENT IMMUNOFLUORESCENCE, ENZYME LINKED IMMUNOASSAY AND LATEX AGGLUTINATION TEST

No	CF	IFA	ACIF-1	ACIF-2	ELISA	LA
1	1:32	1:64	1:64	1:128	1:800	*
2	1:32	1:256	1:64	1:64	1:800	1:64
3	1:32	1:256	1:64	1:64	*	1:1024
4	1:32	1:128	*	*	*	*
5	1:32	1:256	1:128	1:64	1:800	1:1024
6	1:32	1:256	*	*	1:1600	*
7	1:32	1:128	1:32	1:32	1:800	*
8	1:32	*	*	*	1:800	*
9	1:32	1:64	1:128	1:128	*	*
10	1:32	*	1:128	1:128	*	1:32
11	1:16	1:128	*	*	1:800	*
12	1:16	1:32	*	*	*	*
13	1:16	1:16	*	*	1:800	*
14	1:16	1:128	1:32	1:32	1:800	1:64
15	1:16	*	*	*	1:800	*
16	1:16	1:32	1:32	1:32	1:800	*
17	1:16	1:64	1:128	1:256	*	*
18	1:8	1:64	1:128	1:64	*	1:64
19	1:8	1:64	1:32	1:32	1:800	1:1024
20	1:8	1:64	*	*	*	*
21	1:8	1:32	*	*	1:800	*
22	1:8	1:64	*	*	1:800	1:64

TABLE IV: SERUM SAMPLES WHICH GAVE LESS THAN 1:8 TITER WITH COMPLEMENT FIXATION AND THEIR CORRESPONDING TITERS WITH INDIRECT FLUORESCENT ANTIBODY TEST, ANTI-COMPLEMENT IMMUNOFLUORESCENCE, ENZYME LINKED IMMUNOASSAY AND LATEX AGGLUTINATION TEST

No LA	CF	IFA	ACIF-1	ACIF-2	ELISA	
1	<1:8	1:64	<1:8	<1:8	*	*
2	<1:8	1:64	<1:8	<1:8	*	*
3	<1:8	1:64	<1:8	<1:8	1:100	1:16
4	<1:8	1:32	<1:8	<1:8	1:100	*
5	<1:8	1:32	*	*	1:100	1:16
6	<1:8	1:32	<1:8	<1:8	<1:100	<1:8
7	<1:8	1:16	<1:8	<1:8	1:100	1:16
8	<1:8	1:16	<1:8	<1:8	1:100	*
9	<1:8	<1:8	<1:8	<1:8	<1:100	*
10	<1:8	<1:8	<1:8	<1:8	*	*
11	<1:8	<1:8	<1:8	*	*	*
12	<1:8	<1:8	<1:8	<1:8	1:100	*
13	<1:8	<1:8	<1:8	<1:8	*	*
14	<1:8	<1:8	<1:8	<1:8	1:100	<1:8
15	<1:8	*	*	*	<1:00	<1:8
16	<1:8	*	1:32	1:32	1:100	*
17	<1:8	*	*	*	1:100	<1:8
18	<1:8	*	*	*	<1:100	*
19	<1:8	*	<1:8	<1:8	*	*
20	<1:8	1:16	<1:8	<1:8	<1:100	1:16
21	<1:8	1:16	*	*	*	*
22	1:4	*	*	*	*	<1:8
23	<1:8	*	*	*	*	1:16
24	<1:8	*	*	*	*	<1:8
25	<1:8	*	*	*	*	<1:8
26	<1:8	*	*	*	*	<1:8
27	<1:8	*	*	*	*	<1:8

TABLE V: SERUM SAMPLES WHICH GAVE LESS THAN 1:8 TITER WITH COMPLEMENT FIXATION AND THEIR CORRESPONDING TITERS WITH INDIRECT FLUORESCENT ANTIBODY TEST AND INDIRECT FLUORESCENT ANTIBODY TEST WHICH THE SLIDES WERE TREATED WITH 10% GLACIAL ACETIC ACID FOR 5 MINUTES

No	CF	IFA	IFA***
1	<1:8	1:64	1:8
2	<1:8	1:64	<1:8
3	<1:8	1:32	<1:8
4	<1:8	1:32	1:8
5	<1:8	1:32	<1:8
6	<1:8	1:16	<1:8
7	<1:8	1:16	<1:8
8	<1:8	<1:8	1:8
9	<1:8	<1:8	<1:8
10	<1:8	<1:8	<1:8
11	<1:8	<1:8	<1:8
12	<1:8	<1:8	<1:8
13	<1:8	<1:8	<1:8
14	<1:8	1:16	<1:8
15	<1:8	1:16	<1:8

IFA*** Commercial IFA slides treated with 10% glacial acetic acid for 5 minutes

TABLE VI: COMPARISON OF COMPLEMENT FIXATION TEST RESULTS WITH LATEX AGGLUTINATION TEST

No	LA	CF	
1	1:1024	1:8	
2	<1:8	<1:8	**
3	≥1:1024	1:64	
4	1:512	1:128	
5	1:16	1:64	
6	1:32	1:64	**
7	1:64	1:64	**
8	1:256	1:64	
9	1:128	≥1:256	**
10	1:256	1:128	**
11	1:256	1:64	
12	<1:8	<1:8	**
13	<1:8	<1:8	**
14	1:4	<1:8	**
15	1:8	<1:8	**
16	1:16	<1:8	
17	<1:8	<1:8	**
18	<1:8	<1:8	**
19	<1:8	<1:8	**
20	1:16	<1:8	
21	<1:8	<1:8	**
22	<1:8	<1:8	**
23	1:64	1:32	**
24	1:16	<1:8	
25	1:1024	1:32	
26	1:64	1:8	
27	1:64	1:16	
28	1:1024	1:32	
29	1:256	1:16	

30	1:64	1:64	**
31	<1:8	<1:8	**
32	≥1:32	<1:8	
33	<1:8	<1:8	**
34	1:1024	1:16	
35	1:64	1:8	
36	<1:8	1:4	**
37	1:16	<1:8	
38	1:32	<1:8	
39	1:128	1:32	
40	1:64	1:64	**
41	1:256	1:128	**
42	1:128	1:256	**
43	<1:8	1:8	**
44	1:256	1:64	
45	1:512	1:64	
46	1:512	1:64	
47	1:32	1:32	**
48	<1:8	<1:8	**
49	1:4096	1:64	
50	<1:8	<1:8	**
51	1:16	1:8	**
52	1:1024	1:64	
53	1:1024	1:64	
54	<1:8	<1:8	**
55	1:128	1:16	
56	1:128	1:16	
57	1:64	1:32	**
58	<1:8	<1:8	**
59	<1:8	<1:8	**

** 100% correlation with CF.

P = 0.0039

TABLE VII: COMPARISON OF CF, IFA, ACIF, ELISA, AND LA TEST BY Sensitivity, specificity, false-positive rate, false-negative rate, time requires to get the results from the laboratory, labor intensity.

CRITERIA	CF	IFA	ACIF-1	ELISA	LA
Sensitivity	94%	100%	97%	100%	100%
Specificity	100%	57%	100%	38%	90%
False positive	0%	43%	0%	69%	0%
False negative	6%	0%	3%	0%	10%
Time (hours)	30-36	2-4	3-5	4-6	1
Labor intensity	++++	++	+++	+++	+

CF = Complement Fixation Test
IFA = Indirect Fluorescent Antibody Test
ACIF = Anticomplementary Immunofluorescent Test
ELISA = Enzyme-Linked Immunoassay Test
LA = Latex Agglutination Test
++++ = Very heavy labor
+++ = Heavy labor
++ = Moderate labor
+ = Light labor
Time = Time requires to get the result of the test from the laboratory

FIGURE I: Comparison of Complement Fixation Test with Indirect Fluorescent Antibody Test. The correlation is weak, and IFA titers remained higher.

COMPARISON OF IFA WITH CF

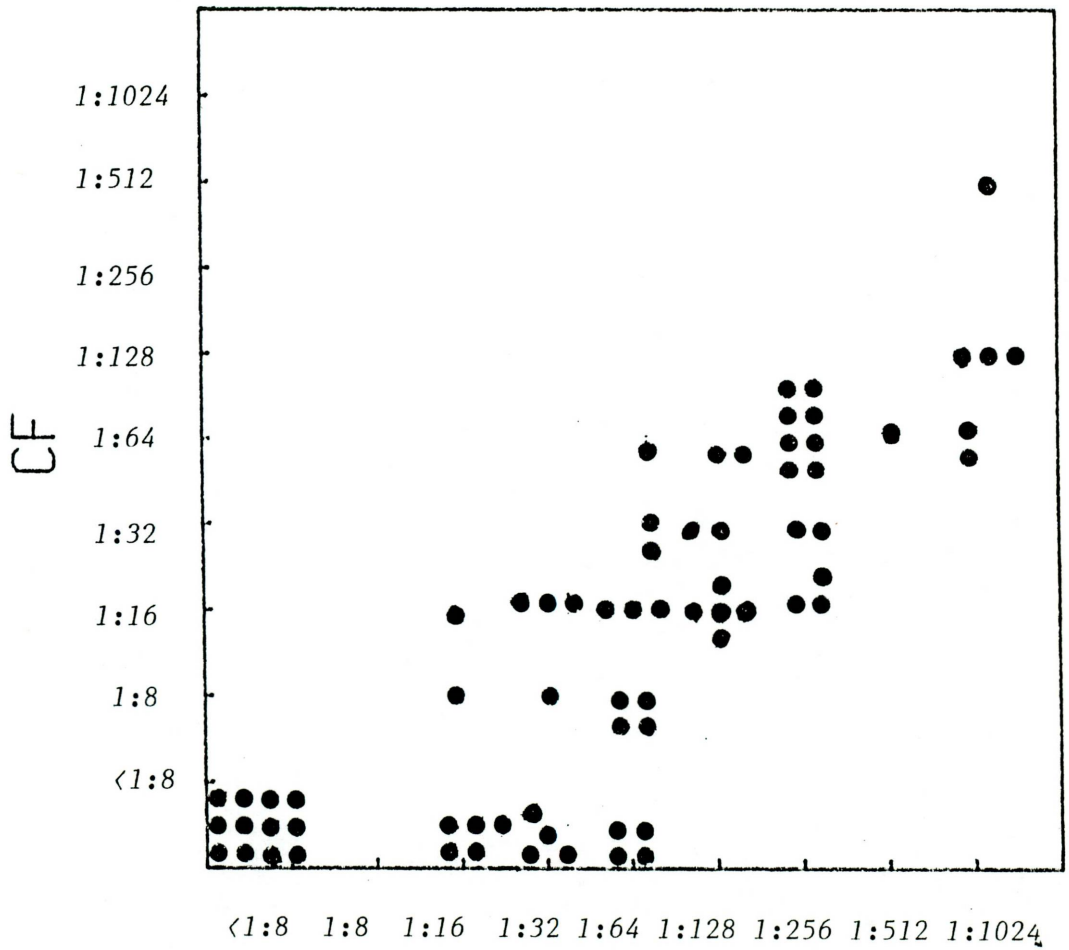


FIGURE II: Comparison of Complement Fixation Test with Anticomplementary Immunofluorescence Test. The correlation is high.

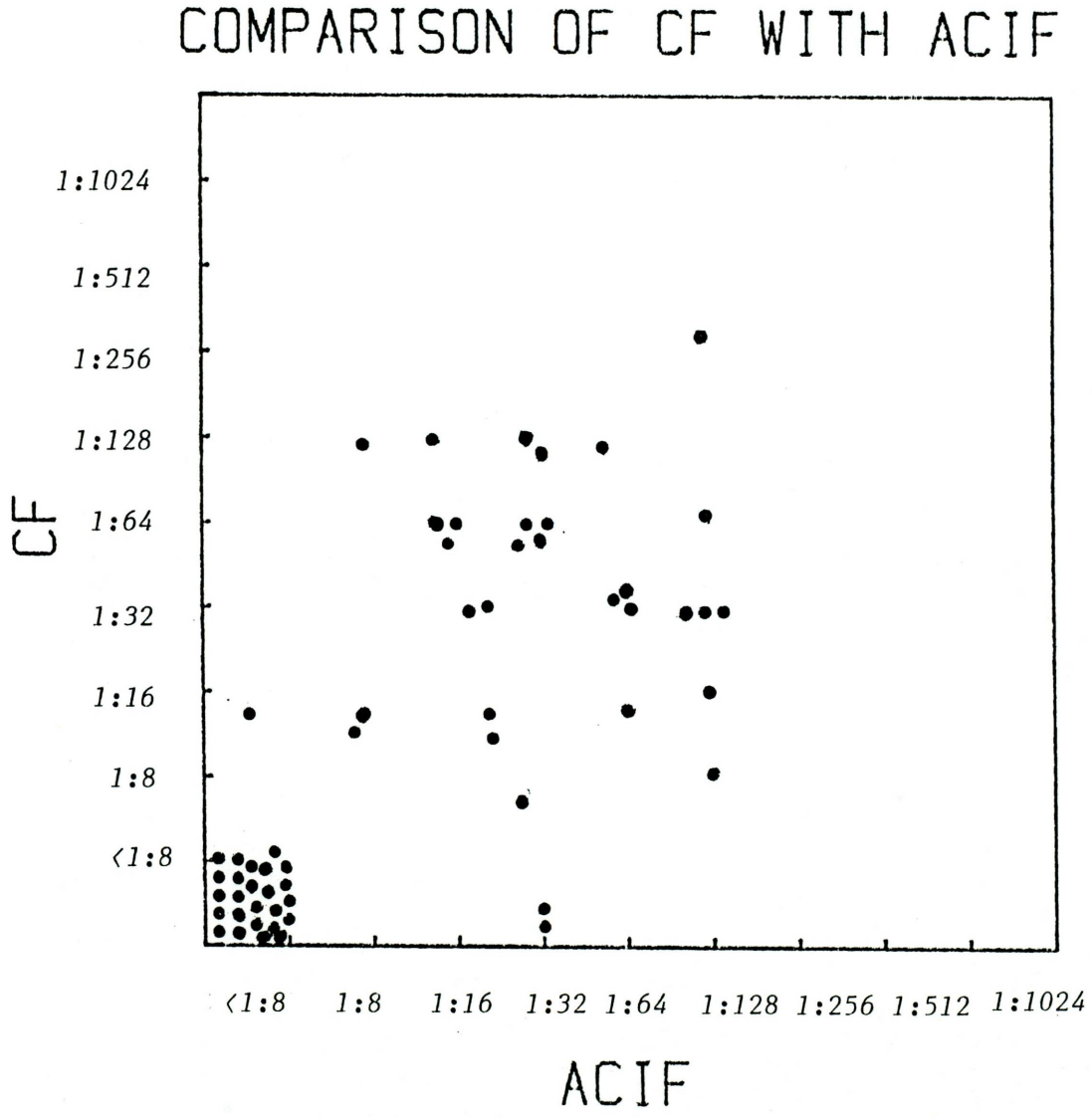

COMPARISON OF CF WITH ACIF
CF
1:1024
1:512
1:256
1:128
1:64
1:32
1:16
1:8
<1:8
<1:8
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
ACIF

FIGURE III: Comparison of Complement Fixation Test with Enzyme Linked Immunoassay Test. The correlation is weak.

COMPARISON OF CF WITH ELISA

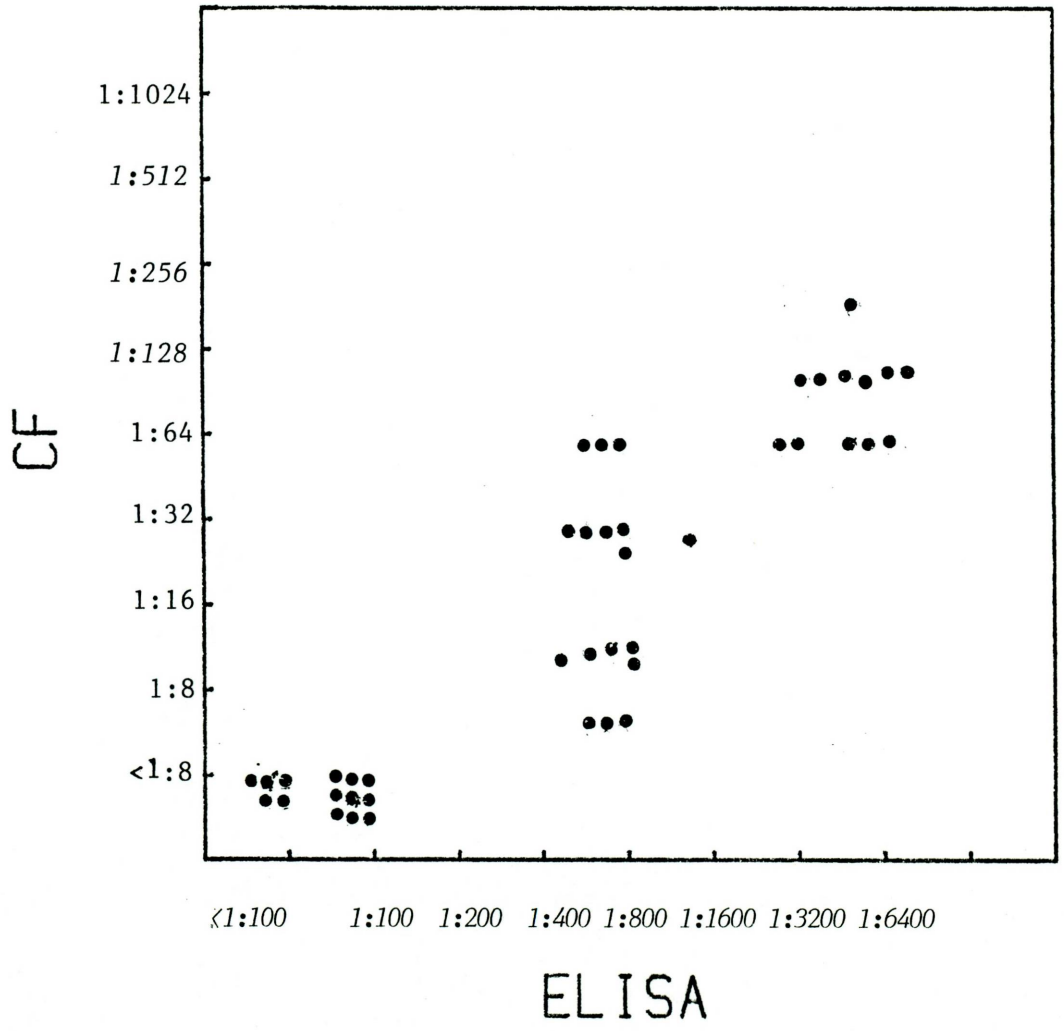


FIGURE IV: Comparison of Complement Fixation Test with Latex Agglutination Test. The correlation is good. The LA test shows higher sensitivity.

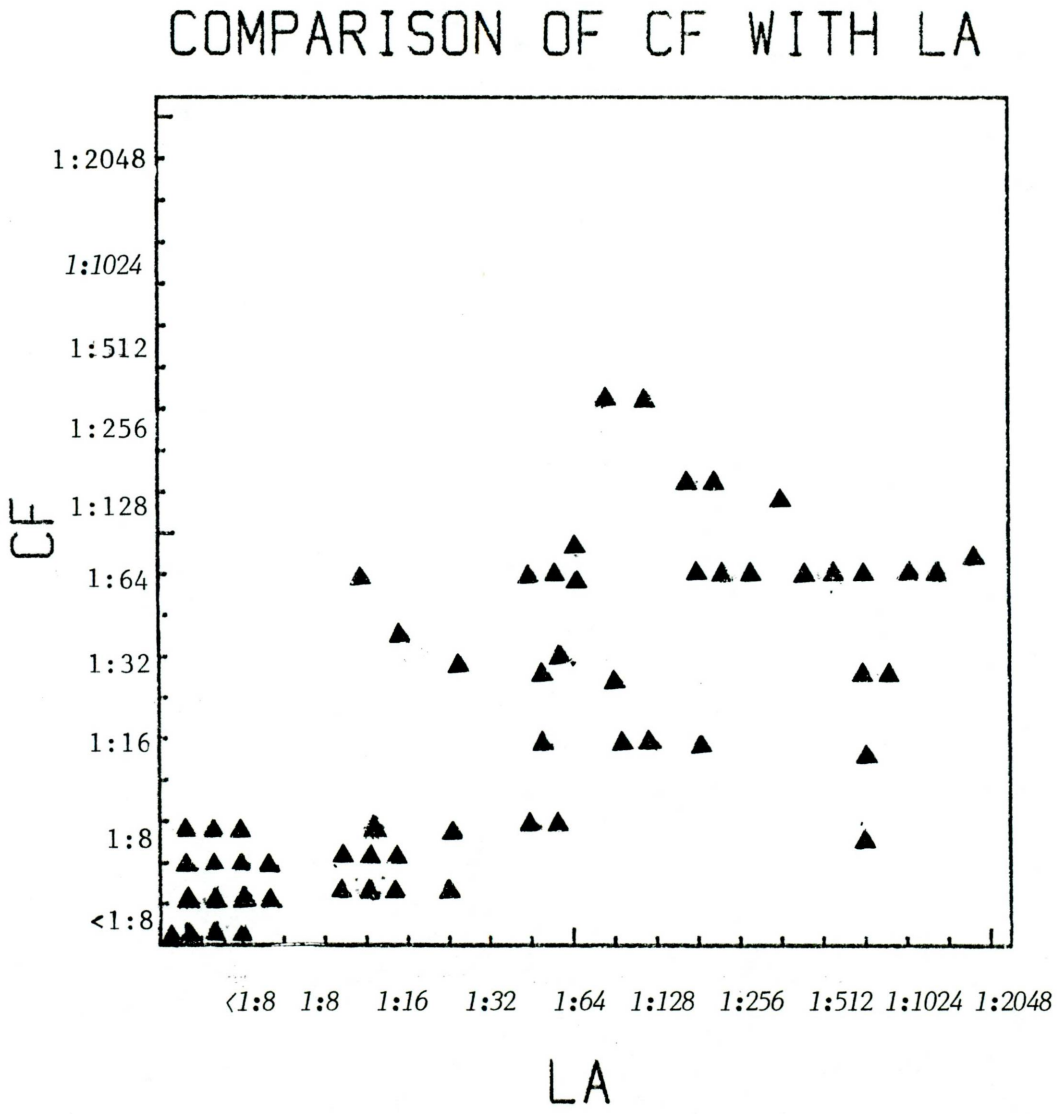

COMPARISON OF CF WITH LA
CF
1:2048
1:1024
1:512
1:256
1:128
1:64
1:32
1:16
1:8
<1:8
<1:8
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
1:2048
LA

FIGURE V: Comparison of Indirect Fluorescent Antibody Test with Anticomplementary Immunofluorescence Test. The correlation is weak. The IFA test shows higher sensitivity.

COMPARISON OF IFA WITH ACIF

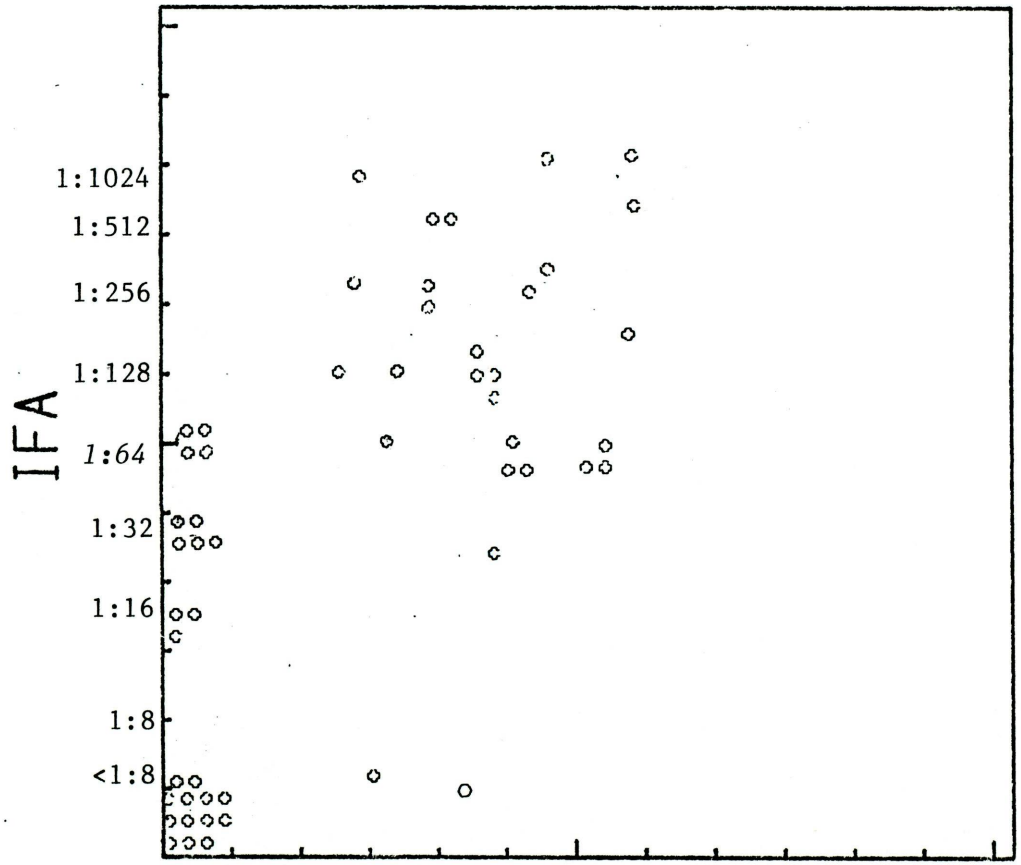


FIGURE VI: Comparison of Indirect Fluorescent Antibody Test with Enzyme Linked Immunoassay Test. The correlation is high.

COMPARISON OF IFA WITH ELISA

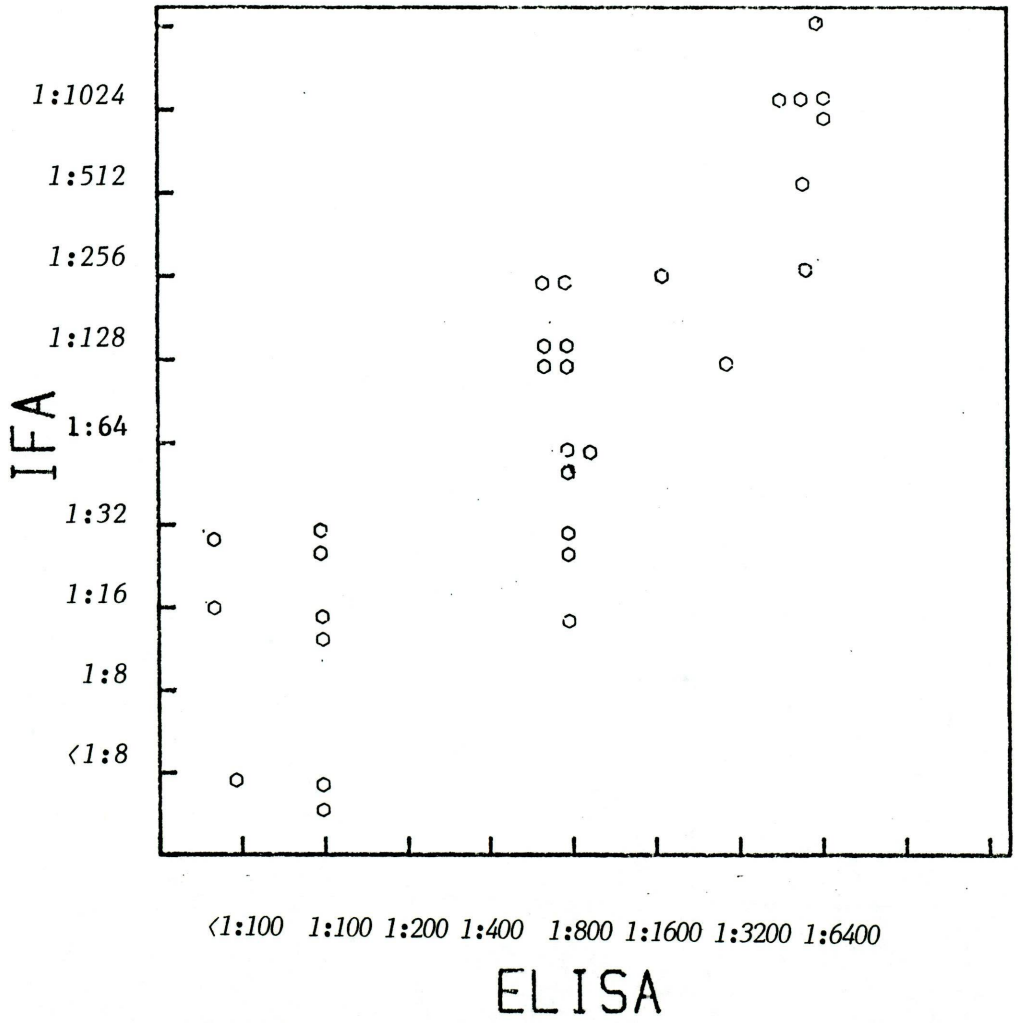


FIGURE VII: Comparison of Indirect Fluorescent Antibody Test with Latex Agglutination Test. The correlation is good. The IFA shows higher sensitivity.

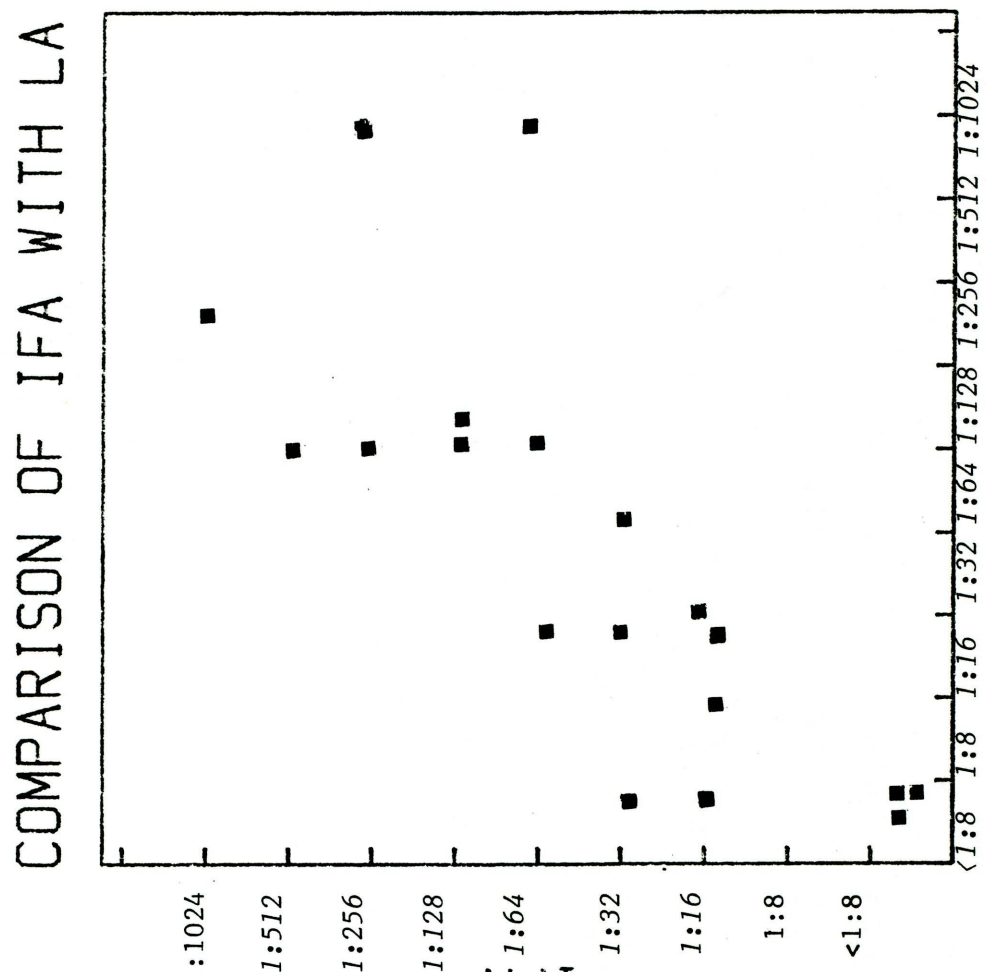

COMPARISON OF IFA WITH LA
IFA
1:1024
1:512
1:256
1:128
1:64
1:32
1:16
1:8
<1:8
<1:8
1:8
1:16
1:32
1:64
1:128
1:256
1:512
1:1024
LA

FIGURE VIII: Comparison of Anticomplementary Immunofluorescence Test with Enzyme Linked Immunoassay Test. The correlation is weak..

COMPARISON OF ACIF-1 WITH ELISA

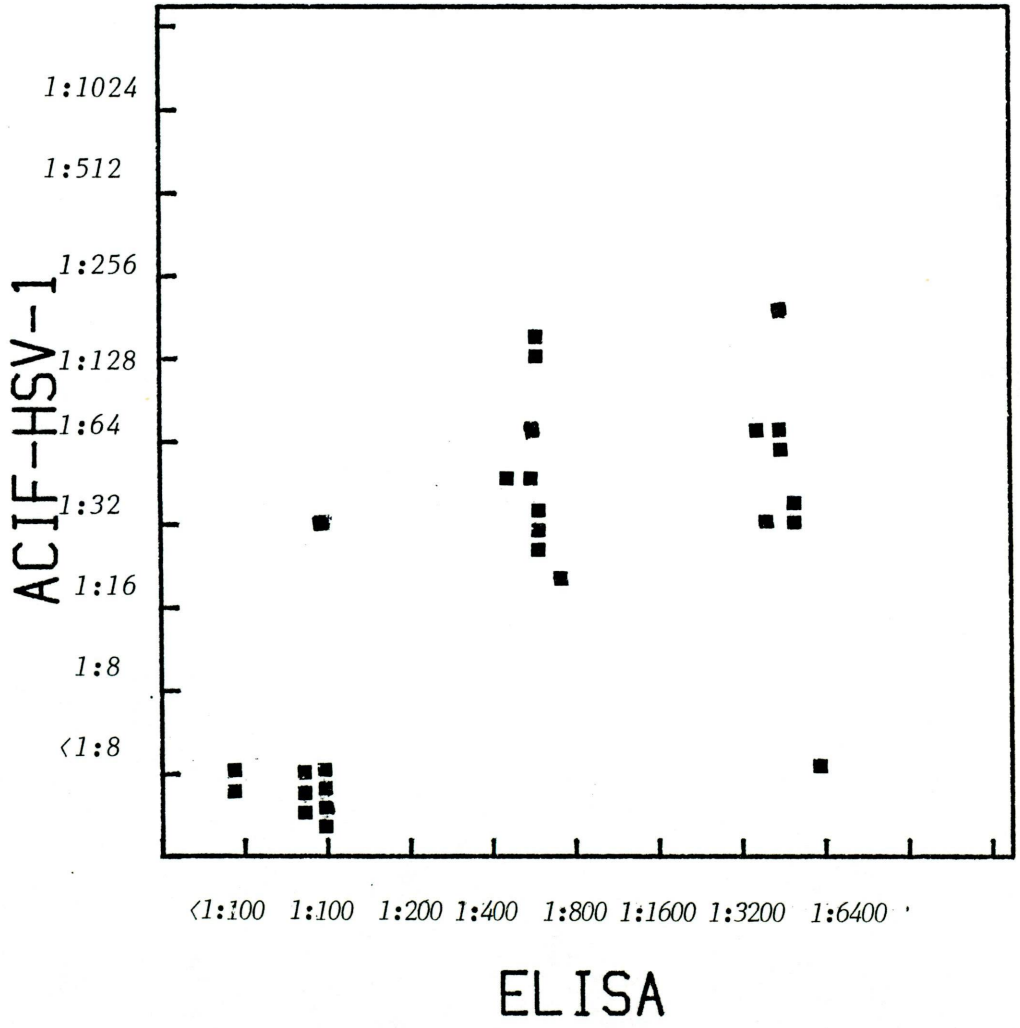


FIGURE IX: Comparison of Anticomplementary Immunofluorescence Test with Latex Agglutination Test. The correlation is good. The LA test shows higher sensitivity.

COMPARISON OF ACIF-1 WITH LA

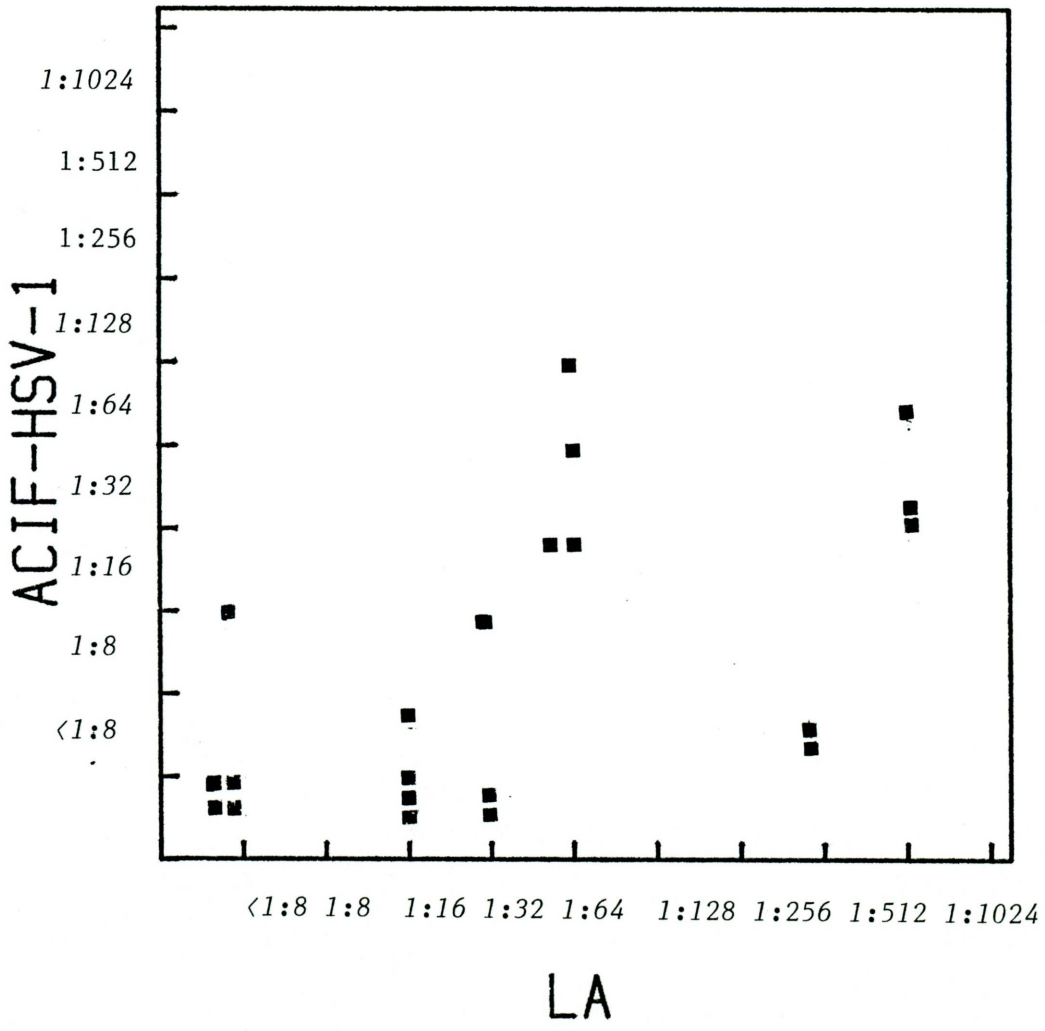
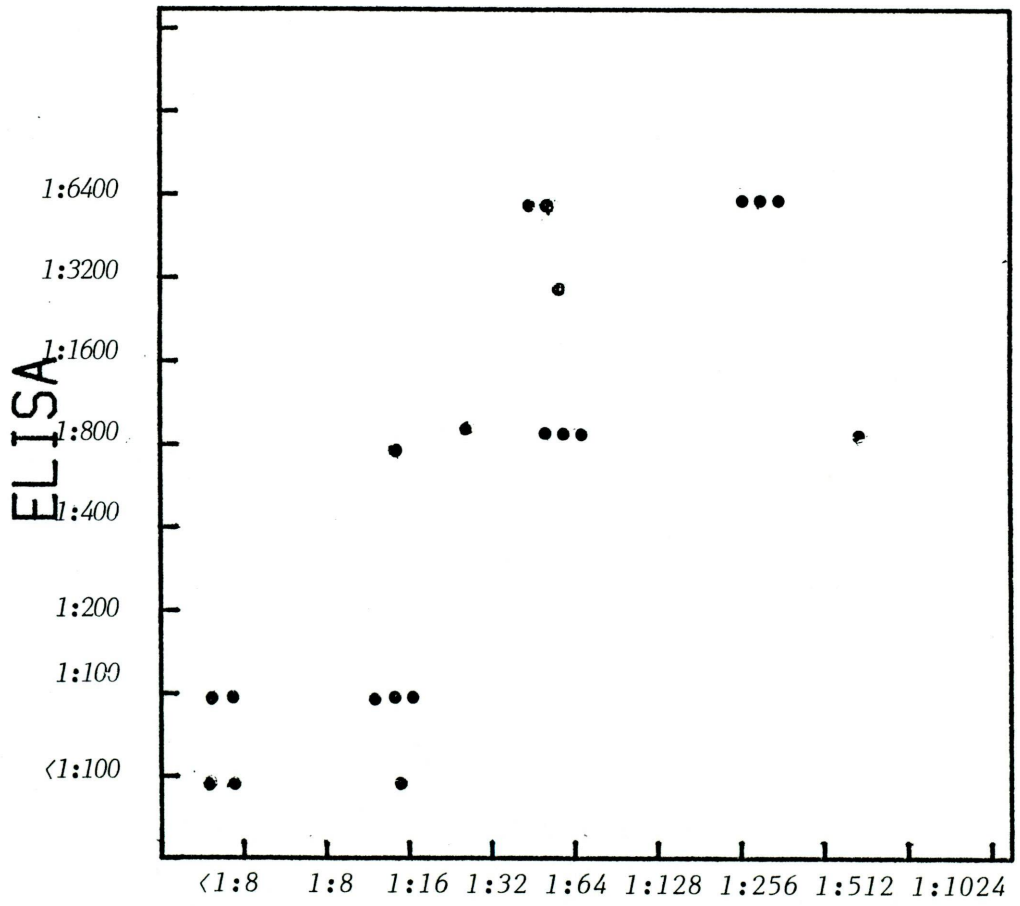


FIGURE X: Comparison of Enzyme Linked Immunoassay Test with Latex Agglutination Test. The correlation is good.

COMPARISON OF ELISA WITH LA



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APPENDIX SECTION

PREPARATION OF VERONAL BUFFERED SALINE (VBS):

Solution A

a. 85.0 gr NaCl

b. 3.75 gr Na 5,5 diethylbarbiturate

c. 1400 ml distilled H_2O

Solution B

a. 5.75 gr 5,5 diethylbarbituric acid

b. 500 ml hot distilled H_2O

Solution C

a. 10 gr $MgCl_2$ .6 H_2O

b. 4 gr $CaCl_2$ .2 H_2O

c. 100 ml H_2O

Mix Solutions A and B, cool to room temperature, and add 5 ml of a stock solution C. Bring the total volume to 2000 ml. (This **stock solution** will keep indefinitely at 4°C.)

Veronal-Buffered Saline (VBS) working solution - Make a precise five-fold dilution which results in an isotonic solution. This working solution should be at pH 7.3. Store at 2°-5°C. It will be stable for 6 weeks.

ANTIGEN TITRATION:

Standardize antigens by testing serial two-fold dilutions of antigen against serial two-fold dilutions of immune serum to determine the optimal dilution of antigen which gives fixation. This procedure is often referred to as a "box" titration.

1. Mark plate such that the serum dilutions are across the

plate and the antigen dilution are down. Mark serum control (SC) wells, complement (CC) wells, previous lot antigen wells, and nonspecific antigen (NS) well.

2. Make 1:8 dilution of positive serum, inactivate at 56°C for 30 minutes.
3. Add 25 μl VBS to all wells in the test except the first well in each row.
4. Add 50 μl diluted serum to each of the first wells and 25 μl to the SC well.
5. Place 25 μl (using a diluting pipettes) in first well of each row of test. Serially dilute rows for test. Two-fold dilution will be 1:8 -1:512. Remove pipettes, discard them.
6. Dilute antigen in tubes (1:2 - 1:64). Add 25 μl of diluted antigen to the designated rows of test.
7. Dilute previous lot antigen (dilution previously obtained) and add 25 μl to each well of designated row. Dilute NS antigen (tissue control antigen) and add 25 μl to each well in designated row.
8. Add 25 μl VBS to all SC wells. Place plate at 40°C for 30 minutes.
9. Make complement dilution of 1:5, 1:0 and 0.5U
   Add to the appropriate wells of the C control.
10. Add 25 μl complement (diluted to 2 U) all wells of test.
11. Incubate at 4°C for 16-18 hours.
12. Remove plate from 4°C, place at room temperature for 10

minutes.

13. Add 50 μl sensitized sheep RBC's (sensitized 15 minutes earlier) to every well of plate. Mix with vibrator for 40 seconds.

14. Incubate at 37°C for 15 minutes. Read.

15. Record results in log book as:

4+	75-100% C′ fixation or no lysis
3+	50-75% C′ fixation
2+	25-50% C′ fixation
+	0% C′ fixation complete lysis

16. Complement control well should read:

2.0 U	0
1.5 U	0
1.0 U	2+
.5 U	4+
SC	0
NS	0

Previous lot: should give titer within $\pm$ one two-fold dilution of earlier results.

COMPLEMENT TITRATION:

NOTE: Complement must be titrated each time tests are performed. Whenever complement is involved, all the test materials should be refrigerated at 4°C for 30 minutes or until cold before complement is added.

1. Label 1 row in a plate for each antigen to be tested. Mark

last well in each row as cell control well. Add VBS (25 μl) beginning at well #1 in each row, and ending at well #10; in cell control add 75 μl VBS.

2. Add 25 μl antigen (see antigen dilution) to each well of test except cell control well. Place plate at 4°C for 30 minutes.
3. Make 1:60 dilution of complement (0.1 ml complement + 5.9 ml VBS)
4. Perform the titration in 12 x 75 ml tubes, using the volumes of reagents shown below:
5. Add 25 μl complement in reverse order to plate beginning with 1:600 dilution of complement in well #10 to 1:60 dilution of complement in well #1.
6. Incubate 30 minutes at 37°C.
7. Add 50 μl sensitized sheep RBC to each well of test and cell control wells. Mix well with vibrator for 40 seconds.
8. Incubate for 30 minutes at 37°C, and then read. The wells containing the least amount of complement showing complete hemolysis represents 1 U. Use 2 exact U in the test. (Example: if 0.6 ml of a 1:60 dilution of complement equal 1 exact U, then 1.2 ml of the 1:60 dilution of complement equal 2 exact U.)

SHEEP RED BLOOD CELL PREPARATION:

1. Place 4 ml of sheep RBC in a 15 ml graduated centrifuge tube.

2. Fill to the 15 ml mark with cold VBS.
3. Centrifuge the blood at 900'g for 10 minutes in a refrigerated centrifuge.
4. Remove the supernatant fluid by suction.
5. Add cold VBS to fill the tube to the 15 ml mark.
6. Mix gently to resuspended the cells, and centrifuge at 900 G's for 10 minutes.
7. Repeat step 4, 5, 6, at least 3 times or until the supernatant is clear.
8. Read and record the volume of packed cells.
9. To determine the volume of VBS to be removed from the suspension, divide the volume of packed cells by 0.2. (20% sheep erythrocyte suspension.)
   For example: If the volume of packed cells is 1.0 ml, divide 1.0 ml by 0.2, so that the total volume of 20% sheep RBC suspension should be 5 ml.
10. Carefully remove the supernatant fluid by suction to the desired mark.

HEMOLYSIN PREPARATION:

1. Prepare a 1:100 stock solution of hemolysin as follows: Mix 94.0 ml of saline and 4.0 ml of 5% phenol in 0.85% saline.(To a 100 ml volumetric flask to the 100 ml mark with saline. Mix by inverting several times.) Add 2.0 ml of hemolysin, 50% in glycerol (store at 4°C).
2. Hemolysin titration

a. Label 2 rows of 12 wells. Mark the last well (12) in each row as cell control (cc).

b. Add 25 μl VBS to wells 2 through 10 in each row.

c. Add 0.1 ml VBS to cell control wells.

d. Add 50 μl Hemolysin (1:100) to the first well in each row.

e. Prepare a serial two-fold dilution with 25 μl diluting pipette. The dilution series is 1:100, 1:200, 1:400, etc.

f. Add 50 μl VBS to wells 1 through 10.

g. Add 25 μl 1:30 complement (0.2 ml of complement plus 5.8 ml of VBS) to each well of test except the cell control well.

h. Prepare a 2% sheep RBC suspension by making a 1:10 dilution of the 20% sheep RBC.

i. Add 25 μl of 2% sheep RBC mixture to all wells, including cell control wells. Mix with vibrator for 40 seconds.

j. Incubate at 37°C for 30 minutes and then read. The highest dilution of hemolysin which shows complete hemolysis represents 1 U. Use 2 U in the test. (Example: If the 1:3200 dilution shows complete hemolysis and the 1:6400 dilution shows partial hemolysis, 1 U would be represented by a 1:3200 dilution. Therefore, 2 U would be contained in a

1:1600 dilution of hemolysin.

SENSITIZED CELL SUSPENSION:

1. Prepare on optimal hemolysin dilution from the 1:100 stock hemolysin solution. (Example: from the above example of hemolysin titration, a 1:1600 dilution of hemolysin is optimal, so make a 1:16 dilution of stock hemolysin solution.)
2. Prepare an equal volume of 2% sheep RBC (make 1:10 dilution of 20% sheep RBC).
3. Sensitize cells by pouring diluted hemolysin into the cell suspension and rapidly pouring the mixture back and forth.
4. Allow the sensitized cells to remain at room temperature for at least 10 minutes before use.
5. Make enough cells for the test.

INDIRECT FLUORESCENT ANTIBODY TEST (IFA)

PRINCIPLE OF TEST

The **HSV-CHECK** test kit utilizes the indirect immunofluorescent antibody (IFA) method for the detection of antibody to HSV. HSV-infected cells are immobilized on a glass slide and any addition of patient serum containing antibodies to HSV will bind to the antigen substrate and will not be rinsed off. Subsequently, when fluoresceinated anti-human globulin is added to the reaction site it will bind to the antibodies, causing the HSV-infected cells to fluoresce when viewed through a fluorescence-equipped microscope. The presence of viral related antibody to HSV is demonstrated by a greenish-yellow fluorescence in the virally infected cells. Negative serum will not show this fluorescence.

INSTRUCTIONS FOR USE

Kit storage: 2°-8°C

Reagents and antigen slides should not be used after printed expiration date.

Reagents are ready for use after reconstitution.

The components of this kit have been tested and standardized as a unit. Use of components from other lots or other manufacturers may yield unsatisfactory results.

MATERIALS SUPPLIED

1. HSV-Antigen Substrate Slides - 10 x 10 well slides.
   individually foil wrapped. HSV-2 infected cells and uninfected control cells are fixed into each well at optimum proportions.
   Storage and Stability: Stored at -20°C or below, slides are

stable to expiration date. Individual slide packets should remain sealed until just before use.

2. Anti-human IgG Globulin (goat) - 1 vial. FITC - labeled, affinity purified conjugate containing Evans Blue counterstain: pretitered.

   Lyophilized - reconstitute with 3 ml distilled or deionized water.

   Stability: Unopened, stored at 2°-8°C. Stable to expiration date.

   Reconstituted aliquots, store at -20°C. Aliquots should not be stored in a frost-free freezer. Stable for 8 months.

   Thawed aliquots should not be re-frozen.

3. HSV Positive Control Serum - 1 vial.

   Lyophilized - reconstitude with 1 ml distilled or deionized water to yield a 1:10 working solution.

   Stability: Unopened, store at 2°-8°C. Stable to expiration date.

   Reconstituted, store at 2°-8°C. Stable for 6 weeks.

   Reconstituted aliquots, store at -20°C. Aliquotsshould not be stored in a frost-free freezer. Stable for 8 months. Thawed aliquots should not be re-frozen.

4. HSV Negative Control - 1 vial

   Lyophilozed - reconstitute with 1 ml of distilled or deionized water to yield a 1:10 working solution.

   Stability: Unopened, store st 2°-8°C. Stable to expiration date.

   Reconstituted, store at 2°-8°C. Stable for 6 weeks.

Reconstituted aliquuots, store at -20°C. Aliquots should not be stored in a frost-free freezer. Stable for 8 months. Thawed aliquots should not be re-frozen.

5. Phosphate Buffered Saline (PBS Powder) - 1 vial

   Add contents of PBS vial to 1 liter volumetric flask. Q.S. with distilled water to 1 liter. Yields 1 liter PBS, 0.01 M, pH 7.5, containing 0.01% merthiolate preservative.

   Stability: Store at 2°-8°C, stable indefinitely.

6. Mounting Fluid - 1 dropper bottle

   Buffered glycerol, pH 8.0. Ready for use.

   Stability: Store at ROOM TEMPERATURE.

TEST PROCEDURE

1. Prepare a 1:10 dilution of the patient specimen using the prepared PBS.
2. Remove slide from foil packet and label appropriately.
3. Using a Pasteur pipette, add a sufficient quantity (approx. 15-20 μl) of the appropriately diluted serum sample so as to just cover each reaction site.
4. Place the slide in a humidity chamber and incubate at 37 °C for 30 minutes.
5. Remove slide from chamber and briefly rinse with a gentle stream of PBS; avoid directing PBS at wells. Immerse slide in PBS for 5 minutes.
6. Very briefly (approx. 1-2 seconds) dip slide into distilled water so as to prevent the formation of any PBS crystal between wells

when drying.

7. Allow the slide to air dry by standing on end on paper toweling.
8. Add just enough of the appropriately reconstituted conjugate to cover each reaction site (approx. 15-20 μl).
9. Incubate slide at 37°C in a humidity chamber for 30 minutes.
10. Rinse slide as described in Steps 5,6, and 7.
11. Place a small drop of mounting fluid on each reaction site just before reading and carefully cover with a No. 1, 22 x 50 mm cover slip; avoid trapping air bubles.
12. Read slide as soon as possible at 150X to 200X magnification.

SUGGESTED FINAL DILUTION METHOD FOR PATIENT SERUM

Use fresh serum dilution with PBS supplied.

Well#	Specimen	Dilution Rate
1	Patient's serum	1:10
2	" "	1:20
3	" "	1:40
4	" "	1:80
5	" "	1:160
6	" "	1:320
7	" "	1:640
8	" "	1:1280
9	" "	1:2560
10	" "	1:5120

SUGGESTED DILUTION METHOD FOR CONTROL SERA

Well#	Specimen	Dilution Rate
1	PBS (conjugate control)	----
2	Negative control serum	1:10
3	Negative control serum	1:20
4	Positive control serum	1:10
5	Positive control serum	1:40
6	Positive control serum	1:160
7	Positive control serum	1:320
8	Positive control serum	1:640
9	Positive control serum	1:1280
10	Positive control serum	1:2560

QUALITY CONTROL

Positive and negative controls should be included in each test run. The negative control must yield a negative result at the screening dilution. The positive control serum should exhibit a 4+ reaction at the screening dilution and diminish to a 1+ reaction at its stated titer. A day-to-day variance within one two-fold dilution from the stated titer is considered acceptable.

INTERPRETATION OF RESULTS

A negative result was obtained when cells do not fluoresce greenish-yellow and the red color of the counterstained cells is predominant. In addition, a negative reaction at the screening dilution indicated no

detectable antibody to HSV.

A positive reaction was indicated by the presence of approximately 10% of the cells in each field that exhibit a greenish-yellow fluorescence. The intensity of the fluorescence was judged as follows: ± to 1+, weak;2+, moderate; 3+, strong; 4+, brilliant. The remaining cells would provide a reddish background. The HSV titer was considered to be the highest dilution of serum which produced a 1+ fluorescence in the infected cells.

EXPECTED VALUES

Positive HSV results can be seen in children and adults; primary infection in either children or adults who have no HSV antibody is commonly mild or inapparent but may be quite sever.

HSV-1 infections can occur early in life and may reach a high incidence in adults, while HSV-2 infections seldom occur until the age of sexual activity and rise to an incidence of 20% in adults.

Since reactivated infections can raise and maintain titers in excess of 1:640, no positive means of recognizing titers of significance in activate disease have been established. However, any four-fold or greater rise in titer can be considered definitive in the diagnosis of active disease.

TEST LIMITATIONS

It is not intended to distinguish HSV-1 from HSV-2 antibodies with the HSV-CHECK procedure. HSV-1 and HSV-2 have specific antigens and because of the extensive cross-reactivity between HSV-1 and HSV-2 antibodies, differentiation between the two using this method is not practical.

ENZYME-LINKED IMMUNOASSAY

"HSV BIO-ENZABEAD TITRATION KIT"

MATERIAL SUPPLIED:

Reconstitution and Storage

A. Beads (Store at 2-8°C.)

1. Antigen Control Beads - 6 vials (26 beads/vial)

2. Negative Control Beads - 2 vials (15 beads/vial)

Store bead vials tightly capped at 2-8°C. The beads are stable for a least 28 days after opening.

B. Control Sera (Store at 2°-8°C.)

1. POSITIVE Control Serum (Human) HIGH TITER, to HSV - 1 vial

2. POSITIVE Control Serum (Human) LOW TITER, to HSV - 1 vial

3. NEGATIVE Control Serum (Human) to HSV - 1 vial

To reconstitute, add 0.8 ml of 1X Diluent to each vial. Swirl gently to dissolve. Each reconstituted vial contains human serum at a 1:17 dilution and is stable for 28 days when stored at 2°-8°C.

C. Reagents (Store at 2°-8°C.)

1. Diluent Concentrate (5X) For Sera and EnzAbody - 2 vials (15 ml/vial). Contains normal goat serum in phosphate buffered saline. Contains Thimerosal as a preservative. To prepare 1X Diluent, add contents to 60 ml of reagent water (distilled or deionized) in a clean glass or plastic screw cap bottle. Add 0.75 ml Of 5% Tween-20 and mix gently by inverting the bottle several times to avoid excessive foaming. Label, date and store the 1X

Diluent tightly capped at 2°-8°C. The 1X Diluent is stable for 28 days when stored at 2°-8°C. If crystals have formed, resolubilize by warming at 37°C before using.

2. Tween-20, 5% - 1 vial (2,5 ml/vial). Used to prepare 1X Diluent. Contains Thimerosal as a preservative.

3. Peroxidase Conjugated Goat Anti-Human Immunoglobulins (EnzAbody) - 2 vials (lyophilized). Each vial contains 24 ml (after reconstitution with 1X Diluent) of the specific antibody fraction of goat anti-human immunoglobulins labeled with horseradish peroxidase. To reconstitute, add 24 ml of 1X Diluent and mix gently by inverting the bottle several times to avoid excessive foaming. Date and store the EnzAbody at 2°-8°C. The reconstituted EnzAbody is stable for 28 days when stored at 2°-8°C.

4. Wash Solution (50X) - 1 vial (5 ml). Contains 2.5% Tween-20. Prepare 1X Wash Solution fresh daily. Add 1.5 ml to 73.5 ml reagent water (distilled or deionized) in a clean glass or plastic screw cap bottle to yield sufficient quantity of 1X Wash Solution to fill two 96- well plates. (For lesser quantities, calculate sufficient solution required based on 0.5 ml of 50X Concentrate yielding 25 ml of 1X Wash Solution). Mix by inverting the bottle several times. Discard unused solution.

5. ABTS Substrate - 2 vials (lyophilized). After reconstitution each vial contains 0.03% 2,2-Azino-di(3-ethyl-benzthiazoline-sulfonate). To reconstitute each vial, add contents of Subtrate Diluent and mix gently by inverting the bottle several times. The reconstituted substrate is stable for 14 days whenstored tightly capped at 2°-8°C.

6. Substrate Diluent for ABTS - 2 vials (30 ml/vial). Contains hydrogen peroxide.

7. "Stop" Solution - 2 vials (5 ml/vial). Contains 1.25% Sodium Fluoride.

D. Accessory Box (Store at room temperature.)

Contains fourteen 96-well plastic plates and four cover sheets. Avoid conditions which might result in abrasion or breakage.

E. Package Insert

F. Data Record Sheet

OUTLINE OF PROCEDURE

1. Dispense solutions of control sera and patient's serum into wells of a 96-well plate or prepare sera dilutions in the plate.
2. Add antigen coated beads and negative control beads and incubate.
3. Wash beads to remove unbound serum proteins.
4. Add beads to enzyme conjugated anti-human immunoglobulins (EnzAbody) and incubate.
5. Wash beads to remove unbound EnzAbody.
6. Add beads to substrate and incubate.

7. Add "Stop" Solution and remove beads.

8. Read and record results.

PROCEDURE

A. General Instructions

1. Review Precautions and Operation of Magnetic Transfer Device sections before starting test.

2. Include the control sera and both negative control and antigen coated beads in each test procedure.

3. Use the enclosed record sheets to identify the location of each serum and the type of bead used.

4. Control sera - see step B below for preparing initial 1:100 dilution.

(a) Negative Control Serum - A negative control bead and an antigen coated bead are used in separate wells containing a 1:100 dilution.

(b) Low Titer Positive Control Serum

(1) A negative control bead is used in the well containing the 1:100 dilution.

(2) Two 2-fold dilutions are made (1:200 and 1:400). Antigen coated beads are used in the wells containing dilutions 1:100, and 1:800 through 1:6400.

NOTE: Do not add antigen beads to the 1:200 and 1:40 dilutions.

5. Patient's Serum

(a) A negative control bead is used in the well containing

the 1:100 Dilution.

(b) Six 2-fold dilutions (1:200 through 1:6400) are made of each patient's serum. Antigen coated beads are used in the wells containing each serum dilution. (1:100 through 1:6400).

B. Preparing Sera Dilutions

1. Prepare 1X Diluent according to instructions in Materials Supplied Section.

2. Utilizing the Tube-titration Method:

(a) Reconstitute the High, Low and Negative control sera with 0.8 ml of 1X Diluent to give a 1:17 dilution. Using test tubes, add 0.2 ml of each of the reconstituted control sera to 1.0 ml of 1X Diluent to yield a starting dilution of 1:100.

(b) Prepare a 1:100 dilution of patient's serum by adding 0.01 ml of serum to 1.0 ml of 1X Diluent.

(c) Starting with the 1:100 dilution, make six serial 2-fold dilutions (1:200 through 1:6400) of the high titer positive control serum and the patient's serum specimen in test tubes. This is done by using six tubes containing 0.5 ml of 1X Diluent and transferring 0.5 ml of the 1:100 dilution to the first tube, 0.5 ml from this tube to the second, and so on. Starting with the 1:100 dilution, make two serial 2-fold dilutions (1:200 and 1:400) of the low titer

positive control serum.

(d) Following your record sheet, add 0.2 ml of each dilution to the appropriate well of a 96-well plate.

(e) Label the plate "HSV Serum."

C. Assay

1. Place the negative control beads and the antigen coated beads in the wells of one of the empty plates according to the record sheet. This may be done with forceps, magnetic tweezers, etc.

2. To start the assay, simultaneously transfer the beads to the "HSV Serum" plate using the magnetic transfer device as follows:

(a) Assure that a 96-well plate is inverted in the top of slot of the transfer device.

(b) Assure that the magnet is in the UP position.

(c) Slide the plate containing the beads under the top plate

and lower the magnet (all beads should now be in the inverted plate).

(d With magnet in DOWN position (without disturbing the inverted plate), remove the lower plate and slide the plate containing the diluted sera under the inverted plate.

(e) Raise the magnet to allow all the beads to drop into the wells of the "HSV Serum" plate.

3. Remove the bottom plate containing the sera and beads, cover the plate with a plastic cover sheet and incubate for 90 minutes at 37°C. During the incubation period, proceed to steps 4 and 5.

4. While beads are incubating (step 3) prepare the 1X Wash Solution according to the Materials Supplied Section and aliquot 0.3 ml of the 1X Wash Solution into the appropriate wells of the 96-well plate from step 2d (based on the record sheet). Label the plate "Wash Plate 1." Similarly, fill a second plate with 1X Wash Solution and label it "Wash Plate 2."

5. While the beads are incubating (step 3) prepare the EnzAbody solution according to the Materials Supplied Section. Aliquot 0.2 ml of the reconstituted EnzAbody into the appropriate wells of a separate plate (based on the record sheet). Label the plate "HSV EnzAbody."

6. After serum incubation, remove cover sheet and wash the beads as follows:

   (a) Assure that the 96-well plate used in step 2a is inverted in the top slot of the transfer device.

   (b) Assure that the magnet is in the UP position.

   (c) Slide the "HSV Serum" plate containing the beads under the top plate and lower the magnet (all beads should now be in the inverted plate).

   (d) With magnet in DOWN position, remove the bottom plate,

(without disturbing the top plate) replace cover sheet and place "HSV Serum" plate in container for proper disposal.

(e) Slide "Wash Plate 1" (from step 4) under the top plate and wash the beads 12 times by raising and lowering the magnet to the extreme UP and full DOWN position (count UP and DOWN as 1 wash).

(f) With magnet in the DOWN position (beads in top inverted plate) remove the wash plate without disturbing the top plate and save for step 10e.

7. Slide the "HSV EnzAbody" plate from step 5 under the top plate and raise the magnet to allow the beads to drop into the wells.

8. Remove the bottom plate containing EnzAbody and beads, cover the plate with a plastic cover sheet and incubate for 90 minutes at 37°C. Leave the inverted top plate in position for use in step 10. During incubation proceed to step 9.

9. While beads are incubating, prepare the ABTS substrate solution according to the Material Supplied Section and aliquot 0.2 ml into each appropriate well of an unused plate (based on the record sheet). Label the plate "HSV Substrate."

10. After EnzAbody incubation, remove cover sheet and wash the beads as follows:

(a) Assure that the 96-well plate used in step 8 is

inverted in the top slot of the transfer device.

(b) Assure that the magnet is in the UP position.

(c) Slide the "HSV EnzAbody" plate containing the beads under the top plate and lower magnet (all beads should now be in the inverted top plate).

(d) With magnet in DOWN position, remove the bottom plate (without disturbing the top plate). Replace cover sheet and place in container for proper disposal.

(e) Slide "Wash Plate 1" (saved from step 6f) under the top plate and wash 3 times by raising and lowering the magnet to the extreme UP and full DOWN position.

(f) With magnet in DOWN Position, (beads in top plate) remove "Wash Plate 1" and insert " Wash Plate 2."

(g) Wash the beads 9 times by raising and lowering the magnet to the extreme UP and full DOWN positions. (Count UP and DOWN as 1 wash).

(h) With the magnet in the UP position (beads in the Wash Plate) replace the top inverted plate with an unused plate. This change must be made to avoid contamination of the substrate with EnzAbody.

(i) Lower the magnet (all beads should now be in the unused inverted top plate) and remove the bottom "Wash Plate 2" without disturbing the top plate.

11. Slide the "HSV Substrate" plate under the top plate and raise the magnet to allow all the beads to drop into the

wells.

12. Remove the bottom plate containing substrate and beads and incubate without a cover sheet at room temperature (20°-25°C) for 10 minutes. Do not agitate the plate.

13. After 10 minutes, add 25 µl of "Stop" Solution to each well and gently swirl the plate to disperse the colored reaction product forming at the surface of the beads. Remove the beads (be sure that the 96-well plate from step 10 is inverted in the top slot of the Transfer Device) by placing the Substrate plate in the transfer device and lowering the magnet.

14. Remove the Substrate plate and place on a white background. Record the amount of color from negative to 4+ on the record sheet. A test reading mirror, with a piece of white paper on top of the plate, will facilitate reading the results. Results can be read immediately or within 2 hours. Leave plate uncovered.

15. After reading and recording the results, release the beads into a used plate by sliding the plate into the transfer device and raising the magnet.

16. Sterilize all used plates, tubes, beads and pipettes prior to disposal.

17. Tightly close and return unused reagents to 2°-8°C storage.

RESULTS

A Positive Reaction is indicated by a green coloration surrounding the bead after the appropriate incubation period. Initially, a green halo forms around the periphery of the bead and upon agitation, the color is dispersed into solution.

A Negative Reaction is indicated by a negligible coloration after the stated incubation period.

Valid Test: A valid test requires that the control sera reacts as follows: The coloration obtained with the Negative Control Serum should be negligible or should have negligible color difference between the antigen coated bead and the negative control bead wells at the 1:100 dilution. The Low Titer and High Titer Positive Control Sera should yield a 1 to 2+ green color (definite but light green) in the antigen coated bead well at the stated titers. The High Titer Positive Control Serum should yields a 3 to 4+ green color (dark green) in the antigen coated bead well at the 1:100 dilution.

Endpoint: The antibody titer is determined as the reciprocal of the highest dilution of serum in which a 1 to 2+ color develops with the antigen coated bead. The 1 to 2+ color intensity is that yielded by the Positive Control Sera at the stated titers.

VIROGEN HSV ANTIBODY TEST

REAGENTS

* Reagent Latex [Inactivated herpes simplex virus sensitized latex]; contains buffer, stabilizer, and preservative: sodium azide 0.1%. Resuspended latex particles by gently shaking before each use.
* Sample Diluent; contains buffer, stabilizers, and preservative: sodium azide 0.1%
* Positive Control [Herpes simplex virus antiserum (human)]; contains normal serum (human), buffer, stabilizer, and preservative: sodium azide 0.1%.
* Negative Control [Normal serum (human)]; contains buffer, stabilizer, and preservative: sodium azide 0.1%.

PROCEDURE

Quantitative Protocol

The quantitative protocol consists of preparing serial dilutions of sera on the slide, adding Reagent Latex, mixing, rotating the slide on a serologic rotator for 10 minutes, and observing for agglutination. An antibody titer is obtained by determining the reciprocal of the dilution in the last well containing a positive reaction. A four-fold (or greater) increase in antibody titer between acute and convalescent paired serum samples tested in parallel is diagnostic of recent infection.

Quality Control

The Positive and Negative Controls included in the kit should be tested with each run in accordance with the established quality control protocol for the testing laboratory to ensure that the reagents are functioning properly during testing.

Procedural Notes:

* Allow all reagents to reach room temperature before use and perform all

  procedures at room temperature (21° to 25°C).

* Keep the Reagent Latex at room temperature only during the test procedure. Return to refrigerated temperature (2° to 8°) when testing has been completed.

* The Sample Diluent is provided in a dropper vial (3.0 ml) and in bulk vial (30.0 ml). For convenience, the user may choose the dropper vial to dispense Sample Diluent and to refill it from the bulk vial as needed. To refill the dropper vial, pour 3 to 5 ml of Diluent into the cap of the bulk vial (or a small sterile cup). Hold the dropper vial upright, gently squeeze out the air, then place the dropper tip into the Diluent fluid and release. Any remaining Diluent may be returned to the bulk vial. Alternatively, the user may prefer to use a sterile pipet to deliver Diluent directly from the bulk vial.

Materials Provided

Reagent Latex

Sample Diluent

Positive Control

Negative Control

Disposable Slides

Disposable Stirrers

Quantitative Protocol:

1. Identify one pair of wells for the Positive Control and one pair for the Negative Control (if tested). Then identify up to 10 wells for each specimen using the first and second rows for the acute sample (and one Control) and the third and fourth rows for the convalescent sample (and one Control.) Holding the slide horizontally, the wells would contain a 1:4 dilution well and the following test wells: 1:4, 1:8, 1:16, 1:32, 1:64 on the first (and third) row of six wells, then 1:128, 1:256, 1:512, 1:1024 on the second (and fourth) row. For each control, use one well to make the 1:4 dilution and the second well for testing 25 μl of the 1:4 dilution (on the second and forth rows).
2. Remove the cap from the Sample Diluent and hold the bottle in an inverted position. With gentle pressure-
   a. Add 3 drops (75 μl) of Sample Diluent to the 1:4 dilution well for both samples and Controls.
   b. Add 1 drop (25 μl) of Sample Diluent to the 1:8 test well and each additional well to be used in each serial two-fold titration. Omit the 1:4 test well(s).
3. Prepare a 1:4 dilution of each serum and Control to be tested by

adding 25 µl of serum to the 1:4 dilution well; using a pipet, mix thoroughly but gently, taking care to avoid the formation of bubbles and transfer 25 µl into the 1:4 test well. Remove another 25 µl from the 1:4 dilution well and transfer to the 1:8 test well for each sample...(Repeat the 25 µl transfer for the remaining additional wells in the dilution series for each serum). Discard 25 µl from the last well in the series.

4. Mix the Reagent Latex thoroughly by gently shaking the bottle several times, taking care to avoid the formation of bubbles. Remove the cap and firmly tap the vial against a flat surface. Then, carefully wipe the dropper tip using an absorbent tissue or equivalent. Hold the bottle in an inverted position, and using gentle pressure - Add one drop of well-mixed Reagent Latex to each well containing a serum dilution to be tested (and to the 1:4 test well for each Control.) Omit the 1:4 dilution well(s).

5. Using a new disposable stirrer for each specimen, begin with the most dilute well and mix the contents of each series of wells by spreading the mixture over the entire well. Without delay, place the slide on a serologic rotator for 10 minutes at 100 rpm.

6. Immediately observe for agglutination with the aid of a light held above the slide while holding and gently rocking the slide. When testing has been completed, return the reagents to refrigerated temperature (2-8°C).

RESULTS: Quantitative Protocol

The reaction are graded as follows:

A Negative result is indicated by a fine granular background or a milky background with absence of agglutination.

A Positive result is indicated by distinct large clumps against a clear or slightly milky background, or small but definite clumps against a milky background. At the 1:4 dilution, the Positive Control should be distinctly reactive, and the Negative Control should be nonreactive. If these results are not obtained, the test is invalid and should be repeated.

Interpretation of Reactivity:

A negative result at the 1:4 dilution and greater indicated the absence of HSV antibody or antibody level below the 1:4 dilution. A negative result presumes the individual has not been infected with HSV or that the sample has been collected too early in the course of the disease to exhibit a detectable level of antibody.

When positive results are observed, the HSV antibody titer is the reciprocal of the last dilution which demonstrates agglutination. When paired sera are properly collected, stored, and tested, a four-fold (or greater) difference in antibody titer is diagnostic of recent HSV infection (reactivation, reinfection, or a primary infection where the acute specimen was obtained too late to demonstrate seroconversion.) Seroconversion in paired specimens (acute sample negative, convalescent, positive) is indicative of primary infection.

A difference of one two-fold dilution in antibody titer is not clinically significant. High antibody titers may yield weaker reactions

at 1:4 and 1:8 serum dilutions than at higher dilutions.

LIMITATIONS OF THE PROCEDURE

As in the case of other diagnostic procedures, the results obtained by the use of this kit yield data that must be evaluated by the physician in light of clinical information and other diagnostic procedures, especially viral isolation. Immunological assays should not be used as the sole basis of diagnosis. This test is not intended to replace viral isolation and/or identification.

This test is not intended for the diagnosis of herpes simplex virus infection in pregnant women. The presence of herpes simplex virus should be determined by viral isolation.

Neonatal and cord blood may contain HSV IgG antibody passively transferred from the mother. Viral isolation should be performed in order to diagnose neonatal HSV infection.

The titer of a single specimen should not be used to aid in the diagnosis of a recent infection. Paired samples (Acute and convalescent) should be collected, then tested in parallel to determine seroconversion or a significant rise in titer.

Proper timing in sample collection is critical when determining recent primary herpes simplex virus infection via demonstration of a four-fold antibody titer rise between paired acute and convalescent sera. The seroconversion characteristic of recent or active infection may not be seen if the first (acute phase) sample is taken too late or the second (convalescent phase) sample is taken too early.

A rise in antibody titer is not sufficient to diagnose HSV encephalitis. HSV reinfection or recurrent infection may occur in the presence of serum antibody. Thus, the presence of detectable herpes simplex virus antibody in patient sera is not indicative of immunity or protection from infection by HSV type 1 or 2.

A significant rise in titer to HSV may be associated with infection by VZV and other viruses in persons previously infected with herpes simplex virus. The higher titering antibody may not always indicate the causative agent. Frequently a differential diagnosis may be made when antibody to the infecting viral type is absent or at a very low titer in the acute phase specimen, whereas antibody to the viral heterotype is already present.

In atypical cases, it is important to test for antibody with a battery of likely agents rather than to test for antibody to only one suspected agent. A definitive diagnosis for typical cases demonstrating significant rises in titer both herpes simplex virus and some other agent must be made by isolation and/or direct identification of virus or viral antigen from a lesion.

When determining recent or active infection, the absence of a four-fold rise in titer does not necessarily rule out the possibility of infection. A significant (four-fold) rise in antibody titer does not always accompany a recurrent infection or reinfection. Antibody titer rises are more likely to accompany primary infections and severe or systemic recurring episodes and, thus are of limited value in diagnosing recurrent disease or reinfection.

The absence of measurable serum antibody or the lack of seroconversion does not exclude the possibility of infection; some persons may fail to develop antibody titers after infection. This test does not differentiate between antibody to HSV 1 and HSV 2; both are detected by this test. Specimens showing obvious microbial contaminations, gross hemolysis, lipemia, or turbidity may yield incorrect results and should not be used.