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Premarket Evaluation of the POckit HSV-2 Type-Specific Serologic Test in Culture-Documented Cases of Genital Herpes Simplex Virus Type 2

[Original Articles]

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Abstract [TOP](#)

Background and Objectives: The genital herpes epidemic continues, in part, because patients with subclinical or atypical presentations cannot be identified by most herpes simplex virus (HSV) antibody tests. A new product, POckit HSV-2, has been developed to rapidly and accurately detect antibodies to HSV type 2 (HSV-2) in capillary blood or serum.

Goal: Sera from patients with culture-documented genital or oral herpes were tested to determine the sensitivity and specificity of the POckit HSV-2 rapid point-of-care antibody test (Diagnology, Belfast, Northern Ireland).

Study Design: Sera from 50 patients with culture-documented HSV type 1 (9 oral, 41 genital) and from 253 patients with genital HSV-2 were tested by POckit HSV-2 for HSV-2 antibodies. Each subject had a positive culture for HSV within 6 months of serum collection. Sera were preselected to include only those that were seropositive to the respective virus subtype by University of Washington Western blot.

Results: Compared with viral culture and Western blot analysis, sensitivity of the POckit HSV-2 test for HSV-2 antibody was 96%; specificity was 98%.

Conclusion: This test provides rapid, accurate identification of HSV-2 antibody in subjects with established HSV infections.

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GENITAL HERPES continues to spread among sexually active individuals and from women to newborns. Between 75% and 90% of herpes simplex virus type 2 (HSV-2) seropositive persons do not have a history of genital herpes,[1-3](#) and most transmission events occur in the absence of diagnosed infection in the source partner.[4,5](#) Increasing evidence suggests that genital herpes infections can facilitate acquisition and transmission of HIV and can adversely affect the clinical course of HIV infection.[6-9](#)

Potential strategies to control the spread of genital herpes include behavioral changes and antiviral drugs to suppress viral shedding.[10-13](#) Both strategies depend on accurate identification of infected persons; however, most patients present with mild or nonspecific symptoms that may be attributed to other sources.[14-17](#) Conversely, "typical" herpetic lesions actually may be caused by pathogens or noninfectious conditions.[17](#) Thus, even highly directed history and examination are inadequate to diagnose most genital herpes infections.[18](#)

In addition to diagnosing infections in patients with subclinical or unusual presentations, detection of antibodies allows diagnosis of established HSV-2 infections when virologic methods are impractical or yield negative results.[16](#) Available serologic tests can detect HSV antibodies but most cannot differentiate between herpes HSV type 1 (HSV-1) and HSV-2.[19](#) To be definitive, tests for HSV-2 antibodies must be based on HSV-2 type-specific antigens, such as those contained on glycoprotein gG-2. Studies indicate that patients attending sexually transmitted disease (STD) clinics in the United Kingdom desire HSV type-specific testing.[20](#) However, availability, cost, and turnaround time remain legitimate obstacles for such testing.[21](#)

A number of type-specific HSV tests are currently under development.[22-26](#) POckit HSV-2 (Diagnology, Belfast, Northern Ireland), uses gG-2 and membrane-based technology to detect antibodies to HSV-2 in capillary blood or serum in 6 minutes. The test compared favorably with Western blot analysis (WB) in a premarket evaluation,[27](#) and is comparable to WB for the speed of detection of seroconversion to HSV-2.[25](#) The rapid, point-of-care format makes this test particularly appealing for testing patients in STD-clinic settings. This study was designed to assess the sensitivity and specificity of POckit HSV-2 in subjects with culture-documented HSV-1 or HSV-2 infection in whom serological status for HSV was confirmed by WB.

Methods [TOP](#)

Sera [TOP](#)

Sera were banked at -20°C, with informed consent, from 303 subjects enrolled in studies of the natural history of genital herpes at the University of Washington Virology Research Clinic. All subjects were infected with culture-documented HSV-1 or HSV-2. WB was

performed on each sample as previously described.²⁸ WB on these sera were all concordant with the culture subtype.

Culture [TOP](#)

Viral cultures were obtained from lesions, if present, and from throat, oral, rectal, and urethral mucosa; vulva and cervix (women); or penile skin (men). Samples were sent to the University of Washington Virology Laboratory and inoculated in duplicate 24-well plates containing monolayers of human diploid fibroblasts. Plates were inspected every other day for 14 days for cytopathic effect. HSV isolates were confirmed and subtyped with monoclonal antibodies.^{29,30} Fifty subjects had cultures that were positive for HSV-1, and 253 subjects had anogenital cultures that were positive for HSV-2. By WB, 153 of the 253 HSV-2-positive subjects (60%) had only HSV-2 antibody, whereas 100 HSV-2-positive subjects (40%) had both HSV-1 and HSV-2 antibodies.

Serologic Testing [TOP](#)

POCkit HSV-2 Rapid Test is composed of a membrane solid phase, containing a spot with semi-purified HSV glycoprotein gG (gG-2). Human immunoglobulin is bound to the membrane as a separate control spot. Each serum was thawed, diluted into buffer included in the test kit, and passed through the membrane. Developing reagent was then added to the membrane to react with bound human antibody. The presence of two red spots in the test window was scored as a positive result. A faint-pink test spot with a red control spot was scored as negative result. A single spot on the control portion on the left of the test window was scored as a negative result.

Sera that had POCkit HSV-2 results that were discordant with culture subtype were repeated by WB and by a gG-1 and gG-2 type-specific enzyme-linked immunoabsorbent assay (Gull gG ELISA; Gull Laboratories, Salt Lake City, UT; Meridian Diagnostics, Cincinnati, OH). The profiles of reactive antibody on the repeat HSV-2 WB were reviewed by one author (RLA). "Limited" profiles contained low-intensity distinct bands corresponding to four or fewer HSV-2 proteins (gB, gG, gD, VP5). Full profiles contained bands corresponding to these proteins plus gG, gE, VP16, and ICP35. POCkit HSV-2 and Gull gG ELISA test results were obtained and entered into a computer database without knowledge of the culture or WB results of the subjects.

Results [TOP](#)

Performance of POCkit HSV-2 [TOP](#)

Overall, POCkit HSV-2 results were concordant with culture in 294 of 303 cases (97%). When sera from 50 subjects with culture-documented oral HSV-1 infection (n = 9) or anogenital HSV-1 (n = 41) were tested, 49 sera (98%) were negative ([Table 1](#)). POCkit HSV-2 results were positive for 245 of 253 sera (97%) from HSV-2 culture-positive patients ([Table 1](#)). Against culture as a gold standard, sensitivity of the POCkit HSV-2 test was 96% and specificity was 98%. Sensitivity was 97% in subjects with only HSV-2 antibodies by WB and 96% in subjects with dual antibodies to HSV-1 and HSV-2.

Culture Site	HSV Subtype	N	POCkit HSV-2 Result	
			-	+
Oral	HSV-1	9	8	0
Anogenital	HSV-1	41	40	1
Anogenital	HSV-2	253	8	245

HSV = herpes simplex virus; HSV-2 = herpes simplex virus type 2;
HSV-1 = herpes simplex virus type 1.

TABLE 1. Performance of POCkit HSV-2 in Patients With Culture-Documented HSV-1 or HSV-2 Infections

Discordant Results [TOP](#)

One of 50 samples (2%) from a woman with vulvar HSV-1 infection was positive by POCkit HSV-2. This specimen was confirmed as HSV-1 with no detectable HSV-2 antibodies by repeat WB and by Gull gG-1 and gG-2 ELISA. Sera drawn 2 months and 5 months later were also negative for HSV-2 antibody by WB and ELISA tests ([Table 2](#)). It is unlikely that this patient was undergoing HSV-2 seroconversion at the time of POCkit HSV-2 testing; rather, the test most likely gave a false positive result.

Subject	Culture Site	HSV Subtype	POCkit HSV-2 Result	HSV-2 gG-ELISA Result	HSV-2 WB Profile
1	Genital	HSV-1	+	-	HSV-1
2	Rectal	HSV-2	-	-	Limited
3	Genital	HSV-2	-	+	Limited
4	Genital	HSV-2	-	-	HSV-2
5	Urethra	HSV-2	-	-	Limited
6	Genital	HSV-2	-	+	Limited
7	Genital	HSV-2	-	+	HSV-2
8	rectal	HSV-2	-	equivocal	Limited
9	Genital	HSV-2	-	ND	Limited

HSV = herpes simplex virus; HSV-2 = herpes simplex virus type 2;
HSV-1 = herpes simplex virus type 1; gG = glycoprotein G;
ELISA = enzyme-linked immunosorbent assay; WB = Western blot analysis.

TABLE 2. Cases With Discordant POCkit HSV-2 Results Discordant With Culture Subtype

Eight sera obtained from patients with HSV-2 isolates was negative by POCkit HSV-2. All eight sera were available for repeat testing by WB, and seven were available for testing by Gull gG-ELISA ([Table 2](#)). Six of the eight sera had limited WB profiles with four or fewer bands, as is seen with early seroconversion, whereas two had more complete antibody profiles with more than six bands. Four sera were negative (n = 3) or had equivocal reactivity (n = 1) by gG-2 ELISA. Thus, seven of eight of the falsely negative sera by POCkit HSV-2 had evidence of low titer antibodies by either gG-2 ELISA, WB profile, or both tests.

Discussion [TOP](#)

These results confirm early reports of accuracy of detection of HSV-2 antibodies by POCkit HSV-2.^{25,27} Sensitivity was 96% and

specificity was 98% in a population that was well defined clinically and preselected for WB serostatus. Sensitivity for HSV-2 was not affected by the presence of HSV-1 antibodies. Gull gG-2 ELISA tests were performed on samples with falsely positive or falsely negative POCkit HSV-2 results, but did not necessarily give concordant results with either WB or POCkit HSV-2 ([Table 2](#)). The Gull test has more than 95% sensitivity and specificity for HSV-2 antibody [23,24](#); thus, test outcome of a single sample may differ because of the nature and presentation of antigen or because of different cut-off criteria for positive versus negative determinations. This phenomena in gG-2 based tests has been described in larger populations and in sequential sera from the same patient. [25,26,28,31](#)

Significant demand for HSV type-specific antibody testing is likely to occur, especially in high-prevalence settings once these tests become commercially available. The high sensitivity and specificity of POCkit HSV-2 against the gold standards of culture and WB suggest that it will be useful to identify asymptomatic carriers of genital HSV-2. The test also provides a rapid in-clinic means of diagnosing patients with mild or atypical symptoms. False-negative results occur mainly in sera with low titers of HSV-2 antibodies and may be associated with seroconversion. [25](#) Clinical trials of POCkit HSV-2 have recently been completed in general practice and STD clinic settings, and the test has been approved by the Food and Drug Administration. With market availability of POCkit HSV-2 and other type-specific tests, clinicians are advised to consider the impact of such testing for their patient population and for the individual patient, [1,10,32](#) and to counsel patients about the implications of positive or negative test results before testing. [33](#)

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