

Review

Sorting out the new HSV type specific antibody tests

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This review will delineate performance characteristics and limitations, as far as they are known, of the new glycoprotein G based, type specific HSV serologies. Several of these tests have been FDA approved in the United States for use in adults. With the departure of Gull/Meridian from the HSV serology market, it is important for clinicians to understand the sources and claims of the remaining type specific tests. Moreover, inaccurate tests using crude antigen preparations remain on the market. These tests are identified based on product insert information provided by company representatives.

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Applications of HSV type specific testing

With the new millennium, type specific herpes simplex virus (HSV) antibody tests based on the type specific proteins, gG-1 and gG-2, are now on the market for clinicians who wish to use them and for patients who desire to be tested.^{1,2} These new tests can legitimately claim to discriminate antibodies to HSV-1 from those to HSV-2. Many feel the commercial availability of these tests is a significant advance for patient care and for public health efforts to control the spread of genital herpes.³⁻⁸

HSV type specific antibody testing may be considered in a variety of clinical settings (table 1). Such tests can supplement culture or antigen detection methods to diagnose patients with lesions.^{9,10} Accurate serology is the only practical way to identify HSV-2 infected people with otherwise unrecognised genital herpes. Conversely, these tests can be useful in ruling out genital herpes in uninfected patients who have symptoms suggestive of herpes.^{4,11}

Tests based on glycoprotein G may also be essential to distinguish antibody responses to HSV infections from those to subunit vaccines containing other, unrelated HSV glycoproteins. However, recipients of other vaccine formulations containing gG should be advised that a positive gG based type specific serology will not be useful in diagnosing HSV should they become infected.

Accurate type specific serology can also characterise the nature of risk that a pregnant woman has for exposing a neonate to genital HSV shedding at term.^{7,12} In most neonatal

herpes cases the mother has no history of herpes. Identifying unrecognised HSV-2 seropositive women allows directed follow up for indications of herpes shedding in the genital tract at labour and delivery. A more controversial use of serology is for screening women and their partners to identify those women at risk of acquiring genital HSV-1 or HSV-2 late in pregnancy. Third trimester genital infections with HSV-1 or HSV-2 in the seronegative mother or HSV-2 in the HSV-1 seropositive mother pose a considerable risk of peripartum transmission to the infant.¹³

Studies showing an association between genital herpes and risk of HIV acquisition suggest another patient population that may benefit from diagnosing unrecognised genital HSV infection.^{8,14-16} Controlling genital herpes may help slow the spread of HIV.

As experts in the field have suggested, the public health benefits and psychosocial impact of widespread HSV antibody screening in low prevalence populations remain to be determined by further, directed study.^{5,17-21} However, for the individual patient, accurate tests can provide the basis for proper clinical management, timely treatment, and appropriate counselling relating to the natural history and transmission risks of the disease.^{18,22,23} Patients may request testing because they feel they may have contracted genital herpes, either because of their own sexual history or because their partner has been diagnosed with herpes. One study of genitourinary clinic attendees in the United Kingdom found that a majority believed HSV-2 type specific antibody determinations to be part of an STD examination.²

Gold standard non-commercial tests for HSV type specific antibody

A number of tests have established track records but have not been developed as commercial kits (table 2). The performance of these tests is uniformly high with respect to sensitivity and ability to discriminate between HSV-1 and HSV-2 antibodies.²⁴ These tests should be used to establish performance of

Table 1 Potential applications for HSV type specific serology

Symptomatic genital disease
Lesions are negative or not sampled for virus
Lesions appear herpetic but may have other aetiology
Patients without distinctive genital herpetic lesions
Partner has genital herpes
Patient has a history of other STDs
Recurring symptoms suggest atypical or undiagnosed herpes
Patients at risk of HIV infection
Pregnant women
To identify those with unrecognised HSV-2 genital herpes
To identify those at risk for acquiring HSV-1 or HSV-2

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Table 2 HSV-2 type specific serology gold standard tests

Test	Antigen	Location
Western blot ^{26 31 32}	Infected cell proteins (HSV-1 and HSV-2)	Seattle, Australia, Italy
Immunodot enzyme assay for gG-1, gG-2 ^{26 31 32}	Immunoaffinity purified gG-1, gG-2	Atlanta
CPHL monoclonal antibody blocking EIA ^{39 40}	Infected cell lysates (HSV-1 and HSV-2)	London
Indirect gG-2 ELISA ⁴³⁻⁴⁵	Lectin purified gG-2	Australia, Sweden
Recombinant gG immunoblot ⁴⁶	Baculovirus recombinant gG-2	Atlanta
gG-1 and gG-2 capture ELISA ⁴⁷	Infected cell proteins (HSV-1, HSV-2)	Japan

future HSV type specific tests, if at all feasible. It should be noted, however, that these tests are offered in academic or reference laboratory settings. While the technologies are published and can be developed for use in other laboratories, the gold standard tests are not available, as kits, for other laboratories to purchase.

WESTERN BLOT (WB)

In WB, sera are reacted against separated, fixed protein arrays ("blots") from either HSV-1 or HSV-2 infected cell lysates.²⁵⁻²⁷ The patterns of antibody binding bands on the two blots are highly predictive of infection with either HSV-1 or HSV-2. Sera from patients with both HSV-1 and HSV-2 infections are also readily identified. Interpretation of WB results is subjective and profiles may not always be definitive. For this reason, the test is unlikely to be developed for widespread commercial use. Further, the test is highly complex to perform and includes a number of timed incubation steps, including overnight exposure of the sample to the blots. These are serious limitations for use of WB in forensic applications where maintaining a chain of custody for the sample is required.

The University of Washington test ("UW WB") has been used to define the spectrum of clinical manifestations of genital herpes and to study the natural history of unrecognised genital herpes infections.²⁸⁻³⁰ It was the gold standard test for FDA trials of the commercial assays described below. Similar WB tests have been described in Australia,³¹ Italy,³² and Germany.³³

IMMUNODOT ENZYME ASSAY (IEA)

This test uses immunoaffinity purified gG-1 and gG-2 immobilised on nitrocellulose discs.^{34 35} In other respects it is similar to an enzyme immunoassay (EIA) and is appropriate for high volume testing. The IEA was validated against culture and UW WB²⁶ and has been used to track HSV-2 seroprevalence trends in the United States between 1979 and 1990.³⁶⁻³⁸

MONOCLONAL ANTIBODY BLOCKING ASSAYS

The Central Public Health Laboratory (CPHL) in London uses a method that gains type specificity from HSV-1 and HSV-2 monoclonal antibodies against type specific gG epitopes. The original radioimmunoassay format was validated against culture and UW WB.³⁹ The EIA version of the test⁴⁰ has high concordance with western blot⁴¹ and is the major type specific reference test for the United Kingdom.^{10 42}

INDIRECT gG-2 ELISA

Lectin purified gG-2 is used as antigen for enzyme immunoassays developed in Australia and in Scandinavia.⁴³⁻⁴⁵ Against culture, this

format is highly sensitive and specific for HSV-2 antibodies⁴³

gG-2 IMMUNOBLOT

This test uses baculovirus recombinant gG-1 and gG-2 that have been denatured and electrophoresed to separate the target proteins from unrelated proteins.⁴⁶ Test sensitivity is somewhat less than IEA.²⁴

gG-CAPTURE ELISAS

Type specificity in these tests is conferred by monoclonal antibodies bound to microwell plates. Comparison tests showed slightly lower sensitivity for HSV-1 (89%) and HSV-2 (90%) than the IEA test.⁴⁷

Commercial HSV type specific gG based serology

Three companies, Meridian Bioscience Inc, MRL Diagnostics (now called "Focus Technologies"), and Diagnology, have received approval from the US Food and Drug Administration for a total of six gG based diagnostic test kits; two for HSV-1, three for HSV-2, and one that combines both in one kit (table 3). Until March 2001, Meridian Bioscience offered gG based HSV-1 and HSV-2 ELISAs, under the "Premier" label (table 3). The tests were originally developed by Gull Laboratories using affinity purified gG-1 and gG-2. Unfortunately these discontinued Premier HSV tests have the largest fund of performance data of the commercial tests.

Focus Technologies has three tests: HSV-1 and HSV-2 ELISAs and an immunoblot test combining HSV-1 and HSV-2 antibody detection. All three tests use baculovirus recombinant gG constructs. All are FDA approved tests and can be purchased as kits. In addition, Focus Technologies' reference laboratory also tests sera that are sent to their facility in Cypress, California.

Diagnology (Belfast, Northern Ireland) offers the only point of care or "near patient" test for HSV-2 antibodies that is designed for clinic use. The antigen for their "POCKit-HSV-2" test is lectin affinity purified gG-2. Quidel Corporation (San Diego, CA, USA) has trials under way for FDA clearance of second point of care antibody test (table 3).

The patient populations for which these tests have been approved vary according to the design of the clinical trials since the FDA requires population specific proof of efficacy for each indication. Diagnology's POCKit-HSV-2 is approved for use in adult men and women while the Focus ELISAs and immunoblot tests are approved for use in pregnant patients, as well.

Table 3 Selected commercial HSV type specific antibody assays based on glycoprotein G-2

Test	FDA approved	Sens/spec for HSV-2	Gold standard (citation)	Type of test
HSV-2 ELISA IgG (Focus/MRL, Cypress, CA)	Yes	96/97 100/98	UW WB (FDA trial results) WB ⁵³	ELISA (HSV-1 ELISA also available)
HSV-1 and HSV-2 IgG differentiation immunoblot (Focus/MRL, Cypress, CA)	Yes	100/ND 97/98	Culture ³³ UW WB (FDA trial results)	Strip immunoblot (HSV-1 and HSV-2)
Premier type specific HSV-2 IgG (Meridian; Cincinnati, OH) (formerly Gull Laboratories tests)	Yes	98/97 81/99 91/96	UW WB ⁴⁹ UW WB ⁵⁰ Culture ⁵²	ELISA (HSV-1 or HSV-2) Tests no longer available
POCkit HSV-2 (Diagnology; Belfast, Northern Ireland)	Yes	93/95 96/98 96/97	CPHL MAb blocking ¹⁸ UW WB/culture ⁵⁵ UW WB ⁵⁴	Membrane point of care
Cobas Core HSV-2 IgG EIA (Roche, Basel, Switzerland)	No	93/98	Culture/(see note) ⁵²	Automated ELISA (HSV-2 only)
Captia Select HSV-2 EIA (Centocor; Malvern, NY)	No	90/99 92/91	Consensus ⁵⁶ Culture/(see note) ¹⁰	ELISA (HSV-2 only)
ETI-HSVK-G2 (Sorin Diagnostics Biomedica)	No	91/100	Culture/(see note) ⁵²	ELISA (HSV-2 only)
Quickvue HSV (Quidel; San Diego, CA)	In trials			Membrane point of care (HSV-1 and HSV-2)

Cobas and Sorin kits were tested for specificity with non-typing HSV ELISA to identify antibody negative samples. Captia Select specificity was determined with paediatric sera to identify HSV-2 negative sera. Focus/MRL data from FDA clinical trials reported with permission.

Sorin Diagnostics Biomedica (ETI-HSVK-G2), Centocor (Captia Select HSV-2 EIA; marketed by Trinity Biotech and by Wampole Labs), and Roche (Cobas Core HSV-2 IgG EIA) produce gG-2 based tests in formats that are cost effective and easy to perform. None is FDA approved. Further, HSV-1 type specific antibody detection is not offered by these companies (table 3).

The commercial tests differ in their format and, in turn, in their most likely application. The ELISAs from Focus, Roche, Centocor, and Sorin are appropriate for high volume testing on automated platforms. The immunoblot from Focus resembles a western blot with gG-1, and gG-2 bands, a type common HSV band, and a control band all arrayed on a single strip. The test is read visually for HSV-1 and HSV-2 results so that optical density instrumentation is not required. It is well suited to low volume laboratory applications.

The POCkit and Quidel tests use capillary blood from a fingerstick or serum. Quidel provides HSV-1 and HSV-2 testing on the same membrane while POCkit tests only for HSV-2. Both companies' tests are performed in minutes with no equipment and little laboratory expertise needed. However, the reading of colour change indicating antibody binding can be subjective. In a recent large scale study of POCkit using banked sera and three independent readers, 5–10% of tests had discordant readings.⁴⁸

Performance of gG based commercial tests

Published data, to date, suggest that all of the tests listed in table 3 are preferable to crude antigen based tests in accuracy. The tests are comparable to each other and to gold standard tests such as western blot for specificity (lack of falsely positive results for HSV-2).

SENSITIVITY IN COMPARISON TESTS

Sensitivity of the commercial tests is more variable across kits than is specificity. In contrast with premarket evaluations of prototype Gull kits that showed high sensitivity,⁴⁹ the Gull/Meridian HSV-2 test has shown lower

sensitivity (81–90%) in recent studies against WB^{50 51} and for diagnosis of culture documented cases.⁵²

The Focus HSV-2 ELISA is very sensitive when compared with WB (96%–100%)⁵³. The Focus immunoblot test has had very promising performance (97–100% sensitivity) against culture³³ and UW WB. The POCkit test has shown high sensitivity (93–96%) against WB, culture, and the CPHL assays.^{54 55}

The non-FDA approved commercial assays appear to have a somewhat lower range of sensitivity (90%–93%) when compared with culture^{10 52} or a test consensus standard.⁵⁶ Scientists at CPHL evaluated five commercial assays and its in-house monoclonal antibody blocking assay against a consensus standard (five of six assays in accord providing an inferred "true" positive or negative). Kappa statistics showed comparable agreement among results from Cobas, Focus/MRL HSV-2 ELISA, Focus/MRL immunoblot, and POCkit; agreement was substantially lower with the Gull/Meridian assay.⁵⁷ This may be a reflection of the relatively lower sensitivity of the Meridian test for HSV-2.

SENSITIVITY AS A FUNCTION OF TIME TO SEROCONVERSION

Another measure of sensitivity is the time required for a test to become positive after a patient becomes infected. Very limited data are available on the commercial assays. We found that seroconversion by POCkit after HSV-2 primary or HSV-2 non-primary first episodes occurred a median of 2 weeks after onset of symptoms. This was comparable in speed with early profiles by WB and about a month faster than required to develop full WB profiles.⁵⁸ Seroconversion time by Gull gG-2 ELISA was notably slow; only 38% were positive by 3 months.²⁴ Other, non-commercial gold standard tests require a median of 2–6 weeks with 80–100% of newly infected patients becoming positive by 3 months.²⁴

Limitations of type specific tests

DETERMINING DURATION OF INFECTION

As described above, limitations include potential slow time to seroconversion to gG-2. In

addition “staging” the disease as being recently acquired cannot be accomplished reliably by serology. Approximately 20% of those reporting first episodes of genital symptoms are not, in fact, newly infected but are presenting with first clinically apparent recurrences.^{59, 60} Most gG-2 based tests will register as positive in such cases; however, a negative result does not guarantee recent infection nor does a positive result rule out primary or non-primary episodes, especially with highly sensitive tests such as POckit.

HSV-1 GENITAL HERPES

HSV-1 is causing an increasing proportion of new genital herpes infections as indicated in recent surveys from Scandinavia,⁶⁰ the United Kingdom,⁴¹ and the United States.⁶¹ The POckit, Cobas, Captia Select, and ETI-HSVK-G2 tests do not detect type specific antibodies to HSV-1. The Focus tests can detect HSV-1 specific antibodies. However, no test can distinguish between HSV-1 antibodies that are generated in response to oral infection and those arising after a genital HSV-1 infection. Moreover, it should be noted that type specific tests for HSV-1 tend to be 5–10% less sensitive than their HSV-2 counterparts and may require longer to reflect seroconversion. Seroconversion to HSV-1 around the time of new genital lesions is presumptive evidence of genital HSV-1 infection; however, virus detection tests are advised.

USE OF TYPE SPECIFIC TESTS IN PAEDIATRIC SERA

Only the HSV-1/HSV-2 gG based Premier type specific test combination from Meridian was tested in paediatric populations in FDA clinical trials. The results prompted a warning in the kit against use of the tests for herpes diagnosis in children. Recently we conducted a blinded study between UW WB and Meridian Premier HSV-1 and HSV-2 kits in sera from 97 children ages 1–14. The Meridian kits had a 54% specificity (70% positive predictive value) for HSV-1 and a 47% specificity (6% positive predictive value) for HSV-2.⁶² The NHANES survey revealed an HSV-2 seroprevalence of 0.25% in children using immunodot enzyme assay.³⁸ Eis-Hubinger *et al* found an HSV-2 seroprevalence of 4% among children with the Gull test and 3% with the Cobas test. Paediatric sera accounted for nearly all of the false positive results in this large comparison study.⁵² Performance in paediatric sera by the Focus, POckit, and other commercial tests are not known. Thus, these tests should be used with caution, if at all, in children under 14.

SEROLOGY IN MEDICOLEGAL CASES

It is important to note that the performance characteristics of gold standard tests, including WB, have not been determined in children. HSV-2 infections that may have occurred as a result of sexual abuse of children should be diagnosed by culture or PCR, not by serology. No test for antibodies to HSV-1 or HSV-2 can be considered to be completely accurate in determining whether a person has or has not

been infected with HSV. Because every serological test has a potential for false positive or false negative results, use of type specific serology in criminal cases to link an alleged perpetrator of abuse or assault with a victim of any age by matching antibody types is not recommended. Similarly, use of serology to infer transmission links for civil lawsuits involving herpes acquisition is not recommended since even the most accurate test cannot reveal when and by whom an individual became infected.

“SEROREVERSION” OR LOSS OF gG-2 ANTIBODIES

The outcome of glycoprotein G based type specific tests may change over time from positive to negative.^{63, 64} This phenomenon has been termed “seroreversion” and implies that the immune response to gG-2 wanes to undetectable levels over time. This possibility has caused concern about the long term reliability of these tests. We examined nearly 300 sera from 32 patients with long term clinic follow up for HSV-2 genital herpes (6–22 years; median 12 years) by western blot and by the Gull gG based HSV-1 and HSV-2 ELISAs.⁶⁵ Sera were drawn at least once a year for a median of 9 years (3–20 years). We found no evidence for change in HSV-1 or HSV-2 western blot profiles that would suggest loss of antibody titre. In contrast, the Gull gG-2 test resulted in sporadic reversal from positive to negative in two subjects. The gG-1 test revealed sporadic reversals from HSV-1 positive to negative in two of 13 HSV-1 seropositive subjects. Based on western blot profiles, these changes probably represent normal fluctuation of the test itself rather than dramatic reduction in amounts of antibody produced. Type specific tests that turn negative over time should be questioned and the sera involved should be repeated, in parallel, on the same day and with the same reagents to reduce run to run variation in test results.

TYPE SPECIFIC IgM TESTS

Very few testing formats have been adapted to detect type specific IgM to gG-2.⁴³ As described elsewhere, Gull Laboratories developed prototype gG based IgM that could detect seroconversion much faster than could the Gull type specific IgG tests. However, the IgM ELISA was not useful for discriminating primary episodes from recurrent episodes since 35% of recurrent HSV-2 episodes elicited IgM to HSV-2.²⁴

“Type specific” tests to avoid

Tests purporting to identify type specific antibodies have been commercially available for some time. When based on crude antigen preparations, such tests are inaccurate and misleading because the extensive cross reactivity between HSV-1 and HSV-2 generate indistinguishable antibody responses.^{51, 66} Two of the tests that we found to be unacceptably inaccurate in 1991 (from Sigma and IncStar) are still on the market. A recent comparison of tests from Diamedix, Zeus, and Wampole revealed HSV-2 specificity values of 61%, 79%, and

Table 4 Tests based on crude antigen (not recommended)

Company	Test name	Location
Diasorin (formerly Incstar)	Herpes 1 or 2 IgG Clin-ELISA	Stillwater, MN
Zeus	HSV-1 and/or HSV-2 ELISA	Raritan, NJ
GenBio	Immuno FA Herpes simplex virus IgG test	San Diego, CA
Sigma	Herpes 1-IgG Herpes 2-IgG	St Louis, MO
Wampole Labs	HSV-1 IgG ELISA HSV-2 IgG ELISA	Cranbury, NJ
Trinity Biotech	Captia HSV-1 IgG Captia HSV-2 IgG	Jamestown, NY
Diamedix	Immunosimplicity HSV-1 and 2 IgG	Miami, FL

85%, respectively.⁵¹ Although the most pervasive problem with these tests is in their inability to detect HSV-2 antibodies in HSV-1 seropositive patients, the tests also mistakenly type antibodies in patients with only HSV-1 infection or only HSV-2 infection.⁶⁶

The American companies that market tests based on crude antigen are listed in table 4. The kit inserts provide instructions for determining HSV-1 versus HSV-2 antibodies and the catalogue descriptions may include the term "type specific." These instructions are misleading; the recent study of Martins *et al*⁵¹ revealed cross reactivity rates of 82% in positive samples by the Diamedix test; 54% by the Zeus tests, and 47% by the Wampole tests. For practical use, the cross reactivity rates indicate that a positive test for HSV-1 or HSV-2 by these manufacturers' kits can be due to HSV-1 infection, to HSV-2 infection, or to infection with both types.

Summary

Clinicians and patients now have a choice of FDA approved laboratory based tests from Focus (ELISA or immunoblot formats) or point of care testing from Diagnology for accurate detection of HSV-2 antibodies. Quidel is seeking FDA approval for an HSV-1 and HSV-2 types specific point of care test. Other companies (Roche, Sorin, Centocor) offer HSV-2 (not HSV-1) tests based on gG-2. While not subjected to the closely controlled clinical trials required for FDA approval, these companies' tests appear to perform reasonably well; albeit with lower sensitivity than tests from Focus or Diagnology. HSV type specific tests differ in their sensitivity and in their time to seroconversion and should be interpreted with great caution if used for paediatric sera. These tests are not recommended for determining transmission links in medicolegal cases.

Many test kits based on crude antigen remain on the market and continue to provide more confusion than value. Those that attempt to discriminate HSV-1 from HSV-2 responses should be pulled from the market or reformatted to include both HSV-1 and HSV-2 antigens in the same test well. Until companies adjust their HSV product lines to reflect performance data, clinicians and laboratory managers are advised to insist on tests that are based on glycoprotein G.

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