INVITED ARTICLE

THE RATIONALE FOR THE USE OF DIAGNOSTIC TESTS IN HERPES SIMPLEX VIRUS INFECTION

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ABSTRACT

All suspect herpes simplex lesions should be diagnosed by isolation methods where possible. Rapid tests utilise antigen detection methods. The enzyme immunoassay method is the most sensitive of these, and the immunofluorescence method has the advantage of being able to type the virus, but has slightly lower sensitivity. Serological methods are only useful to exclude a herpes simplex infection in the past, and to establish a primary infection. They cannot be used to type an infection or give evidence of past infection to a particular type.

Keywords: Herpes simplex virus infection, Diagnosis

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Many new tests and techniques have been developed in the diagnosis of herpes simplex virus infection. An attempt will be made, in this article, to help the clinician make a rational choice from the range of diagnostic tools available to him. Limitations of the various tests and pitfalls in interpretation of the various available tests will also be discussed.

ISOLATION AND ANTIGEN DETECTION METHODS

The principle of diagnosis of herpes simplex virus infection is simple. It is to use isolation techniques wherever possible, and in all suspect lesions. Suspect lesions include atypical lesions thought to be due to trauma, chancroid and linear fissures, all of which have, on occasion, had herpes simplex isolated from them. Sensitive cell lines are available and the virus is usually isolated in a few days. A positive culture from the lesion is the 'gold standard' for herpes simplex diagnosis and is the method against which all other methods are measured. It enables confirmation of diagnosis and typing of the virus and picks up low-titred specimens. It is the only method which enables diagnosis in asymptomatic excretion. Culture confirmation and typing are important for management of the patient, prognosis and institution of long-term suppression of recurrences with acyclovir.

Isolation results can be obtained within one week. The use of cover-slip cultures with spin amplification frequently enables results to be obtained after 1 day[1]. Even in conventional tube cultures, 85% and 95% of positives can be reported on the 2nd and 3rd day, respectively[2]. The amount of virus excreted is greater and the duration longer in primary infections and in immunosuppressed patients. Specimens must be collected in virus transport medium, and for best results, taken early in the infection (within 72 hours of onset), and transported on ice as soon as possible to the virus laboratory. Should transport not be available, specimens should be kept at 4-8°C until it can be sent. Specimens should never be frozen at -20°C as herpes simplex virus becomes rapidly inactivated at this temperature. Adequate numbers of antigen bearing cells from the base of the ulcer or vesicle should be present in the sample.

Cultures should always be taken:

(1) on the first visit to the clinic in a case of suspected herpes,

(2) during delivery in a patient with a history of genital herpes, from the area of recurrence and from the cervix,

(3) repeatedly from a neonate with suspected herpes from the oropharynx, eyes and suspect lesions during the first 4 weeks and especially during the first 2 weeks. If there is a possibility of central nervous system involvement or disseminated herpes, urine, cerebrospinal fluid and buffy coat specimens should also be sent.

Where culture facilities are unavailable, antigen detection methods are the next method of choice. Table I summarizes their usefulness, specificity, sensitivity, typing ability and speed with which results can be obtained. These tests constitute the 'rapid' methods of diagnosis for herpes simplex. If they are used for speed, it is recommended that they be used, wherever possible, with isolation to gain the advantages of both speed and sensitivity. As shown, the test which correlates best with culture is the enzyme immunoassay (EIA) for antigen detection. This is sensitive, specific, picks up even dead antigen which will not grow on cultures but may miss out specimens with very little virus. The disadvantages are that it requires a number of controls, which makes isolated testing expensive, while batching of specimens would

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Table I
Comparison of rapid methods of herpes simplex diagnosis with isolation of the virus

Method	Sensitivity	Specificity	Typing	Time taken
ISOLATION				
1) Tube culture	'Gold' standard	'Gold' standard	Yes	1-7 days
2) Coverslip with spin amplication	95%	100%	Yes	16-24 hours
ANTIGEN DETECTION				
EIA	87-100%	100%	No	4 hours
IF	80-95%	100%	Yes	40 mins
HISTOCHEMICAL STAIN				
Tzanck test	50%	Relatively non specific	No	30 mins

delay testing and nullify the 'rapidity' of the method. Most test kits do not type specimens either, which is a further disadvantage.

The immunofluorescence test (IF) is more rapid and has the advantage of being able to type specimens, but the sensitivity of the test depends on the number of antigen bearing intact cells in the sample. A carefully taken specimen ie. one containing many basal cells from the ulcer or vesicle, would make the sensitivity of this method approach that of culture. However, an immunofluorescence microscope and trained microscopist must be available. As with other 'rapid' tests, virus culture should be done simultaneously.

The Tzanck test is the traditional histocytochemical method of staining with Giemsa for eosinophilic intranuclear inclusions in basal cells. It has the advantage of being cheap and quick (1/2 hour) and only requires the use of an ordinary light microscope. However, the test is relatively non-specific as both varicella zoster and herpes simplex infections cause identical changes. Sensitivity is only 50% that of culture[3] and typing is not possible. It is the least satisfactory of the rapid tests.

SEROLOGICAL TESTS

There is a 50% homology in the genomes of the two herpes simplex types so that they share many common antigens. As a result of this, none of the current routinely used serological tests, eg. the complement fixation test and the EIA can distinguish between type 1 and type 2 antibodies with any degree of certainty. A person who has had a type 1 herpes simplex infection in childhood will develop antibodies to type 1 herpes which will also cross-react with herpes simplex type 2. A significant rise in titre is seen in true primary infection (no past history of herpes simplex virus infection) and first episode genital herpes infection, (first time genital infection after a previous infection at an extra-genital site). However, only 5% of recurrences give rise to a concomitant rise in antibody titre - indicating that antibodies do not confer immunity but only confirm past infection with one or other virus type or both.

A laboratory report such as *Patient has herpes Type II IgG antibody of high titre,* encourages the following incorrect assumptions:-

1) That the EIA test can accurately distinguish between type 1 and type 2 antibodies. This is incorrect; the predictive value of EIA for typing is only 30%[2].
2) That knowledge of the type of antibody is of diagnostic value, ie. that genital herpes is only caused by the type 2 virus. In fact, local figures indicate that 40% of primary genital herpes and 33% of first-episode genital herpes are type 1 infections[4].
3) That a single 'high' titre is significant. Serological testing is of significance only when acute and convalescent specimens are available for simultaneous testing and show a rise of titre.

The only information gained from a report such as the one quoted is that the patient has had a past infection with one or other of the herpes simplex viruses.

In herpes simplex virus infection, IgM estimations are only useful in neonatal infection, as IgM antibodies reappear with most reinfections. Again, type specific IgM antibodies cannot be reliably detected with the currently available serological tests.

The latex agglutination test is relatively insensitive and is not satisfactory for the screening of asymptomatic patients.

New serological tests such as the Western Blot are being developed and evaluated for their ability to distinguish between type 1 and type 2 antibodies. They appear to be promising but are not commercially available at present.

Serological tests therefore are only useful for the exclusion of a herpes simplex infection in the past, and for establishing a primary infection. They should not at present be used to type an infection or give evidence of past *genital* infection.

In summary, all herpes infections should be documented by viral culture and/or antigen detection and typed. Serological tests can confirm a primary infection and exclude past infection by a herpes simplex virus. The current tests cannot distinguish between the two types.

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