

# Direct Immunofluorescence Test for the Diagnosis of Genital Herpesvirus Infections

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Herpetic lesions of the genitalia may be confused clinically with other ulcerative, genital lesions. Direct immunofluorescence (FA) provides a rapid method of diagnosis, and the utility of this method for the diagnosis of genital ulcers was examined. One hundred and ten patients with genital lesions were examined by darkfield for syphilis and by FA and culture for herpes simplex virus (HSV) infections. Satisfactory samples were obtained from 102 patients, of which 81 were clinically suspected cases of HSV. Acetone-fixed slides of scrapings of ulcerative lesions were stained with conjugated antiserum prepared in rabbits against HSV type 2. HSV was isolated from 73% of specimens of suspected herpetic lesions, and 77% of these specimens were positive by FA. Nine percent were positive by FA only and these were not thought to represent false positives. Five percent were positive by culture only. A comparison of clinical diagnoses with laboratory findings revealed that 4% of the cases were misdiagnosed when only the clinical evaluation was considered. The data suggest that the inclusion of a diagnostic FA test for HSV along with the darkfield examination may be useful for differentiating the etiological agents of ulcerative, genital lesions.

Immunofluorescence (FA) of clinical specimens has been compared with virus isolation as a means of rapid diagnosis of various manifestations of herpes simplex virus (HSV) infections (7). These studies were concerned with differentiating the etiology of oral, skin (1, 2), corneal (6), and genital (7) lesions. Herpetic lesions of the genitalia may be confused clinically with ulcerative lesions of other causes, especially those of primary syphilis. Since the darkfield (DF) examination is often repeated on 3 successive days on patients with darkfield-negative lesions, the inclusion of a rapid diagnostic test for HSV with the initial physical examination could reduce the number of visits to the physician as well as aid in proper treatment. The present study was undertaken to determine the utility of a diagnostic test for HSV, in a venereal disease clinic, and the applicability of direct FA on scrapings from lesions as a rapid test for diagnosis of genital lesions.

## MATERIALS AND METHODS

**Selection of patients.** Specimens were obtained consecutively, 1 day per week, from all patients with ulcerative, genital lesions seen in a venereal disease clinic at the Houston City Health Department. One hundred and ten patients with genital lesions of

various etiologies on whom a darkfield examination for syphilis was done were tested for HSV infection. A history of the lesion including duration and number of occurrences was obtained from each patient.

**Treatment of specimens.** Two samples were taken from each lesion after the DF examination was completed. One was obtained with a cotton swab and placed in Eagle medium containing 2% fetal bovine serum, penicillin (100 U), streptomycin (100 µg/ml), and sodium bicarbonate, (2.25 g/liter) and kept on ice up to 4 h before inoculation into primary rabbit kidney tissue culture. The second specimen was taken from the base of the lesion by light curettage. These scrapings were placed on each of two ringed areas on a nonfluorescing glass slide. After air-drying, the specimens were fixed in acetone for 15 min at -20 C and frozen at -70 C. The FA procedure was done within 1 month of freezing.

**Virus isolation.** Primary rabbit kidney tissue culture was used for virus isolation. Procedures described by Figueroa and Rawls (5) were used for both isolation and typing of the viruses.

**Preparation and titration of fluorescein-labeled antiserum.** Antiserum was prepared by using the Sav strain of HSV type 2. Antigens were prepared as 10% suspensions of infected rabbit kidney cells which were ruptured by sonication. A young adult New Zealand white rabbit was injected twice in the foot pads at 2-week intervals with 0.2 ml of antigen mixed with an equal amount of complete Freund adjuvant. The inoculations were followed at 2-week intervals by

three injections of 0.5 ml of antigen intramuscularly. The animal was exsanguinated 1 week after the last injection. The globulin fraction of the serum was labeled with isothiocyanate as described (3). The conjugate had a titer of 1:80 when titered on infected rabbit kidney and Vero cells. No fluorescence was observed in uninfected rabbit kidney and Vero cells at a dilution of 1:5, the lowest dilution tested. Fluorescence was not observed in cells infected with vaccinia virus or Pichinde virus. This conjugate did not differentiate between HSV type 1- and type 2-infected cells. Before use, the conjugate was adsorbed with mouse liver powder, diluted 1:10, and stored up to 30 days in the dark at  $-70^{\circ}\text{C}$ .

**FA procedure.** Frozen slides were allowed to air-dry before specimens were covered with one drop of the conjugate (about 0.03 ml). The slides were placed in a humidified incubator at  $37^{\circ}\text{C}$  for 45 min. The incubation was followed by three 2-min washes in tris(hydroxymethyl)aminomethane buffer, pH 7, and one wash in distilled water. Air-dried slides were then mounted with elvanol and examined independently by two of the authors. Each test included cover slips of infected and noninfected Vero cells, which served as controls. The FA slides were given a code number and specimens for virus isolation were identified by the patients' name. The slide numbers were not decoded until isolation attempts were completed. An AO fluorescent microscope with a BG12 exciter filter and an OG1 barrier filter was used to read the slides.

## RESULTS

The patient population consisted of 92 males and 18 females ranging from 16- to 49-years-old with a mean age of 22 years. There were approximately equal numbers of Negroes and Caucasians.

Eight of the slide specimens did not contain enough cells for a definitive reading and are not included in these results. Table 1 shows that of 81 clinically suspected cases of genital herpetic lesions, 59 (73%) were culture positive and 62 (77%) were FA positive. Although three isolates were lost, of the remaining 56 which we were able to type, 50 (90%) were type 2. All controls (DF-positive lesions and those due to other causes) were negative for virus by culture and FA.

The slides were read with 98% agreement between the two independent examiners. Of the 59 culture-positive specimens, 55 were FA-posi-

TABLE 2. Culture and FA methods for the diagnosis of HSV in specimens from patients with genital lesions

Culture/FA results	Suspected HSV	Controls
Positive/positive	55 (68%)	21
Positive/negative	4 (5%)	
Negative/positive	7 (9%)	
Negative/negative	15 (19%)	
Total	81 (100%)	

tive, indicating a 93% sensitivity (Table 2). However, of the eight unsatisfactory slides, five were culture positive, and, if these are included, the sensitivity is only 86%. Five percent of the specimens were culture positive and FA negative, and about 9% were FA positive and culture negative (Table 2).

To determine whether diagnoses based solely on the clinical presentation were missed, the initial clinical diagnosis was compared with the laboratory diagnosis (Table 3). At least four of the 102 lesions were misdiagnosed by clinical data alone. Of the 81 patients diagnosed as having herpetic lesions, at least 3 (4%) were shown to have primary syphilis, and of the 14 patients diagnosed as being syphilitic, one (7%) was shown to have HSV and not syphilis. This patient's positive VDRL was later interpreted as a biological false positive.

## DISCUSSION

Nahmias et al. (7) report that direct FA staining on clinical material may be used to diagnose and type HSV infections. In our study there was no attempt to type by FA, but we found a sensitivity of 93% for our diagnostic FA test as compared with the previously reported 80% sensitivity for diagnosis (7). This discrepancy may be due to differences in the preparation of the conjugates or in the staining technique.

The main problem with the FA for HSV, as with any diagnostic laboratory procedure based on cytological changes, was in obtaining smears with an adequate number of cells. The type of experience of the physician seemed related to the adequacy of the specimen. For example, dermatologists consistently took specimens with large numbers of cells, whereas most of the inadequate specimens were taken by physicians whose primary interest was not dermatology. Nahmias et al. (7) suggest that the best specimens are those from vesicular lesions before ulceration, but in our clinic, patients are rarely seen with vesicular lesions. We found the dermatologists could get adequate smears from

TABLE 1. Clinically suspect genital herpetic infections confirmed by culture or FA

Infection	No. of patients	Percent
Suspect	81	
Culture positive	59	73
FA positive	62	77
Total positive	66	82

most lesions. Adequate slides for examination may be more dependent on the expertise with which the specimen is taken rather than the stage of the lesion.

It is important that 4% of the cases studied were misdiagnosed when based solely on clinical evaluation because of the possibility of subsequent improper treatment. The three lesions that were actually DF-negative syphilitic chancres were detected when standard tests for syphilis were positive (VDRL and FTA-ABS) and viral tests were negative. Another lesion was clinically described as a DF-negative chancre. This patient had a VDRL of 1:16 and was treated for primary syphilis. Careful study revealed that the patient was a drug user, had a negative FTA-ABS, and his lesion was both FA- and culture-positive for HSV. Even this small percentage of problem diagnoses suggests the usefulness of a diagnostic test for HSV in the evaluation of genital lesions.

Genital HSV infections may be more prevalent than syphilis among infectious genital lesions (8). Although infections with both agents could occur in the same patient, this does not appear to happen often, and the FA test for HSV may be used to differentiate the etiological agent responsible for the ulcerative lesions of most patients. Since many patients often do not return to the clinic after an initial visit, a rapid, accurate test for HSV would be useful. In addition, patients with a DF-negative, HSV-positive lesion need not be repeatedly examined by darkfield, although serological studies in the convalescent period would be needed to absolutely exclude syphilis. The FA test may also be useful for dermatological clinics since as many as 2% of all attendances at these clinics may be due to HSV infections (4). Black et al. (2) point out that primary herpetic infections may be difficult to recognize, and their recognition is becoming more critical as anti-viral treatment becomes available.

Nahmias et al. (7) noted that in mice, the FA

may have become positive before virus could be successfully isolated. Since virus was eventually isolated from these mice, they felt that the FA-positive, culture-negative results were not false positives. Although the number of FA-positive, culture-negative specimens in our study was too small to make such a conclusion, there did appear to be a similar trend since all of these specimens which were FA-positive, culture-negative were taken from lesions of less than 4 days' duration (data not shown). If the FA is positive before virus can be isolated, the use of FA would be more sensitive and would enable an earlier diagnosis than do standard cultural methods.

The recognition of HSV as a venereal disease, its role in newborn disease, and its association with cervical cancer makes improved and rapid laboratory tests a necessity. The good correlation between the FA and culture methods and a 4% incidence of misdiagnoses indicate that the inclusion of this test along with the DF examination may be useful for differentiating the etiological agent of ulcerative, genital lesions.

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TABLE 3. Comparison of clinical and laboratory diagnosis of genital lesions

Clinical Diagnosis/ laboratory diagnosis	No.	Laboratory Results				
		DF	VDRL	FTA-ABS	FA	Culture
HSV/HSV	66	-	-	-	+	+
HSV/syphilis	3	-	+	+	-	-
HSV/unknown	12	-	-	-	-	-
Syphilis/syphilis	10	+	+	+	-	-
Syphilis/HSV	1	-	+	-	+	+
Syphilis/unknown	3	-	-	-	-	-
Others	7	-	-	-	-	-
Total	102					