Comparison of cell culture with an amplified enzyme immunoassay for diagnosing genital herpes simplex infection

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Abstract

An amplified enzyme immunoassay (EIA) for herpes simplex virus (Novo Nordisk) was compared with cell culture in 853 genital specimens from a genitourinary medicine clinic. The sensitivity of the EIA was 86% and its specificity 99.6%. The sensitivity increased to 94% for lesion swabs but decreased to 68% for cervical swabs. Sensitivity for urethral and vulval swabs was 83% and 82%, respectively. It is concluded that the EIA is specific and quick and easy to perform. It will be suitable for testing for genital herpes simplex infections in laboratories without access to local cell culture facilities.

Herpes simplex virus (HSV) infections are common. It is estimated that in the United Kingdom there are 12 000 cases of genital HSV infection every year.¹ Neonatal herpes simplex is a serious complication of maternal, genital HSV infection. Some people have suggested that routine viral cultures from high risk women may help to prevent neonatal HSV infection, caesarian section being advocated in women found to be shedding virus at term.^{1,2} Others have associated neonatal herpes simplex with only primary maternal genital HSV infection.³ Effective antiviral treatment is now available for genital HSV infection,⁴ however, and trials are in progress to assess the efficacy of prophylactic acyclovir in high risk women in the last four weeks of pregnancy to reduce the risk of neonatal herpes simplex. Accurate and rapid laboratory diagnosis of HSV infection is therefore very important. The traditional method of cell culture may take up to 10 days for diagnosis; we therefore assessed a rapid amplified enzyme immunoassay (EIA) (Novo Nordisk) for the diagnosis of genital HSV infection and compared it with cell culture.

Methods

A total of 853 genital specimens were submitted from the genitourinary medicine clinic at Sheffield between January and June 1990. Of these, 312 were cervical swabs, 211 vulval, 75 urethral, 159 skin (lesion) and the rest were

from other sites. These swabs were taken as part of routine investigation of all patients at risk of HSV infection and included both symptomatic and asymptomatic patients. Vulval swabs in the asymptomatic women were taken from the inner mucus membrane, and in the symptomatic women the vulval lesions were swabbed.

Viral culture and EIA were performed on the same swab. Swabs were taken with cotton-tipped plastic swabs which were broken in 2 ml of virus transport medium. Specimens were transported to the laboratory daily where a portion was separated for EIA.

Viral culture Vero cells were inoculated with 0.2 ml of the specimen. The tubes were rolled at 37°C and examined daily for cytopathic effects. If such effects were observed the isolates were confirmed and identified using type specific fluorescein isothiocyanate conjugated monoclonal antibody for HSV1 and HSV2 (Novo Nordisk).

EIA The IDEIA (Novo Nordisk) HSV test relies on HSV specific monoclonal antibodies and an enzyme amplification system. HSV antigen (common to types 1 and 2) from a clinical specimen was bound by monoclonal antibody adsorbed to the surface of the plastic well. After adding the enzyme conjugated monoclonal antibody and amplifying agent the final reaction was measured at 492 nm in a spectrophotometer. Appropriate positive and negative controls were included. The cut off was calculated by adding 0.15 to the negative control mean. Results with an absorbance value greater than the cut off were considered to be positive. Results between 0.1 below the cut off and cut off were regarded as equivocal according to the manufacturer's instructions.

The EIA was performed two to three times a week; meanwhile, the aliquots were stored at 4°C for not longer than three days. The EIA was performed according to the manufacturer's instructions after "spiking". The spiking reagent consists of HSV extract buffer which has to be added to the specimen before testing. This step is not required if specimens are collected in the EIA medium provided by the manufacturer. The sensitivity of the EIA was further studied by testing 10-fold dilutions of an HSV1 laboratory strain (F strain)

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Table 1 Comparison of culture and EIA results

	EIA		Total
	Positive	Negative	
Culture positive	114	18	132
Culture negative	3	718	721
Total	117	736*	853
EIA sensitivity	$\frac{\text{EIA} + \text{culture} +}{\text{culture} +} = 86.4\%$		
EIA specificity	$\frac{\text{EIA} - \text{culture} +}{\text{culture} -} = 99.6\%$		
Positive predictive values	$\frac{\text{EIA} +, \text{culture} +}{\text{EIA} +} = 97.4\%$		
Negative predictive values	$\frac{\text{EIA} -, \text{culture} -}{\text{EIA} -} = 97.5\%$		

*Includes 19 equivocal EIA results, three of which were culture positive and 16 culture negative.

and one HSV2 (strain 333) by both EIA and culture. The reproducibility was determined by testing 10^{-1} , 10^{-2} , and 10^{-3} dilutions of HSV1 and HSV2 isolate on three different occasions.

Results

The culture results were taken as the true results. Of 853 specimens, 132 (15.5%) were culture positive; 36.4% were HSV1, and 63.6% were HSV2. Of these, 114 (86.4%) were EIA positive (table 1). An analysis of the HSV type showed that of 114 EIA positive and culture positive specimens, 41 (36%) were HSV1 and 73 (64%) were HSV2. In the 18 EIA negative and positive culture the distribution of HSV1 and HSV2 was similar, being, respectively, 7 (39%) and 11 (61%). These formed 14.6% and 13.1% of total HSV1 and HSV2 isolates; therefore there was no difference in the proportion of HSV1 and HSV2 isolates missed by the EIA. The sensitivity of the EIA varied with the type of specimen, being 68% with cervical swabs and 94% with lesion swabs (table 2). The specificity of the EIA was 99.6%. Of the three EIA positive and culture negative specimens, two were from patients who had herpes simplex isolated from swabs taken from another genital site, and these were probably true positives. The specificity of the EIA was therefore 99.9%.

A distribution of the EIA index obtained (specimen optimal density cut off) showed that although 94 (82%) of the 114 EIA specimens had an index of more than 2, the indices of the EIA positive specimens were spread over a wide range (table 3). There were 17 (15%) specimens with an index value below 1.5; of these, 12 had an OD of only 0.01 to 0.09 above the cut off. The two HSV laboratory strains were culture positive at 10^{-11} (HSV2 333 strain) and 10^{-5} (HSV1, F strain). The EIA was

Table 2 Comparison of culture and EIA according to the specimen site

Specimen type	Total	Culture + EIA +	Culture + EIA -	Sens EIA (%)
Cervical	312	15	7	68
Vulval	211	14	3	82
Urethral	75	15	3	83
Skin/lesion	159	47	3	94

Table 3 Distribution of HSV EIA index of culture positive and negative specimens

Index (%)	Culture positive (n =)	Culture negative (n =)
0.1-0.2	0	1
0.2-0.3	1	19
0.3-0.4	1	119
0.4-0.5	6	260
0.5-0.6	4	217
0.6-0.7	4	86
0.7-0.8	1	14
0.8-0.9	1	4
1.0-1.1	3	0
1.1-1.2	6	0
1.2-1.3	2	0
1.3-1.4	2	0
1.4-1.5	4	0
1.5-1.6	2	0
1.6-1.7	2	0
1.7-1.8	0	0
1.8-1.9	1	0
1.9-2.0	1	0
2.0-3.0	10	1
3.0-4.0	7	0
>4	72	2

positive at 10^{-9} and 10^{-3} fold dilutions, respectively. The coefficient of variation on three different dilutions of the HSV1 and HSV2 laboratory strains, done on three different days, was well within 10%.

Discussion

The EIA performed well with both HSV1 and HSV2, detecting 85% and 87% of cell culture positive HSV1 and HSV2, respectively. The variability in sensitivity with different types of genital specimens (68% with cervical swabs and 94% with lesion swabs) was possibly due to the difference in viral load. Most cervical specimens were taken from women without lesions on the cervix. The heavy viral load in lesion swabs would facilitate detection by EIA and this would also explain the sensitivity of EIA with clinical specimens, in spite of at least a 100-fold difference in sensitivity compared with culture of the experimental strains of HSV.

A correlation between the EIA absorbance value and virus yield on cell culture has been noted with HSV EIA.⁵ We also found a correlation between virus infectivity and EIA absorbance value when 10-fold dilutions of the standard HSV strains at known infectivity were tested (results not shown). Interestingly though, there was no correlation between the strength of the EIA signal and the day the first cytopathic effect appeared in the cell culture. The HSV EIA has good specificity and reproducibility. The "spiking" step, however, is essential if the manufacturer's medium is not used. There were 19 (2.2%) specimens which were repeatedly equivocal in the EIA. These, respectively, formed 2.3% of culture positive (three of 132) and 2.2% of culture negative (16 of 721). The positive predictive value and negative predictive value of the EIA were 97.4% and 97.9% if the equivocal results were discounted from calculations—97.9% and 97.5% if they were regarded as EIA negative, and 86% and 97.9% if they were regarded as EIA positive. We therefore suggest that equivocal results should be considered negative if repeat specimens are not available.

There were only three EIA positive and

culture negative specimens: these were a vulval, a cervical, and a lesion swab, respectively. All had an EIA index of more than 2. The last two are likely to be false negative culture results as HSV2 was isolated from a lesion swab from the patient with an EIA positive and culture negative cervical swab, and HSV1 was isolated from a cervical swab of the patient with the EIA positive and culture negative lesion swab. The EIA is extremely specific, but the fact that at least 12 out of 114 (10%) of positive specimens had an optimal density fairly close to cut off needs to be addressed. The overall sensitivity of 86% makes the replacement of cell culture unlikely, but the higher sensitivity with lesion swabs would make the EIA a suitable diagnostic test to confirm clinical HSV infections in peripheral laboratories without access to cell culture facilities locally. Furthermore, the EIA would

be useful in cases where a rapid diagnosis is required—that is, for management of pregnant women with suspected genital HSV infection near term, or HSV infection in immunocompromised patients.

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