ABVIC: an improved serological test to resolve indeterminate diagnoses for herpes simplex virus 2 (HSV-2)

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Abstract
Current HSV-2 serological tests are imperfect; 5-6% of HSV 2 ELISA IgG tests yield low (+) results that are often false (+) relative to the HSV Western Blot. Even when low (+) samples are run by Western, ~13% of patients obtain indeterminate results. We report a new, flow-cytometry-based method that measures serum antibody-binding to virus-infected cells (ABVIC). Unlike the glycoprotein G (gG)-based HerpeSelect assay, ABVIC tests for antibodies to ~30 HSV-2 antigens. We report that when serum was collected from n=17 patients who had received "HSV-2 indeterminate" results by HSV Western Blot, the ABVIC test offered a clear answer in every case; 16 of 17 were HSV-2 (-) and 1 of 17 was weak HSV-2 (+). The results indicate that the quantitatively superior ABVIC test may be used to clarify ambiguous results returned by the qualitative HerpeSelect and Western blot methods.

New test investigated in this study: ABVIC

Potential Strengths:
- Most epitopes present in fixed, HSV-1 or HSV-2-infected cells
- Ab binding to virus-infected cells quantifiable over 100-fold range.
- Internally controlled; UI (uninfected) cells define background
- Can be run in 96-well format

Potential Weaknesses:
- Is a type-specific test possible?
- Untested in a clinical setting

Introduction to HSV antibody tests

Desired Goal
Rapid blood test that may be used to advise patients if they are infected with herpes simplex virus type 1 (HSV-1) and/or herpes simplex virus type 2 (HSV-2).

Main Constraint
HSV-1 and -2 encode 75 homologous proteins. HSV-1 elicits antibodies against 100s of epitopes; some are "HSV-1-specific" and some are "type-common." Likewise, the antibody response to HSV-2 includes both type-specific and type-common antibodies.

Current 1° Test

HerpeSelect (Focus Diagnostics) is an antibody-capture ELISA that uses glycoprotein G (gG-1 vs gG-2) to detect HSV-1 or HSV-2-specific antibodies.
Strength: gG-1 and gG-2 differ greatly; gG-1 (238 a.a.) and gG-2 (729 a.a.) share only a 160-a.a. region of 55% amino-acid homology.
Weakness: >99% of HSV-1 or -2-specific antibodies may target viral proteins other than gG-1 or gG-2.

HSV Western Blot (Univ of Washington)
Strength: most HSV-1 and HSV-2 proteins present on blot and available to react with HSV-1 or -2-specific antibodies, although only linear epitopes are available.
Weakness: interpretation of qualitative results is subjective and emergence of background bands confounds test, yielding "indeterminate" results.

Current 2° Test

- The adjacent Western stained with known HSV-2+ serum from Focus Diagnostics highlights two critical points:
  i. HSV-2+ sera may cross-react with HSV-1 proteins in Westerns
  ii. gG-2 protein (91 kDa) is not a dominant HSV-2 antigen

Central questions:
Is it possible to develop a better HSV serological test that is:
- type-specific?
- more sensitive?
- able to resolve equivocal HSV Western blot results?

1. Pre-adsorption makes ABVIC type-specific
- Serum pre-adsorbed to HSV-2+ cells depleted of HSV-2-spec. & type-common Abs \(\rightarrow HSV-1\-specific\ Abs\ enriched\) (Fig. 1A,B)
- Serum pre-adsorbed to HSV-1+ cells depleted of HSV-1-spec. & type-common Abs \(\rightarrow HSV-2\-specific\ Abs\ enriched\) (Fig. 1A,C)
- After adsorption, 3-population ABVIC (UI, HSV-1+, HSV-2+ cells) used to quantify HSV-1/2 specific Abs. Test utilizes all HSV epitopes (linear & conformational) in fixed and permeabilized cells.

2. ABVIC is \(>100x\) more sensitive than HerpeSelect
- A known HSV-1+2+ sample (10++) was tested to determine the lowest dilution that yielded a positive Ag-Ab reaction in each HSV Ab assay.
- In HerpeSelect, 10++ serum had a titer of HSV-1 and HSV-2 Abs of 320 and 1,000, respectively (Fig. 2A)
- In HSV Western blots, 10++ serum had a titer of HSV-1 and HSV-2 Abs of \(~200,000\) for each (data not shown)
- In ABVIC, 10++ serum had a titer of total HSV Abs of 1,000,000.
- Corresponding IF microscopy images of HSV-2 plaques stained with 1:10,000 or 1:100,000 dilutions of 10++ serum are shown (Fig. 2B)
- In such comparisons, the ABVIC test is \(>100x\) more sensitive than the gG-1- or gG-2-based HerpeSelect ELISA test (Fig. 2A vs 2B)

3. ABVIC resolves equivocal Western results
- Westover Heights Clinic (Portland, OR) has a long history of counseling patients who receive low (+) results by HSV-2 HerpeSelect ELISA followed by HSV-2 indeterminate Western blot results.
- "HSV-2 Indeterminate" serum typically poses 1 of 2 problems for the Western blot test (Fig 3A):
  \(\rightarrow HSV-1^+\) serum cross-reacts with HSV-2 proteins (left, Fig. 3A), or
  \(\rightarrow\) negative serum produces background bands (right, Fig. 3A)
- Type-specific ABVIC was performed on control sera from individuals whose past Western blot results offered a definitive conclusion:
  - seronegative (n=6; black circles in Fig. 3B)
  - HSV-1+ (n=6; blue circles in Fig. 3B)
  - HSV-2+ (n=6; red circles in Fig. 3B)
  - HSV-1+2+ (n=6; purple circles in Fig. 3B)
- Type-specific ABVIC performed on sera from individuals whose past Western blot results had yielded "Indeterminate" results:
  - HSV-2 Indeterminate (n=17; open triangles in Fig. 3C)
- By ABVIC, 16 of 17 "Indeterminates" were definitively HSV-2 (-) and only 1 of 17 was weakly HSV-2 (+)
- The results indicate that the quantitatively superior ABVIC test resolves most equivocal results produced by HerpeSelect or HSV Western blot.

Fig 1. Type-Specific ABVIC. (A) Pre-adsorption scheme yields enriched populations of HSV-1- or HSV-2 specific Ab. (B,C) ABVIC of (B) HSV-1+ or (C) HSV-2+ serum after adsorption to HSV-2- or -1 infected cells.

Fig 2. Relative sensitivity of HerpeSelect vs ABVIC

Fig 3. ABVIC analysis of "Indeterminate" serum