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## Performance of HSV-2 Type Specific Serological Tests in Men in Kenya

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### Abstract

This study compared five serological tests with Western blot from University of Washington to determine the most accurate method for detecting antibodies to herpes simplex virus type 2 (HSV-2) in a male population in Kisumu, Kenya. A random sample of 250 fishermen from 18 beaches along Lake Victoria underwent serological testing by two generations of the HerpeSelect HSV-2 ELISA ("Focus Gen 1" and "Focus Gen 2"), Kalon HSV-2 ELISA ("Kalon"), Biokit HSV-2 Rapid Test ("Biokit"), HerpeSelect Express Rapid HSV-2 ("Express") against the Western blot test ("WB") as the "gold standard". Sensitivity and specificity of tests in this population with a high prevalence of HSV-2 (58% by WB) were: Focus Gen 1: 98.6% and 63.5%; Focus Gen 2: 99.3% and 52.3%; Biokit: 66% and 90.9%; Express: 99.3% and 44.3% and Kalon: 98.6% and 85.7%. Increasing the positive cut-off value improved the specificity of the Focus Gen 2 to 84.9% and Kalon to 92.2%. Focus Gen 2 offered no improvement in specificity over that of Focus Gen 1. Neither rapid assay could be recommended as either a stand-alone assay or as a confirmatory test. The results of Kalon using a cut-off of 1.5 were the most concordant with those of WB for all the approaches tested. However, low positive Kalon test results should be interpreted with caution as they could reflect early seroconversion or false positive results.

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## Keywords

HSV-2; serology; test performance; Fishermen in Kenya

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## 1.0 Introduction

Strategies to control the spread of genital herpes in Kenya require accurate, inexpensive, and easy to perform tests for HSV antibodies. This study compared five commercial HSV-2 serological tests against Western blot to determine which test or test combination would detect most accurately HSV-2 serum antibodies among men in Western Kenya.

Western blot, considered a “gold standard” test, is expensive (\$156 per test) and difficult to obtain in Kenya. The study compared Western blot to three glycoprotein G-2 (gG-2) based HSV-2 enzyme immunoassays including two versions of the HerpeSelect HSV-2 IgG ELISA (Focus Diagnostics, Cypress CA): the original (“Focus Gen 1”) and the reformulated (“Focus Gen 2”) ELISAs. Although the Focus Gen 1 kits are no longer on the market, the comparison may be useful for investigators analyzing test data from kits obtained prior to September, 2006. The third ELISA was the Kalon HSV-2EIA (Kalon Biological Ltd, Surrey, UK). Although these kits are relatively inexpensive (about \$3 per test), they require special equipment. The fourth test, Biokit HSV-2 rapid assay (“Biokit”), (Biokit USA, Lexington, MA, formerly POCkit-HSV-2 from Diagnology, Belfast Northern Ireland) requires no special equipment, but costs about \$20 per test. Compared to Western blot, the reported sensitivity of these tests ranges from 93% to 100%, with reported specificities between 95% and 100% in United States residents (Ashley et al. 2000; Ashley, 2002; Ashley-Morrow et al. 2003a). The specificity of the ELISAs appear to be lower in African populations (Van Dyck et al. 2004; Morrow et al. 2004; Gamiel et al. 2008; Smith et al. 2009; Delaney et al. 2009). The fifth test, HerpeSelect Express HSV-2 rapid assay, developed by Focus Diagnostics Cypress CA, is a qualitative test for HSV-2 IgG antibodies in human whole blood or serum, and is used for presumptive diagnosis of HSV-2 infection in sexually active adults or pregnant women. Early reports indicate that Express has comparable performance to the Focus ELISA (Laderman et al. 2008; Philip et al. 2008) but no comparisons with Western blot have been published. Like Biokit, Express requires no special equipment, and costs about \$30 per test.

## 2.0 Material and Methods

### 2.1 Study population

This study took place between August, 2005 and April, 2006 during the pre-clinical phase of a pilot study of the acceptability and safety of a topical microbicide for application to the penis to reduce acquisition of sexually transmitted diseases among fishermen along Lake Victoria in the Kisumu district of Kenya. Fishermen in this region earn high-income and many migrate back and forth from their home villages and wives to Lake Victoria. Women who come to these beaches to scale and trade fish often exchange sex for fish or money (Ng'ayo et al. 2008). None of the fishermen in this study had been circumcised, a factor that predisposes them to increased risks of acquisition of HIV (Boerma et al. 2003) and certain other sexually transmitted diseases including HSV-2 (Tobian et al. 2009).

### 2.2 Study design

This study obtained informed written consent and enrolled 250 fishermen from 18 beaches in Kisumu district. Before enrolment, beach seminars were conducted to educate the local population about the forthcoming study. Eligibility criteria, the study's scope and the expectations of the study participants were discussed and their concerns were addressed.

Through these beach seminars, fishermen were preselected to participate in the study. Thus, participation refusal data were not collected. The participation eligibility criteria included men who worked in the fishing industry for at least the past six months; aged  $\geq 18$  years; were sexually active (had sex at least once in the last 2 weeks); and were willing and able to give written informed consent. Three to four boats with up to 4 crew members per boat were randomly selected per beach, using cluster-sampling proportionate to the number of registered boats per beach. Demographic data were obtained using detailed structured behavioral face-to-face interviews. Blood specimens were collected in the morning hours in temporary mobile research clinics erected at each of the 18 beaches. The collected blood specimens were kept in the cooler boxes and transported in the afternoon to the nearby Kenya Medical Research Institute laboratory in Kisumu, where sera were stored at  $-80^{\circ}\text{C}$ . The ethical review committees of the Kenya Medical Research Institute and the University of Washington approved the study.

## 2.3 Serology

The initial HSV-2 serological testing using Focus Gen 1 was done in the Kisumu laboratory. Then sera were shipped in liquid nitrogen to the Virology Laboratory at the University of Washington, Seattle USA, and stored at  $-20^{\circ}\text{C}$ . In Seattle, sera were thawed once, 0.1 ml withdrawn, and the vials were refrozen at  $-20^{\circ}\text{C}$  until batch testing by WB. The withdrawn portion was tested by Focus, Kalon, Biokit and Express within one week of thawing.

**2.3.1 Western blot**—Western blot was performed according to published methods (Ashley et al. 1988). Sera without clear profiles for either HSV-1 antibodies or HSV-2 antibodies were absorbed against HSV-1 or HSV-2 antigen and re-tested on fresh strips. If profiles remained unclear or were insufficient to meet the criteria for a positive test, results were scored as “atypical.” Criteria for a positive HSV-2 test included: presence of four of the seven major viral bands (VP123, gB, gC/gE, gG, VP16, gD, ICP3) or, after pre-absorption, a clear gG-2 band.

**2.3.2 Commercial serology**—The HSV-2 serology performed in Kisumu used the original, first generation Focus HerpeSelect HSV-2 ELISA kits (“Gen 1”) approved by the U.S. Food and Drug Administration. The Focus HSV-2 ELISA performed at the University of Washington used a reformulation (“Focus Gen 2”). Sera were also tested in Washington by Kalon HSV type 2 IgG ELISA (“Kalon;”), the Biokit HSV-2 Rapid Assay (“Biokit;”) and by HerpeSelect Express HSV-2 Rapid Assay (“Express;”). Out of the 250 sera, one had insufficient quantity for testing by Focus Gen 1, Focus Gen 2, Kalon, Express and WB; and two had inadequate volume for testing by Biokit.

Commercial tests were performed according to the kit instructions with minor differences in the bench protocol for the Focus assays performed in the two study laboratories: 1) Only 20 samples at a time were run in Kisumu using dilution tubes, whereas in Seattle, 88 samples were run simultaneously using dilution plates and a 12 channel electronic pipette to add diluted sample to plates. 2) The Seattle laboratory used a semi-automated washer while Kisumu used a fully automated washer. 3) Index values were calculated manually in Kisumu, and by KC4 software in Seattle.

**2.3.2.1 Test interpretation:** Focus Gen 1 and Focus Gen 2 kits and Kalon use the same cut-off for test interpretation; sera with index values  $< 0.9$  are negative, values  $> 1.1$  are positive, and values  $0.9 - 1.1$  are equivocal. Sera with equivocal test results were not retested and were not further evaluated. Biokit and Express rapid tests use the same principle for test interpretation; a test spot (Biokit) or line (Express) clearly colored red is considered to be positive; whereas negative test areas have faint or no color. Sixty-eight tests by Biokit and 47 by Express had faint color and were recorded as negative.

### 2.3.3 Statistical Measures

The test sensitivity was calculated using the following formula; Sensitivity = number of true positive (TP)/sum of the number of TP and number of false negative (FN). Specificity was calculated as follows; Specificity = Number of true negatives (TN)/sum of TN and the number of false positives (FP). The positive predictive value (PPV) which is the proportion of patients with positive test results who were correctly diagnosed was determined as follows;  $PPV = TP / \text{sum } TP+FP$ ; while the negative predictive value (NPV) defined as the proportion of patients with negative test results who were correctly diagnosed was determined as follows;  $NPV = TN / \text{sum } TN+FN$ . The 95% confidence interval for the sensitivity and specificity were calculated using an online exact confidence interval for proportion method available at [http://www.causascientia.org/math\\_stat/ProportionCI.html](http://www.causascientia.org/math_stat/ProportionCI.html). For tests that measured index values (Focus and Kalon ELISA), receiver operating characteristics (ROC) curves and the area under the curve were generated for each test using SPSS version 12 (SPSS Inc. Chicago, IL). The values for the area under the curve approaching 1.0 signify highest accuracy compared with WB the “gold standard” test outcome while values near 0.5 indicate that the test has effectively 50% sensitivity and 50% specificity; using such a test is no better than guessing.

## 3.0 Results

### 3.1 Prevalence of HSV-2 antibody

Prevalence in this high risk cohort varied widely depending on the test used: 58.2% by WB, 73.5% by Focus Gen 1, 79.5% by Focus Gen 2, 42.9% by Biokit, 81.5% by Express and 63.9% by Kalon.

### 3.2 Test performance

Data were used for performance analyses only if the results were definitive. The following indeterminate or equivocal results were not used in the analyses: 15 (6%) sera by WB; 7 (2.8%) by Focus Gen 1; 2 (0.8%) by Focus Gen 2 and 10 (4%) by Kalon. Results concordant with those of WB were obtained in 195 (85.9%) of 227 sera by Focus Gen 1; 189 (81.5%) of 232 by Focus Gen 2; 175 (75.4%) of 232 by Biokit; 183 (78.5%) of 233 by Express; and 210 (93.8%) of 224 by Kalon. Based on WB as the gold standard, the test sensitivities were as follows: Focus Gen 1, 98.6%; Focus Gen 2, 99.3%; Biokit, 66%, Express, 99.3%, and Kalon, 98.6%. The specificities of each test were: Focus Gen 1, 63.5%; Focus Gen 2, 52.3%; Biokit, 90.9%; Express, 44.3%; and Kalon, 85.7%. The positive predictive values (PPV) of the five tests ranged from 74.6% by Express to 92.2% by Biokit. The negative predictive values (NPV) ranged from 62.3% by Biokit to 97.8% by Focus Gen 2 (Table 1).

Both Focus ELISA tests had large numbers of false positive results relative to WB: 31 for Focus Gen 1, and 42 by Focus Gen 2. Of the 31 false positive Focus Gen 1 results, 5 were negative by Focus Gen 2, 25 were negative by Biokit, 2 were negative by Express and 17 were negative by Kalon. Focus Gen 2 had 42 false positive results. Of these 42 sera, 13, 36, 3 and 24 were negative by Focus Gen 1, Biokit, Express and Kalon, respectively.

The Biokit test had eight false positive results. Of these sera, 2, 2, and 6 were negative by Focus Gen 1, Focus Gen 2, and Kalon respectively. The main problem with Biokit was low sensitivity; there were 49 false negative results by Biokit of which 46 were positive by Focus Gen 1, 48 were positive by Focus Gen 2, and 43 were positive by Kalon.

Of the 145 sera positive by WB, 144 were positive by Express, but 48, 1, and 1 of these were negative by Biokit, Focus Gen 1, and Kalon, respectively.

Of 12 sera false positive by Kalon, 11 were also false positive by Focus Gen 1 and all the 12 were false positive by Focus Gen 2, but only two were false positive by Biokit.

### 3.3 Determination of the accuracy of the three ELISAs by ROC curves

A test with perfect discrimination between negative and positive status (no overlap in the two distributions) has a ROC plot that passes through the upper left corner (100% sensitivity and 100% specificity). Therefore the closer the ROC plot is to the upper left corner, the higher the overall accuracy of the test. Examination of the curves reveals that Kalon ELISA had higher accuracy as determined by the above criterion than either Focus Gen 1 or Focus Gen 2. The areas under the curve were 0.975 (95% CI 0.953 – 0.997) for Kalon (Figure 1A); 0.955 (95% CI 0.926 – 0.983) for Focus Gen 1 (Figure 1B); and 0.923 (95% CI 0.888 – 0.959) for Focus Gen 2 (Figure 1C).

### 3.4 Effects of adjusted cut-off values

Increasing the positive cut-off can substantially increase the specificity of the Focus test (Laeyendecker et al. 2004; Laderma et al. 2008). In this study, increasing the cut-off value from an index value of 1.1 to an index value of 3.5 increased specificity of the Focus Gen 1 test from 63.5% to 93% and decreased the sensitivity only slightly from 98.6% to 97.9%. The overall concordance with WB increased from 85.9% to 96.1% (148/154). The altered cut-off value resulted in 90 equivocal results versus 7 with the standard cut-off value, but eliminated 27 false positives. The PPV increased from 82.1% to 96% with no change in N the PV.

When an index value of 3.5 was used as the cut-off for the Focus Gen 2 test, specificity increased from 52.3% to 84.9%, with little effect on sensitivity. Concordance with WB increased from 81.5% to 95% (170/179). This method resulted in 62 more equivocal results (64 versus 2) than with a cut-off value of 1.1, but eliminated 34 false positives. The PPV value increased from 77.4% to 94%.

Adjusting the Kalon cut-off from an index value of 1.1 to 1.5, increased the specificity from 85.5% to 92.2%. Concordance with WB increased from 93.8% to 96.2%. This method resulted in 11 more equivocal results than with a cut-off value of 1.1, but eliminated 6 false positives. The PPV increased from 92.1% to 95.7% with no change in NPV (Table 1).

### 3.5 Effect of Test Combinations

Because Focus Gen 1 tests are no longer available, Focus Gen 2 and Kalon were used in sequence for two test combinations. In the first model, all the sera were tested by Focus Gen 2 and the positive results retested by Kalon. Only dual positive results were counted as positive; sera positive by Focus but negative by Kalon were counted as negative (Figure 2). The sensitivity and specificity of this test combination was 98.6% (139/141) and 85.2% (69/81) compared with WB while the PPV was 92.1% and NPV was 97.2%. While this test combination improved the specificity over that of Focus Gen 2 as the sole test (85.2% vs 52.3%), the combination did not perform better than Kalon, alone, with respect to specificity or PPV. The second model using Kalon as the first test and Focus Gen 2 to confirm Kalon positive results gave identical performance figures to those of Kalon, alone, because 100% of the positive tests by Kalon were also positive by Focus Gen 2 (Figure 2).

An attempt to improve Kalon specificity by confirming positive results with Biokit was not effective. The specificity was improved from 85.7% with Kalon, alone, to 81/83 (97.6%) with a PPV of 95/97 (97.9%). However, sensitivity of the combination was only 95/140 (67.9%) and the NPV was only 81/126 (64.3%).

Because Express was very sensitive but had poor specificity, trying to confirm the positive results by Express with Kalon, using a cut-off value of 1.5, resulted in a slight drop in sensitivity from 99.3% with Express, alone, to 134/140 (95.7%) with a NPV of 52/58 (89.6%) for the test combination. The specificity improved from 44.3% with Express, alone, to 52/58 (89.6%) with the test combination while the PPV increased from 75.8% to 134/140 (95.7%).

#### 4.0 Discussion

This study aimed to determine the most accurate test or test combination for detecting serum antibodies to the HSV-2 type specific glycoprotein G (gG-2) in a high HSV-2 prevalence population of men from Kenya. The HSV-2 ELISA from Focus is the serological test most widely used in Kenya. However, concerns have been raised over its specificity in sera from African countries (Laeyendecker et al. 2004; Morrow et al. 2004; Van Dyck et al. 2004; Gamiel et al. 2008; Delany et al. 2009; Smith et al. 2009; Lingappa et al. 2009). The Kalon ELISA is a commercially available recombinant gG-2-based kit reported to have higher specificity than the Focus HSV-2 ELISA in African populations (Van Dyck et al. 2004; Gamiel et al. 2008; Delany et al. 2009; Smith et al. 2009; Lingappa et al. 2009). The Kalon and Focus tests are very similar to perform as 96-well, indirect ELISAs; with the main differences being in the serum dilution (1: 20 for Kalon and 1:101 for Focus) and enzyme substrate. Cost of the two tests is comparable (about \$3 per serum).

The diagnostic performance of a test or the accuracy of a test to discriminate between infected (seropositive) and uninfected (seronegative) cases can also be evaluated using the ROC curve analysis (Zweig and Campbell, 1993). The ROC curves can also be used to compare the diagnostic performance of two or more laboratory or diagnostic tests (Griner et al. 1981). In this study, Kalon ELISA had higher overall accuracy to detect HSV-2 among this male population compared to the two Focus ELISA tests as determined by the sensitivity and specificity calculations. The ROC plots further confirmed the overall higher Kalon ELISA performance to detect HSV-2 antibodies among this population.

The Biokit HSV-2 rapid assay, formerly on the market as POCKit-HSV-2, requires only 10 minutes to perform and has appeared useful to confirm low positive Focus ELISA results among the American populations (Ashley et al. 2005). At about \$20 per test, the Biokit test would be considered too expensive in Kenya as a screening or first-line diagnostic test, and was evaluated as a possible alternative to Western blot as a confirmatory assay. HerpeSelect Express HSV-2 rapid assay is a newer test that takes less than 10 minutes to perform. Its usefulness has yet to be widely proven, but its performance appears similar to that of Focus ELISA in the U.S population (Ladema et al. 2008; Philip et al. 2008).

This study is one of the only three studies that have reported the use of Biokit tests in Africa. Gamiel et al (2008) showed a sensitivity of 95.8% and a specificity of 56.1% when the Biokit was used in a young population from Rakai district in Uganda while Lingappa et al (2009) showed a sensitivity of 86.4% and a specificity of 97% of the Biokit in an urban, adult population in Kampala, Uganda. The sensitivity in this study of Kisumu fishermen was only 66% with a specificity of 90.9%. Biokit assays were performed in a different laboratory for the Gamiel study while the same laboratory was used for the studies of Kampala adults (Lingappa et al. 2009) and Kisumu fisherman. This could, in part, explain the difference in outcome as the laboratory producing the outlier data. Gamiel et al. (2008) might have had more liberal reading criteria, leading to falsely positive results. It remains unknown why one population would have a very low (66%) sensitivity compared with another population (86.4%) when both sets of sera were evaluated in the same laboratory using the same criteria.

One unique aspect of this study was the ability to compare performance of the original (“Focus Gen 1”) – which is no longer in the market - and the reformulated version (“Focus Gen 2”) ELISAs. Focus Gen 2 appears to improve specificity in U.S. sera (Hogrefe and Morrow, unpublished observations). However, in the Kenyan cohort, specificity (52.3%) and PPV (77.4%) for Focus Gen 2 were actually lower than those obtained with the Focus Gen 1 test (63.5% specificity and 82.1% PPV). The formulation change that is effective in American sera does not appear to address factor(s) in African sera that affect specificity.

This study also allowed analysis of the ability of the test from Biokit USA (“Biokit”) to clarify the status of low positive Focus results. Although this algorithm has been considered effective in American sera (Ashley et al. 2005) and, more recently, in sera from Uganda (Lingappa et al. 2009), somewhat surprisingly, Biokit was so insensitive (66%) that its use in verifying low positive Focus or Kalon results was compromised. The usefulness of Express in clarifying both negative and positive Kalon results also appeared to be limited.

One strategy for optimizing the accuracy of screening assays such as Focus or Kalon ELISA is to raise the cut-off value for positive to a value that reflects high concordance with WB or another confirmatory assay (Gamiel et al. 2008; Hogrefe et al. 2002; Laeyendecker et al. 2004). When the cut-off value was increased to 3.5 for Focus Gen 2, specificity improved from 52.3% to 84.9% with no loss of sensitivity (99.2%). When the cut-off value was increased to 1.5 for Kalon, specificity improved from 85.7% to 92.2% with no loss of sensitivity (97.3%). This strategy, however, increases the number of equivocal test results from 2 to 64 for Focus Gen 2 and from 10 to 21 for Kalon. In practice, the fact that approximately one quarter of results could not be regarded as definitive would seriously reduce the utility of the Focus Gen 2 test. The fact that 8.4% of sera would not have a definitive outcome when a Kalon cut-off value of 1.5 is used may be acceptable, especially in settings that allow repeat testing later to resolve equivocal results (Wald and Ashley, 2002).

This study has two major limitations. First, Western blot could be less sensitive than the other tests leading to erroneously low specificity. Without virological confirmation of infection status, this possibility cannot be entirely dismissed. Seroconversion may be detected earlier in some patients by both ELISAs and Biokit than by WB (Ashley et al. 1999; Ashley-Morrow et al. 2003b). Also, WB can be falsely negative in sera with high titers of HSV-1 antibodies that mask HSV-2 profiles (Golden et al. 2005). In this study, only two sera were negative for HSV-1 antibodies by WB, but positive by the other five tests. Moreover, the WB and another gold standard test, the monoclonal antibody inhibition assay from the Central Public Health Laboratory in the United Kingdom have been shown to have virtually identical results in African sera (Van Dyck et al. 2004). Thus, it seems unlikely that the apparent specificity problems in the ELISA tests are entirely an artifact of WB performance.

The second limitation is that the subjects were from a single region of Kenya. Tests may perform differently in various geographic areas (Morrow et al. 2004). Without more extensive sampling, it is not possible to generalize the outcome of this study to other populations such as women or subjects in other regions.

Given these limitations, a reasonable conclusion that can be drawn from these data is that in this geographically defined, high HSV-2 prevalence, male population, the Kalon test, performed alone, provides results that most closely approximate those obtained by WB. The same results were obtained by testing sera from a nonrandom sample of men and women in Kampala, Uganda (Lingappa et al. 2009). A small increase in the cut-off value for a positive Kalon test result provided very acceptable sensitivity and specificity with very high negative and positive predictive values. Kalon test results with index values between 1.1 and 1.5 had a higher likelihood of being falsely positive (6 of 11 or 54.5%) than those above 1.5; (6 of 140

or 4.3%). The proportion of tests with low positive results was quite low (14 of 159 or 8.8%) compared with Focus Gen 2 (46 of 176 or 26%). Studies of people in the Rakai district of Uganda showed superior sensitivity and specificity of Kalon (95% and 90%, respectively) to Focus (97%, 78%, respectively) when compared with WB; even when the Focus cut-off value was increased to 2.2 (Gamiel et al. 2008). Based on the data from the Kenyan cohort and with the Rakai cohort (Gamiel et al. 2008), a confirmatory assay by either Focus or Biokit adds little value to a single Kalon test.

Until inexpensive and accessible confirmatory tests are available for use in Kenya, it would appear that the Kalon test is the most cost-effective choice of serological assay for HSV-2. It is advisable to interpret low positive Kalon results with caution and to consider repeat testing at a later time to determine whether early seroconversion is a source of the low positive result (Ashley-Morrow et al. 2003a).

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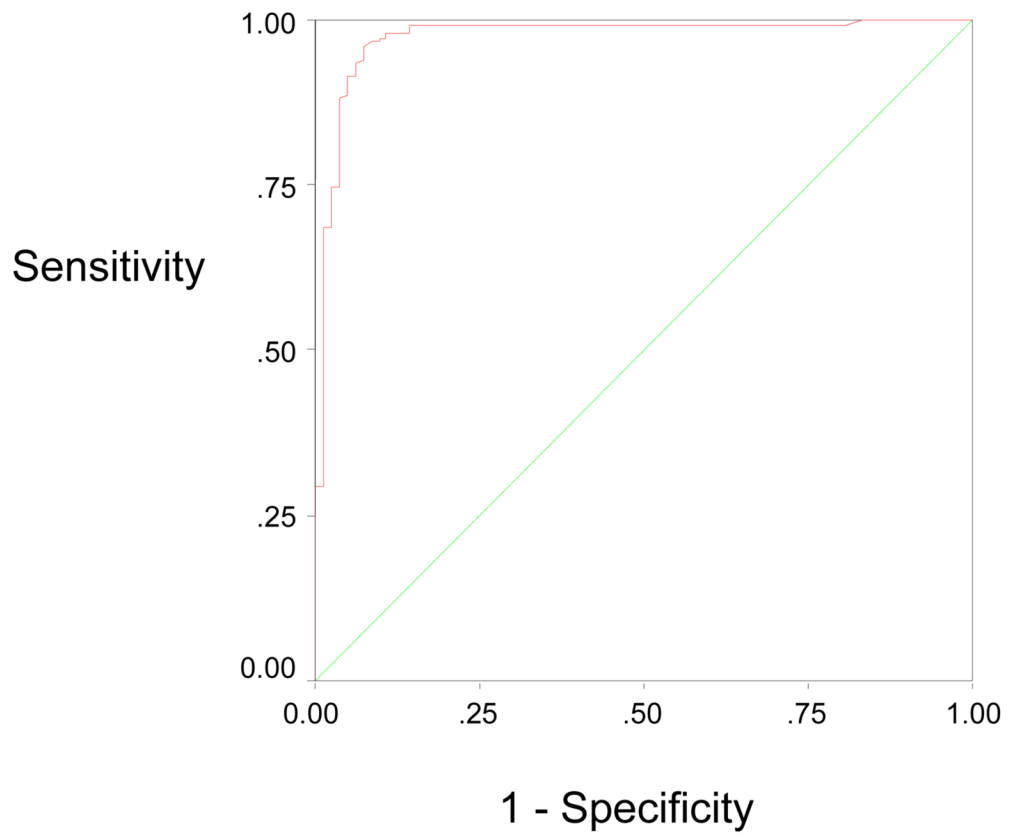


Figure 1A

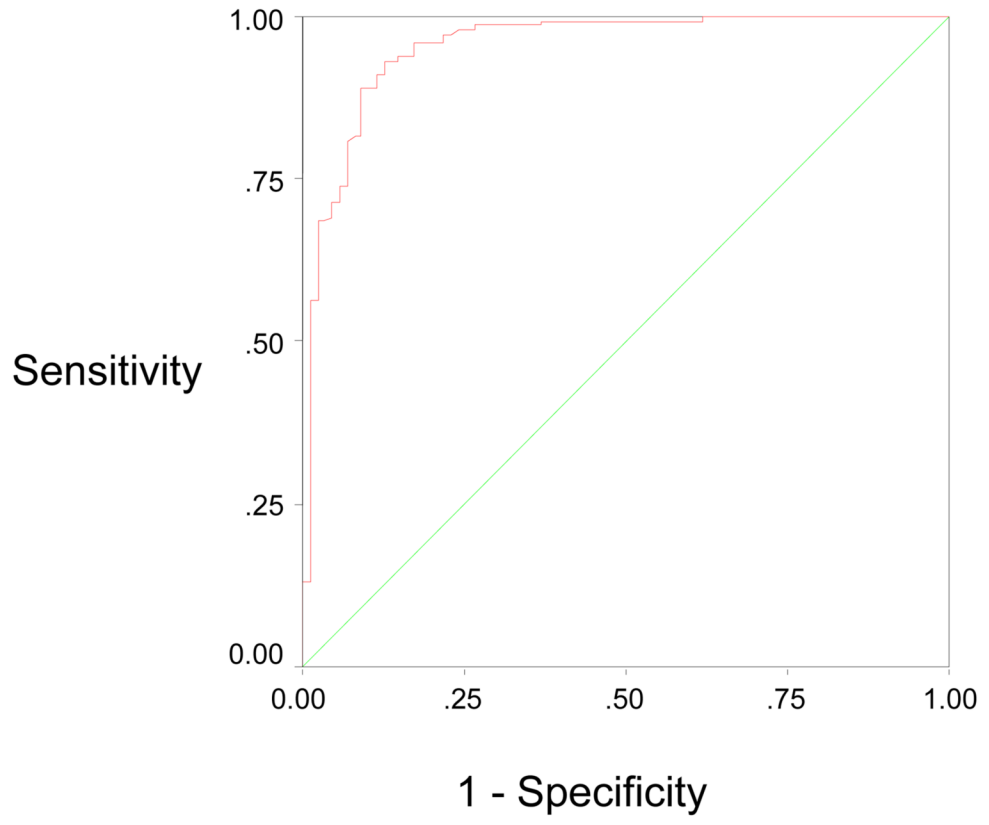


Figure 1B

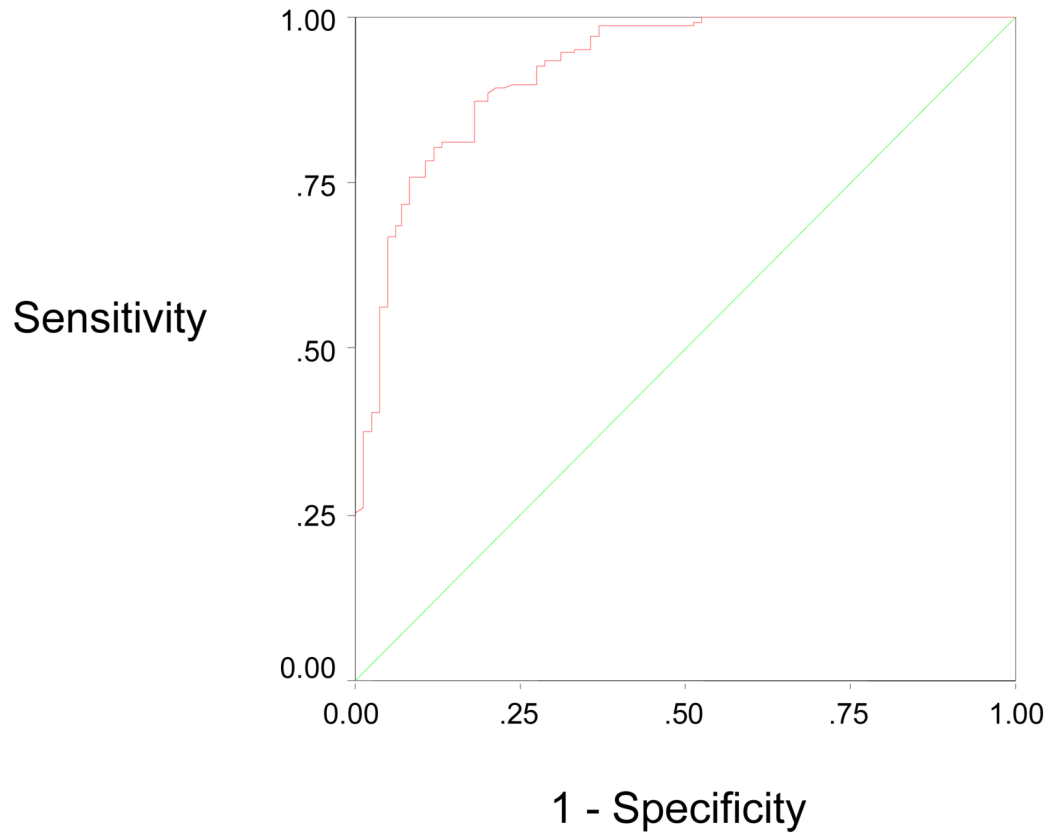


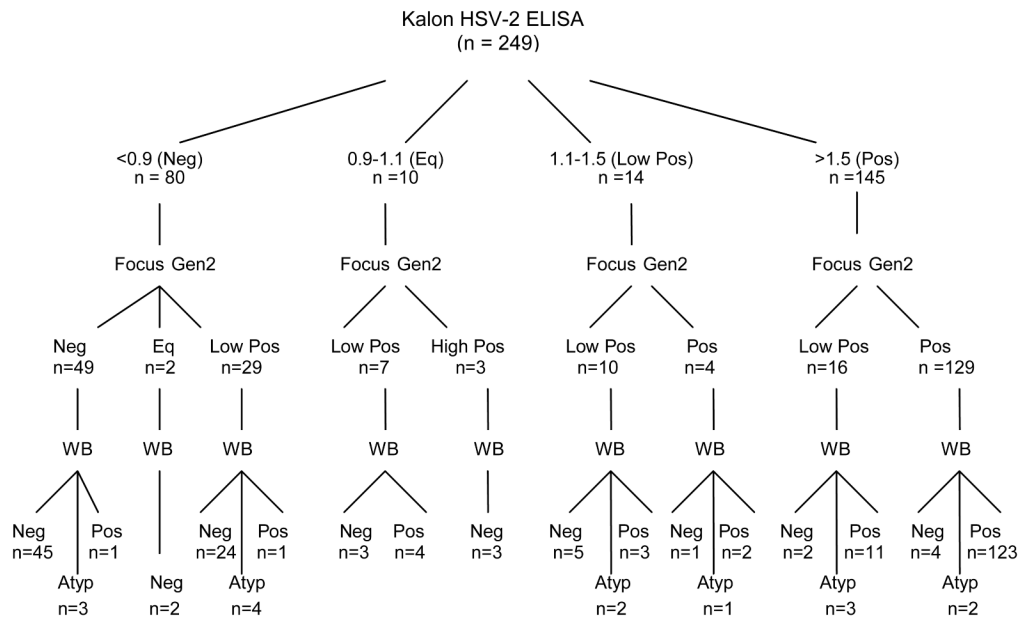
Figure 1C

**Figure 1.**

Figure 1A: The ROC curves for Kalon ELISA: Area under curve = 0.975, Standard error = 0.011, Asymptotic significance = 0.0001, 95% CI 0.953 – 0.997. Note: Values near 1 or 0 mean the test is almost always right or wrong compared to the gold standard, respectively, while values near 0.5 mean that using the test is no better than guessing

Figure 1B: for Focus Gen 1 ELISA: Area under curve = 0.955, Standard error = 0.014, P = 0.0001, 95% CI 0.926 – 0.983

Figure 1C: The ROC curve for Focus Generation 2 HSV-2 ELISA. Area under curve = 0.923, Standard error = 0.018, P = 0.0001, 95% CI 0.888 – 0.959



**Figure 2.** HSV serology results by three type specific antibody tests. Sera from the indicated categories of Kalon index values were tested by Focus Gen 2 and categorized by index value as negative (“neg”, <math><0.9</math>); equivocal (“Eq”, <math>0.9-1.1</math>); low positive (“low pos”, <math>\geq 1.1-3.5</math>) or positive (“Pos”, <math>>3.5</math>). Finally, the WB results are shown for each Focus Gen 2 category. Atypical “Atyp” WB results indicate HSV-2 reactivity that does not meet the criteria for a positive test

**Table 1**  
**Performance Characteristics of HSV-2 Type Specific Serologies Against Western Blot**

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for sera with clearly positive or clearly negative results by both the candidate test method and Western blot. The effect of increasing cut-off values between equivocal and positive interpretations was examined. Increasing the cut-off increases the number of samples with equivocal interpretation as shown in the column labeled “Equivocal” and reduces the number of sample results available for performance calculations as shown in the column labeled “N.”

Tests	Index Value Used for Positive Cut-off	N	Equivocal	Concordant Results with WB (%)	Sensitivity (%)	95% CI	Specificity (%)	95% CI	NPV (%)	PPV (%)
Focus Gen 1	1.1	229	6	85.9	98.6	95.1–99.6	63.5	52.9–73.0	96.4	82.1
Focus Gen 1	3.5	154	90	96.1	97.9	92.8–99.3	93	83.3–97.1	96.4	96
Focus Gen 2	1.1	233	2	81.5	99.3	96.2–99.8	52.3	37.6–58.0	97.8	77.4
Focus Gen 2	3.5	179	64	95	99.2	95.6–99.8	84.9	73.3–92.2	97.8	94
Kalon	1.1	225	10	93.8	98.6	95.0–99.6	85.7	76.7–91.6	97.3	92.1
Kalon	1.5	213	21	96.2	98.5	94.8–99.5	92.2	83.8–96.3	97.3	95.7
Biokit		233		75.5	66	57.9–73.2	90.9	83.2–95.4	62.3	92.2
Express		221		78.5	99.3	96.2–99.8	44.3	34.3–54.7	97.5	74.6