

- 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.**

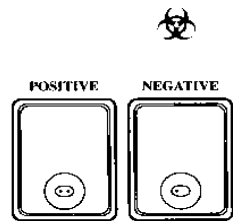


Figure 3

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.

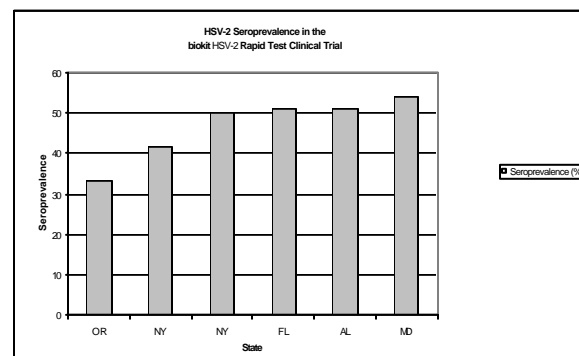
- The capillary is not filled entirely with blood or the blood clots in the capillary tube prior to addition to the diluent.
- All blood from the capillary is not mixed with diluent.
- The pre-filter tip is not clicked securely onto the diluent vial leading to inadequate filtration of the capillary whole 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 "fingerprint" 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% (23/31)	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 intervals 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 intervals 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).