

discrepant tests were retested on the reference Western blot assay and on the **bioKitHSV-2 Rapid Test** assay by using the frozen serum at the central laboratory and at the clinical sites. There were 128 discrepant for CWB and 135 discrepant for serum. After discrepant re-testing there were 117 discrepant for CWB and 87 discrepant for serum. Upon re-testing of all discrepant combinations only, the following results were obtained: For capillary whole blood, there was an increase in true positives from 483/524 to 493/524 and a decrease in true negatives from 582/669 to 585/669. The results for serum were an increase in true positives from 516/537 to 526/537, and an increase in true negatives from 567/683 to 608/683.

Note: Statistical analysis has indicated that serum demonstrates a higher sensitivity with this assay than does capillary whole blood (McNemar Test, p<0.05).

The prevalence of the analyte will affect the assay predictive value. The following table illustrates how the positive and negative predictive value will vary in a population vary in relation to the seroprevalence of HSV-2 in that population based on a sensitivity of 92.2% and a specificity of 87%.

% Seroprevalence	Positive Predictive Value	Negative Predictive Value
55%	89.6%	90.1%
50%	87.6%	91.8%
40%	82.5%	94.4%
30%	75.2%	96.3%
25%	70.3%	97.1%

2. The following information is from a serum panel obtained from the CDC and tested blind by the kit manufacturer. The results are presented to convey further information on the performance of this assay with a masked characterized serum panel. This does not imply an endorsement of the assay by the CDC.

The panel consisted of 36-paired HSV-2 positive and 64 paired HSV-2 negative samples. Only 35 of the 36 paired HSV-2 positive samples were tested on **bioKitHSV-2 Rapid Test**. The **bioKitHSV-2 Rapid Test** demonstrated 98% overall agreement with the CDC results. Of the results obtained with the **bioKitHSV-2 Rapid Test** there was 100% overall agreement with the positive specimens and 97% overall agreement with the negative specimens.

3. The **bioKitHSV-2 Rapid Test** was independently evaluated at the University of Washington, Seattle (13) using 87 stored sera characterized by Western blot at the University of Washington Virology Laboratory. These sera were chosen at random and 85 of the 87 sera gave conclusive results by the **bioKitHSV-2 Rapid Test**. One of the sera were falsely negative (94% sensitivity) and two were falsely positive (98% specificity).

bioKitHSV-2 Results	Number of sera with Western blot result				
	Negative	HSV-1	HSV-2	HSV-1 & HSV-2	Total
Negative	28	22	2	0	52
Positive	0	1	12	20	33
Total	28	23	14	20	85

Relative Sensitivity: 94%
95% CI 81-98%
Two samples gave invalid test results by **bioKitHSV-2 Rapid Test**.

Relative Specificity: 98%
95% CI 89.6-99.6%

Note: 'Relative sensitivity' and 'relative specificity' are used to denote comparison of results of the **bioKitHSV-2 Rapid Test** with those of a reference assay. No attempt was made to correlate the performance of the reference assay with the presence or absence of disease. No judgement can be made of the comparison assay's accuracy to predict disease.

REPRODUCIBILITY & PRECISION

A reproducibility study was conducted at the six clinical sites. Each site tested 20 masked serum samples on one lot of test kits in one day. There were only 6/120 tests where results for the paired samples were discordant, indicating a reproducibility of 95%. Precision over the six sites was 86.9% (see following table).

Inter-Operator Precision Using a Panel of Twenty Masked Samples.

	E (Lab Tech)	B (Nurse)	A (Nurse)	F (Lab Tech)	C (Nurse)	D (Nurse)
Percentage Accuracy for Twenty Masked Samples	78%	89%	100%	72%	94%	100

Mean percentage accuracy was 88.8% (CV = 13.1%)
Precision over the six sites was, therefore, 86.9%

Percentage Accuracy and Precision For All Samples With All Operators.

Site (Type of site; status of operator)	Percentage Accuracy						Mean Intra-operator Accuracy	Intra-operator Precision
	Sample 1 (Pos.)	Sample 2 (Neg.)	Sample 3 (Weak Pos.)	Sample 4 (Cut off, +/-)	Sample 5 (Moderate Pos.)	Sample 6 (Weak Pos.)		
1 (Clinic; Lab Tech)	100%	100%	100%	100%	100%	100%	100%	100%
2 (POL; Nurse)	85.2%	100%	18.5%	100%	80.8%	88.9%	78.9%	61.2%
5 (Clinic; Nurse)	100%	100%	90.9%	100%	100%	100%	98.5%	95.2%
6 (Lab; Lab Tech)	100%	100%	100%	100%	100%	100%	100%	100%
D (Lab; Lab Tech)	100%	100%	100%	100%	100%	100%	100%	100%
Mean Inter-operator Accuracy	97%	100%	81.9%	100%	95.2%	97.8%		
Inter-operator Precision	93.2%	100%	56.5%	100%	91.1%	94.9%		

The second study (see preceding table), involving multiple testing of six blind samples (4 HSV-2 positives, 1 negative and 1 cut off) on three different days and with three different kit lots, at five different sites, was intended to assess both intra- and inter-site precision. The study was performed at two clinics (sites A and C), one physician office laboratory (site B) and two laboratory sites (L1 and L2). The results of the study indicated that both mean inter- and mean intra- site precision is greater than 89% and 91% respectively.
Note: The assay incorporated control is intended to monitor for substantial reagent failure and will not ensure precision at the assay cut off.

Cross Reactivity
Studies were conducted on serum or plasma samples, which were obtained with a variety of possible cross-reacting or interfering substances. These samples were from individuals with human papilloma virus (HPV), cytomegalovirus (CMV), varicella zoster virus (VZV), Epstein Barr virus (EBV), chlamydia, rubella, syphilis, toxoplasmosis, antinuclear antibodies (ANA), bilirubin, rheumatoid factor, biotin, triglycerides and hemoglobin. The results of the cross reactivity studies are summarized in the following table.

Summary of Cross-Reactivity Studies

Disease State	Total Number of Samples Tested	Number of HSV-2 Negative Samples*	Number of bioKitHSV-2 False Positive Samples	Number of HSV-2 Positive Samples*	Number of bioKitHSV-2 False Negative Samples
HPV	32	22	1	10	2
CMV	67	51	8	16	2
VZV	14	12	0	2	0
EBV	8	6	0	2	0
Chlamydia	16	13	1	3	0
Rubella	18	18	3	0	0
Syphilis	14	9	3	5	0
Toxoplasma	14	14	2	0	0
ANA	14	13	3	1	0

*As determined by EIA or HSV-2 Western blot.

Interfering Substances

Addition of rheumatoid factor (up to 148 IU/ml) or of biotin (up to 20 ug/ml) and concentrations of bilirubin (up to 56.7mg/dl), and of triglycerides (up to 2161mg/dl) to HSV-2 characterized serum did not affect the results of the **bioKitHSV-2 Rapid Test**. There was no significant correlation (linear regression) either between hemoglobin or PCV and flow rate through the **bioKitHSV-2 Rapid Test** device. Note the effect of varying levels of hemoglobin on test performance or interpretation of test results has not been determined.

REFERENCES

Note: Mention of these references is not intended to imply any additional performance claims other than those made in the 'Intended Use' section of this Package Insert.

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Note: Any published data on the **POCKIT® HSV-2 Rapid Test** also applies to the **bioKitHSV-2 Rapid Test**.

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bioKitHSV-2*

RAPID TEST

Catalogue No: 3000-28001

Instructions for Use

INTENDED USE

The **bioKitHSV-2 Rapid Test** is a single unit, membrane-based immunoassay for the qualitative determination, either in heparinized capillary whole blood taken by fingerstick or in serum, of circulating IgG antibodies specific for herpes simplex virus type 2 (HSV-2), which arise as a result of infection with HSV-2. It is intended for in-vitro diagnostic use by health professionals in Point of Care testing. The presence of antibodies to HSV-2 may be indicative of a previous infection with HSV-2 and may be of value in determination of previous immunological experience and to aid in the diagnosis of HSV associated disease. This assay will not differentiate whether infection is currently in a latent or active state.

PRECAUTION

The **bioKitHSV-2 Rapid Test** is intended for use in a high prevalence population, e.g. sexually active adults and sexually transmitted disease clinic. If used in a low prevalence population, positive results should be considered presumptive and should be confirmed with an alternate methodology e.g. Western blot. Assay results are not intended for medical/legal use. The **bioKitHSV-2 Rapid Test** is not recommended as a screening procedure for the general population or for testing in a prenatal, pediatric or neonatal population. Performance characteristics have not been established in a low prevalence population e.g. prenatal patients, on immunocompromised individuals, for prenatal or neonatal screening, on other patients with HSV associated diseases, or for early stages of HSV seroconversion.

SUMMARY AND EXPLANATION

Genital herpes is most frequently caused by HSV-2 (1, 2) where it establishes latency in dorsal nerve root ganglia. Intermittent reactivation of virus occurs in most people who are infected with HSV-2. These reactivations cause genital lesions and the shedding of virus on mucosal areas. Genital HSV infection may be sub-clinical, which means that a significant number of patients may not realize that they are infected and may unknowingly transmit the virus to sexual partners (3). Numerous studies (e.g. 1,2) report that, while infection with HSV-2 frequently results in periods of overt disease (expressing "typical" or "atypical" symptoms) followed by periods of viral latency with no overt disease, the disease does not self-cure (4). Neither can it be cured through medical intervention. Furthermore viral shedding in HSV-2 antibody positive patients in the absence of symptoms has been demonstrated (3). Because HSV-2 infection is not expected to be eradicated either spontaneously or by medical intervention, the presence of anti-HSV-2 IgG would be indicative of a previous immunological experience regarding infection with HSV-2, regardless of whether the infection is symptomatic or asymptomatic. However, presence of HSV IgG antibodies would not differentiate whether infection is currently in a latent or active state. Therefore, if a patient has antibodies to HSV-2, it is indicative of infection with the virus regardless of symptomatology. Use of a single sample will not allow discrimination between a true primary or recurrent infection. Seroconversion may be detected by testing samples taken at different time intervals (see below). Determination of a patient's HSV-2 serological status using a type-specific antibody test enables the physician to make appropriate interventions for treatments and professional guidance.

PRINCIPLE OF THE PROCEDURE

The **bioKitHSV-2 Rapid Test** is a rapid immunoassay, which detects the presence of antibodies specific for HSV-2 in capillary whole blood or serum. The test device is composed of a membrane solid phase, which is held in a plastic envelope containing wicking material. The membrane is visible to the user through a TEST WINDOW on the front of the device. The method employs a unique combination of a specific semi-purified antibody binding protein, conjugated to colloidal gold particles (developing reagent) and semi-purified glycoprotein G2 (gG2), a specific antigen derived from the HSV-2 virus (5). The gG2 has been bound to the membrane as a TEST spot on the right side of the TEST WINDOW. Human IgG is bound to the membrane as a CONTROL spot on the left side of the TEST WINDOW. When a pre-diluted (fingerprick) capillary whole blood sample or serum sample is passed through the membrane any anti-HSV-2 antibodies present become bound to the HSV-2 antigen in the TEST spot. Upon addition of the developing reagent, which reacts with human IgG antibody, a pink/red color develops. The developing reagent also reveals the human IgG immobilized on the CONTROL spot, which demonstrates that the reagents are functioning properly. The test device is designed to completely absorb the volume of added reagents. The presence of two spots in the TEST WINDOW indicates a positive result. A single spot on the left of the TEST WINDOW is a negative result.

MATERIALS PROVIDED

Each kit contains everything needed to perform twenty tests. To run an individual test, one of each of the following is required:

- Test Device: One **bioKitHSV-2 Rapid Test** device - The permeable membrane on the device has been coated with two small, circular areas consisting of lectin affinity semi-purified HSV-2 antigen from HSV-2 virus (LoveLace strain) infected Vero cells (TEST spot), and human IgG (CONTROL spot), respectively. The membrane is covered by a protective "peel off" strip.
- Sample Diluent: One plastic vial containing sample diluent (0.4ml) [Tris / HCl buffer containing bovine and goat protein with 0.1% [w/v] sodium azide].
- Developing Reagent: One plastic tube containing developing reagent (0.3ml) [goat anti-human IgG antibodies conjugated to colloidal gold in a tetraborate buffer with 0.1% sodium azide (w/v)].
- Vial 1: One empty plastic tube for the transfer of sample diluent.
 - One plastic pre-filter tip [single use].
 - One heparinized glass capillary tube for the collection of the capillary whole blood sample.
 - One sterile automatic lancet - single use, disposable needle for fingerprick blood sampling.
 - One alcohol swab - For disinfecting skin surface prior to drawing the blood sample.
 - Tube rack
 - Instructions for use

MATERIALS NEEDED BUT NOT SUPPLIED

Protective clothing and disposable gloves should be worn. When using serum samples, a pipette capable of delivering a 50µl sample volume is required, as well as disposable tips for the pipette.

* **bioKitHSV-2 was formerly known as POCKIT® HSV-2 Rapid Test**

PRECAUTIONS

- For *in vitro* diagnostic use.
- DO NOT pipette any material by mouth. Do not smoke, eat or drink in areas where specimens or kit reagents are handled.
- Use suitable protective clothing and gloves when handling the test components or the patient samples, and while performing the assay.
- WARNING:** The human source components were tested and found negative for anti-HIV (types 1 and 2) and HbsAg by FDA approved tests. Because no test method can offer complete assurance that laboratory specimens do not contain HIV, Hepatitis B virus or other infectious agents, specimens should be handled as recommended for any potentially infectious human serum or blood specimen in the CDC-NIH manual, Biosafety in Microbiological and Biomedical Laboratories, 1984 pages 12-16. The liquid reagents in the kit contain 0.1% sodium azide which is toxic if ingested. It should not be allowed to come into contact either with skin or with mucous membranes.
- Do NOT use the test components beyond the expiration date that appears on the package label.
- This test is for single use only. All test components are for single use only.
- DO NOT use the test kit if package seals have been broken on the lancet, alcohol swab, or the test device, or if it is evident that leakage has occurred from the developing reagent or diluent vials.
- Handle the glass capillary with care.
- The sample diluent and developing reagent contain sodium azide, which may react with lead and copper plumbing to form highly explosive metal azides, therefore any excess should be disposed of with large quantities of water.
- After completion of the test, all components should be disposed of as biohazardous waste (see Point 4).

STORAGE AND STABILITY

The **bioKitHSV-2 Rapid Test** can be stored at any temperature between +2°C and +8°C. DO NOT freeze the test kit. DO NOT use the kit beyond the labeled expiry dating on the box.

SPECIMEN COLLECTION AND STORAGE

- The **bioKitHSV-2 Rapid Test** can be performed on capillary whole blood (obtained by fingerstick) or serum.

Warning: Statistical analysis has indicated that serum demonstrates a higher sensitivity with this assay than does capillary whole blood (McNemar Test, p<0.05).
- Capillary whole blood samples, obtained by lancing the patient's middle finger, should be tested immediately on the test device to avoid clotting in the capillary. Stored, collected capillary whole blood for further testing cannot be utilized with this assay.
- Serum specimens should be stored according to accepted standards and practices e.g., National Committee for Clinical Laboratory Standards (NCCLS) (Approved standard Procedure for the Handling and Processing of Blood specimens, H18-A, 1990). It is recommended that serum samples are stored at room temperature for no longer than 8 hours. If the assay will not be completed within 8 hours, refrigerate the sample at 2-10°C. If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C.
- To detect seroconversion, separate specimens should be drawn during acute and convalescent phases of the illness. The acute phase specimen should be stored (recalled a second acute serum specimen for long term storage if capillary whole blood was initially used) and tested in parallel with the convalescent specimen. The first serum should be obtained as close as possible to the onset of illness and the convalescent specimen obtained 10-21 days later.

TEST PROCEDURE

The **bioKitHSV-2 Rapid Test** procedure must be followed closely.

Perform the test in a room temperature environment (+18°C to +25°C). **Note:** After removal of the test from storage (+2°C to +8°C), it is not necessary to wait until the test components have equilibrated to room temperature before use. It is important, however, if frozen or cold serum specimens are to be tested they must reach room temperature before use.

CAPILLARY WHOLE BLOOD:

- Remove a test device (labeled Test Device) from its foil pouch by tearing along the notch and place on a flat surface.
- Label the device with the patient's name or identification number, and peel back the protective label to reveal the test window.
- Place the empty plastic vial (Vial 1) into the tube rack so that it is in an upright position. Tip the entire contents of the sample diluent vial into the empty vial (Vial 1) and discard the empty sample diluent vial (labeled Sample Diluent).
- Take patient's blood sample: If the patient's hands are cold, it is advisable that the patient washes them in warm water and dries them before proceeding with the following steps.
 - Ensure that the patient is comfortably seated.
 - Wipe the surface of the patient's middle finger with the alcohol swab provided.
 - Apply gentle pressure to the base of the finger so that it becomes pink.
 - Place the puncture hole of the lancet against the patient's middle finger and press the red trigger of the lancet firmly (see Figure 1).



- Remove the capillary tube (50µl) from the plastic vial. Keeping gentle pressure on the base of the patient's middle finger, fill the capillary entirely with the patient's blood by holding one end of the capillary at the droplet of blood (avoid getting air-bubbles into the capillary).
- Place the blood filled capillary into the plastic vial (Vial 1) which now contains the sample diluent buffer.
- Fit the pre-filter tip (see figure 2) onto vial 1 ensuring that the tip clicks into the vial and is secure. Then gently mix the sample and diluent by inverting the sample diluent vial several times.



- Keeping the pre-filter tip on the vial, squeeze all the sample/diluent mixture onto the TEST WINDOW, ensuring that the vial is squeezed in the middle, away from the pre-filter tip. Allow the mixture to drain completely into the test device.
- After the sample/diluent mixture is absorbed into the test device, gently mix the developing reagent in the plastic tube (labeled Developing Reagent) by inverting several times. Remove the lid and pour all the contents of the developing reagent onto the TEST WINDOW.
- Allow the developing reagent to absorb into the test device. If a residue appears on the TEST WINDOW, gently wipe the surface of the TEST WINDOW with the cotton bud provided.
- Interpret the result of the test as described below.

SERUM SAMPLE:

Follow steps 13 above, then add 50µl of serum by pipette into vial 1 which now contains the sample diluent. Complete the test procedure by following steps 7-11.

QUALITY CONTROL:

Control features incorporated into the biokit HSV-2 Rapid Test device.

The biokit HSV-2 Rapid Test incorporates two levels of quality control into each device. The manufacturer's recommendation for daily quality control is that the user records the results of these controls for the first sample run on each day of testing.

Positive Control: The biokit HSV-2 Rapid Test has a two-color result format: red is positive, white is negative facilitating distinction of positive and negative results.

The appearance of the pink/red CONTROL spot provides the following internal controls:

- Flow of assay reagents through the membrane has occurred;
 - The functional integrity of the biokit HSV-2 Rapid Test has been maintained.
- If the CONTROL spot on the left-hand side of the TEST WINDOW fails to appear within 10 minutes the test is invalid.

Negative Control: Incorporated into the biokit HSV-2 Rapid Test device as a negative control is the clearing of red background color in the TEST WINDOW once all the developing reagent has flowed into the device. The background color of the TEST WINDOW should be white-to-pale pink after 10 minutes and not interfere with the interpretation of the test result. If background color appears which interferes with interpretation of the test result, the result should be considered invalid and the test repeated with a new biokit HSV-2 Rapid Test device.

Do not report patient results if either of the two levels of quality control in the device fails out of range.

External Quality Controls.

Additionally, external controls may also be used to indicate that the biokit HSV-2 Rapid Test reagents are performing correctly. Such controls may include patient samples previously characterized as HSV-2 positive or HSV-2 negative.

The manufacturer recommends that external quality controls be used as required by the user's standard Quality Control procedures.

If external controls do not provide expected results, repeat the test or contact biokit Technical Service before testing patient samples.

Warning: Do not report patient results if the results of any additional controls tested are out of range, until the cause of the error has been identified and rectified.

INTERPRETATION OF RESULTS

The biokit HSV-2 test should be used as an aid in the diagnosis of infection with HSV-2, in patient groups where, because of either symptoms or clinical history, the physician suspects infection with this virus.

Establishment of a cut off for the biokit HSV-2 assay.

As the biokit HSV-2 Rapid Test is a membrane based assay which is visually read, the determination of the cut off was part of the product design. Presence of color on the reactive surface of the membrane should indicate a positive result and absence of color should indicate a negative result. The reagents used in the kit were optimized during the development of the assay to ensure that HSV-2 positive samples caused a color reaction on the membrane surface and that negative samples gave no color on the membrane surface. This work was done using serum samples only, of known HSV-1 and -2 serostatus (6, 7). Results can be interpreted as soon as the developing reagent has drained into the test device. **The results must be interpreted within 10 minutes of addition of the developing reagent.**

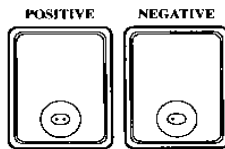


Figure 3

NEGATIVE

Only one pink/red CONTROL spot appears on the left side of the TEST WINDOW. A negative result indicates no detectable level of anti-HSV-2 IgG. Negative results do not rule out the diagnosis of HSV infection as the specimen may be drawn before appearance of detectable antibodies. If a negative result is obtained from a patient suspected to be in early disease, then a second sample should be drawn after 4-6 weeks and re-tested.

POSITIVE

Two pink/red spots appear in the TEST WINDOW. The TEST spot on the right side of the TEST WINDOW may be less intense in color than the CONTROL spot but should be clearly discernible. A positive result means the patient sample contains detectable levels of IgG antibodies to HSV-2.

Warning: Over-interpretation of the results may occur as some HSV-2 seronegative samples may give very faint reactions. Unless color is clearly discernible on the TEST spot when the device is on a flat surface, the result should be considered negative. However, as stated above, a negative result does not rule out an HSV infection.

Warning: Cross reactivity (false positive results) was found with some samples containing antibodies to HPV, CMV, chlamydia, rubella, syphilis, toxoplasma, and ANA.

NOTE: Use of a single sample will not allow discrimination between a true primary or a recurrent infection. If an initial or primary infection is suspected the patient should be tested for HSV-1 or -2, IgM or concurrently tested with a second sample on biokit HSV-2 Rapid Test with a second sample collected 10 to 21 days later.

INVALID TEST

If the CONTROL spot on the left side of the TEST WINDOW fails to react, the test is invalid. If this occurs, repeat the test with a new kit.

Because results from patients exhibiting an elevated hematocrit due to dehydration or a myeloproliferative disorder, e.g. Polycythemia vera, could give erroneous results. Serum testing on these individuals is recommended.

LIMITATIONS OF USE

- The biokit HSV-2 Rapid Test is a qualitative assay for the detection of antibodies specific for HSV-2 in capillary whole blood or serum and is not indicative of the level of antibody titer.
- A positive biokit HSV-2 Rapid Test result indicates the presence of antibodies specific for HSV-2 but does not allow discrimination between a primary or recurrent infection. In primary genital herpes infection, there may be no immunological response to the HSV gG2 for several weeks (5), therefore a negative biokit HSV-2 Rapid Test result at any time does not preclude the possibility of infection with HSV-2 especially if the infection is recent. The biokit HSV-2 Rapid Test should not be performed as a screening procedure of the general population. The predictive value of a positive or negative serologic result depends on the pretest likelihood of HSV-2 being present. This test should not replace viral isolation as the sole basis for diagnosis.
- This assay will not allow discrimination between a latent and current infection.
- Only those interfering substances and disease states indicated in the 'Cross Reactivity' section of this package insert have been evaluated. This, however, does not preclude potential interference from other disease states or interfering substances not evaluated by the biokit HSV-2 Rapid Test.
- The biokit HSV-2 Rapid Test detects antibodies to HSV-2 glycoprotein G. The source of the antigen is HSV-2 (Lovelace strain) cultured in Vero cells. The protein gG was affinity-purified with lectin affinity chromatography. Based on published literature (4), antibodies to this protein, as detected by EIA or Western blot, may not be produced for up to 6 months after acquisition of HSV-2. Seroconversion can vary anywhere from 21 to 40 days (8). Because some individuals may not have detectable levels of the IgG antibody to HSV-2 early in infection and because type specificity may not be evident immediately upon seroconversion, this test system may have limited sensitivity for early seroconversion determination (1). Because glycoprotein G is not an essential protein for viral replication and because infections might occur with a glycoprotein deficient virus, it is possible that some individuals may lack detectable levels of gG 2 after infection. However, in a study evaluating stored serum samples (N=188) from 29 patients with recent primary genital herpes, it was demonstrated that the median time to detection of HSV-2 antibodies by the biokit HSV-2 Rapid Test was 13 days (range 3 to 102 days) in comparison to 13 days (range 2 to 58 days) for Western blot (8).
- The user should be aware that there is no performance data for the biokit HSV-2 Rapid Test on patients with an abnormally high hematocrit.
- Interpretation of the biokit HSV-2 Rapid Test has not been tested on color-blind individuals. There is, therefore, no data available on the level of color vision required to adequately interpret the results of the test. Color-blind users should be aware of this and interpret the test with caution.
- The continued presence or absence of antibodies cannot be used to determine the success or failure of therapy.
- The performance of the biokit HSV-2 Rapid Test has not been established in the following patient groups: pediatric and neonatal patients or patients with HSV-suspected pneumonia, encephalitis or meningitis.

POTENTIAL CAUSES OF ERROR

An erroneous result may be obtained with the biokit HSV-2 Rapid Test if the following occur.

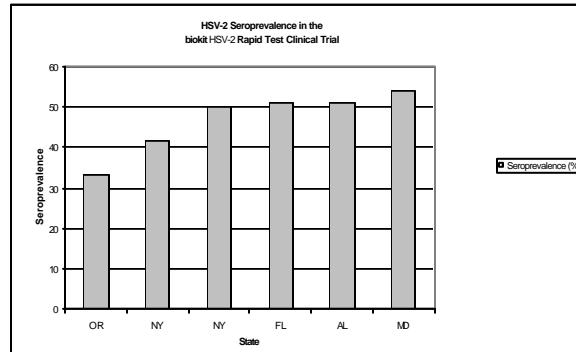
- The capillary is not filled entirely with blood or the blood clots in the capillary tube prior to addition to the diluent.
- All blood from the capillary is not mixed with diluent.
- The pre-filter tip is not clicked securely onto the diluent vial leading to inadequate filtration of the capillary whole blood or serum/diluent mixture.
- Failure to add developing reagent.
- Failure to interpret results within 10 minutes of addition of developing reagent.
- Failure to take into consideration the warning given in the "Interpretation of Results" Section.

EXPECTED VALUES

HSV-2 specific antibodies usually appear in patient serum within a week of infection reaching peak levels in 4 to 6 weeks. The antibody level then declines and usually persists indefinitely at relatively stable levels. Some persons may not develop detectable antibody titers after infection and in others, antibody levels may fall to very low or undetectable amounts, which then may increase, by later infection (9). Therefore the lack of antibodies to HSV-2 does not exclude the possibility that the individual is infected with HSV-2.

Reactivation of the initial infection or infection with HSV-1 usually does not cause a significant change in antibody titer to HSV-2 (9).

The results of the US clinical trial of the biokit HSV-2 Rapid Test indicated that HSV-2 seroprevalence varied at the different geographic locations where the test was evaluated. The results for seroprevalence in serum samples are summarised in the following histogram.



Previous studies have demonstrated that HSV-2 seroprevalence varies with a number of factors including age, race and gender (10, 11, 12). Seroprevalence was found to vary from approximately 20% to higher than 70% for those in high-risk groups (e.g., Sex workers, homosexual men).

PERFORMANCE CHARACTERISTICS

COMPARISON

Independent studies were performed to evaluate the performance of the biokit HSV-2 Rapid Test.

Note that a serum matrix only, not capillary whole blood, was used for cut off determination, precision and reproducibility testing and for cross reactivity and interference studies.

1. A study was performed at six sites within the United States with 1237 patients. Three sites (MD, AL and NY) were STD clinics, the remainder (OR, NY and FL) were physician office laboratories. Numbers of patients from each site are given in Tables 1, 2 and 3. The numbers for both serum and capillary whole blood (CWB) exclude 17 patients for whom 'atypical' Western blot results were obtained and a further 27 results were not reported for CWB (25 biokit HSV-2 test failures e.g. an invalid test result was obtained and two additional patients on whom CWB was not tested on biokit HSV-2 Rapid Test). The final numbers reported for serum are 1220 and 1193 for capillary whole blood. The study evaluated the performance of the biokit HSV-2 Rapid Test versus the reference Western blot method. The patients who participated were identified as asymptomatic or symptomatic based on physical symptoms and clinical history. The patients were tested at the site with the biokit HSV-2 Rapid Test using "fingerprick" capillary whole blood and with serum. At the same time, a serum sample was collected and sent to an independent clinical laboratory for testing on the reference Western blot assay.

Western blots for both HSV-1 and -2 were independently performed for detection of anti-HSV-1 and anti-HSV-2 antibodies. Discrepant test results between the biokit HSV-2 Rapid Test and the reference test required retesting frozen serum from the patient on the biokit HSV-2 Rapid Test at the clinical site and on Western blots at the central laboratory. Only samples obtained from patients recruited into this study were tested. There was no retrospective testing.

The following table (Table 1) presents the performance of the biokit HSV-2 Rapid Test in patients who displayed any of the following symptoms: plaques, vesicles or ulcerated skin on the vagina, vulva, cervix, glans, penile shaft, scrotum, perineum or perianal region; fever; general malaise, or swollen or tender lymph nodes. Those patients who were classed as negative were determined by Western blot.

Table 1 Performance of biokit HSV-2 Test by Clinical Site vs. Western blot (Symptomatic Patients)

Site	Specimen Type*	N	Prevalence (%)	vs. HSV-2 Western blot			
				Sensitivity	95%CI**	Specificity	95%CI**
A (POL ⁺)	Whole Blood	66	53.0%	91.4% (32/35)	76.9-98.2	96.8% (30/31)	88.8-100
	Serum	66	53.0%	94.3% (33/35)	80.8-99.3	87.1% (27/31)	70.2-96.4
B (POL)	Whole Blood	44	40.9%	100% (18/18)	81.5-100	84.6% (22/26)	65.1-95.6
	Serum	46	43.5%	100% (20/20)	83.2-100	92.3% (24/26)	74.9-99.1
C (STD CLINIC)	Whole Blood	37	51.4%	73.7% (14/19)	48.8-90.0	88.9% (16/18)	65.3-98.6
	Serum	41	46.3%	100% (19/19)	82.6-100	90.9% (20/22)	70.8-98.9
D (POL)	Whole Blood	15	86.7%	84.6% (11/13)	66.4-100	50.0% (1/2)	12.6-98.7
	Serum	15	86.7%	84.6% (11/13)	66.4-100	50.0% (1/2)	12.6-98.7
E*** (STD CLINIC)	Whole Blood	--	--	--	--	--	--
	Serum	--	--	--	--	--	--
F (STD CLINIC)	Whole Blood	57	73.7%	97.6% (41/42)	87.4-99.9	66.7% (10/15)	38.4-88.2
	Serum	60	75.0%	100% (45/45)	92.1-100	40.0% (6/15)	16.3-67.6

* Whole blood is used as an annotation for capillary whole blood in this table.

** Confidence Interval

^ Indicates Confidence intervals calculated by normal method All other confidence intervals were calculated by the exact method

*** All patients tested at this site were classified as: 'asymptomatic'

+: POL: Physician Office Lab

In summary, on initial testing, the overall seroprevalence for anti-HSV-2 antibodies in capillary whole blood was 58% in 219 symptomatic patients. The overall sensitivity with capillary whole blood was 91.2% (95% CI[^] 87.6-95.1) and the overall specificity was 85.9% (95% CI[^] 81.3-90.5). With serum, seroprevalence was 57.9% in 228 patients. The overall sensitivity with serum was 97% (95% CI[^] 94.7-99.2) and overall specificity was 81.3% (95% CI[^] 76.2-86.3).

The following table (Table 2) presents the performance of the biokit HSV-2 Rapid Test in patients who displayed no symptoms.

Table 2 Performance of biokit HSV-2 Test by Clinical Site vs. Western blot (Asymptomatic Patients)

Site	Specimen Type*	N	Prevalence (%)	vs. HSV-2 Western blot			
				Sensitivity	95%CI**	Specificity	95%CI**
A (POL ⁺)	Whole Blood	167	37.1%	95.2% (59/62)	86.5-99.7	95.2% (100/105)	89.2-98.4
	Serum	167	37.1%	93.5% (58/62)	84.3-98.2	86.7% (91/105)	80.2-93.2^
B (POL)	Whole Blood	307	32.2%	91.9% (91/99)	84.7-96.4	91.3% (190/208)	86.7-94.8
	Serum	314	32.5%	94.1% (96/102)	87.6-97.8	93.4% (198/212)	89.2-96.3
C (STD CLINIC)	Whole Blood	54	57.4%	93.5% (29/31)	78.6-99.2	100% (23/23)	85.2-100
	Serum	57	57.9%	100% (33/33)	89.4-100	100% (24/24)	85.8-100
D (POL)	Whole Blood	112	46.4%	75% (39/52)	61.1-86.0	98.3% (59/60)	91.1-100
	Serum	112	46.4%	90.4% (47/52)	79.0-96.8	95.0% (57/60)	85.8-99.0
E (STD CLINIC)	Whole Blood	43	51.2%	100% (22/22)	84.6-100	81.0% (17/21)	58.1-94.5
	Serum	43	51.2%	100% (22/22)	84.6-100	95.2% (20/21)	76.2-99.9
F (STD CLINIC)	Whole Blood	288	45.1%	96.9% (126/130)	92.3-99.2	70.9% (112/158)	63.8-78.0^
	Serum	296	44.9%	98.5% (131/133)	94.7-99.8	60.7% (99/163)	53.2-68.2

* Whole blood is used as an annotation for capillary whole blood in this table.

** Confidence Interval

^ Indicates Confidence intervals calculated by normal method. All other confidence were calculated by the exact method

+: POL: Physician Office Lab

In summary, on initial testing, the overall seroprevalence for anti-HSV-2 antibodies in capillary whole blood was 40.8% in 971 asymptomatic patients. The overall sensitivity with capillary whole blood was 92.4% (95% CI[^] 90.8-94.1) and the overall specificity was 87.1% (95% CI[^] 85-91.2). With serum, seroprevalence was 40.8% in 989 patients. The overall sensitivity in serum was 95.8% (95% CI[^] 94.5-97.0) and overall specificity was 83.6% (95% CI[^] 81.3-85.9).

Upon initial testing the biokit HSV-2 Rapid Test gave the following performance results for whole blood and serum by individual clinical site and overall results:

Table 3: Performance of biokit HSV-2 Test by Clinical Site vs. Western blot (Symptomatic and Asymptomatic Patients*)**

Site	Specimen Type*	N	Prevalence (%)	vs. HSV-2 Western blot			
				Sensitivity	95%CI**	Specificity	95%CI**
A (POL ⁺)	Whole Blood	234	41.5%	93.8% (91/97)	87.0-97.7	95.6% (131/137)	90.7-98.4
	Serum	234	41.5%	93.8% (91/97)	87.0-97.7	86.9% (119/137)	81.2-92.5^
B (POL)	Whole Blood	353	33.4%	93.2% (110/118)	87.1-97.0	90.6% (213/235)	86.2-94.0
	Serum	362	34%	95.1% (117/123)	89.7-98.2	93.3% (223/239)	89.4-96.1
C (STD CLINIC)	Whole Blood	91	54.9%	86% (43/50)	73.3-94.2	95.1% (39/41)	83.5-99.4
	Serum	98	53.1%	100% (52/52)	93.2-100	95.7% (44/46)	85.2-99.5
D (POL)	Whole Blood	127	51.2%	76.9% (50/65)	64.8-86.5	96.8% (60/62)	88.8-99.6
	Serum	127	51.2%	89.2% (58/65)	79.1-95.6	93.5% (58/62)	84.3-98.2
E (STD CLINIC)	Whole Blood	43	51.2%	100% (22/22)	84.6-100	81.0% (17/21)	58.1-94.5
	Serum	43	51.2%	100% (22/22)	84.6-100	95.2% (20/21)	76.2-99.9
F (STD CLINIC)	Whole Blood	345	49.9%	97.1% (167/172)	93.3-99.1	70.5% (122/173)	63.7-77.3^
	Serum	356	50%	98.9% (176/178)	96-99.9	59% (105/178)	51.8-66.2^

* Whole blood is used as an annotation for capillary whole blood in this table.

** Confidence Interval

^ Indicates Confidence intervals calculated by normal method. All other confidence were calculated by the exact method

*** Including three patients who were not classified as either symptomatic or asymptomatic

+: POL: Physician Office Lab

In summary, on initial testing, the overall seroprevalence for anti-HSV-2 antibodies in capillary whole blood was 43.9% in 1193 patients. The overall sensitivity in capillary whole blood was 92.2% (95% CI[^] 90.7-93.7) and the overall specificity was 87% (95% CI[^] 85.1-88.9). With serum, seroprevalence was 44.0% in 1220 patients (this number excluded the 17 patients for whom 'atypical' Western blot results were obtained). The overall sensitivity in serum was 96.1% (95% CI[^] 95.0-97.2) and overall specificity was 83.3% (95% CI[^] 81.2-85.4).