Laboratory Aspects of Venereal Disease*

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Introducing the laboratory aspects of venereal diseases, I would like to define the criteria according to which certain infectious diseases are considered to be "sexually transmitted." The expression "sexually transmitted disorder" is less weighted with moral overtones than the term "venereal" and may therefore be more acceptable to both medical practitioners and the general public.

Sexually transmitted diseases are best considered as a group of lower genitourinary inflammations related to sexuality, rather than in the narrower sense of two or three disorders associated mainly with sexual promiscuity. Not all lower genitourinary tract infections are characterized by a discharge, but those which are may be due to a variety of causes. When these infections are caused by a microorganism which exists primarily in the genital epithelium, that infection is rarely transmitted except by sexual intercourse. Intercourse must, therefore, have taken place with an individual already infected who had contracted the disease in the same way. On the other hand, when the responsible microorganism might possibly exist in other sites, although usually sexually transmitted, coitus with an infected individual is not essential. Both conditions, however, are strictly "venereal."

Although many different microorganisms or parasites may be sexually transmitted, only those commonly transferred in this manner can be said to be venereal. A list of microbial agents associated with venereal disease is shown in Table 1. Only the diseases caused by those agents which appear in italics are reportable in this country, according to the U. S. Public Health Service. There is, however, a difference from country to country as to which venereal diseases are notifiable. A few of these diseases are endemic in almost every part of the world. Some of them, notably syphilis, have become sporadic in certain countries.

The disruption of the natural clinical and immunological responses of the infected individuals by the liberal use of antibiotics places a greater responsibility on the interpretation of laboratory results and on the judicious performance of serological tests in the absence of clinical signs. As far as we know, no treponemal resistance has yet arisen to the antibiotics usually recommended. Nevertheless, higher doses of antibiotics are considered more essential today than two decades ago.

Ulcerative and vesiculopapular lesions of the genitalia are commonly secondary to infective causes within the lower genitourinary tract, or are due to such lesions as herpes simplex, thrush or scabies. Chancroid and the conditions discussed along with it are becoming less common; their importance lies in the necessity to exclude syphilis. The cause of urethritis, vaginitis or cervicitis cannot always be determined. Consideration must be given to such factors as trauma, irritation, allergy and primary or secondary infection. It is often possible to make an etiological diagnosis, or at least an assessment of the probable cause, after both partners have been seen and examined. The incidence of these conditions shows no sign of diminishing. The inflammation resulting from sexually transmitted infection varies greatly in severity and extent. The potentially most damaging disease is syphilis, which unless treated, is frequently a life-long disease and may also infect the fetus *in utero*. Other infections presenting as sores which include chancroid, granuloma inguinale and lymphogranuloma venereum result as a rule in few complications. Any infectious discharge from the genital tract may proceed to a variety of complica-

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TABLE 1

MICROBIAL AGENTS OF VENEREAL DISEASE

Protozoon:	*Trichomona vaginalis*
Yeast-like:	*Candida albicans*
Mycoplasma:	*M. hominis*, "*T*" *strain*
Bacteria:	*Treponema pallidum*
	Neisseria gonorrhoeae
	Haemophilus ducreyi
	Donovania granulomatis
Bedsonia:	*Lymphogranuloma venereum*
	Trachoma-inclusion conjuctivitis
Virus:	*Herpes hominis*, type 2

tions, local or general, mild or severe. About 5% of males with gonorrhea seen in the venereal disease clinics are asymptomatic. Eighty percent of females with gonorrhea, however, are asymptomatic and remain in the population as an infectious reservoir. Gonorrhea in the male is predominantly symptomatic, motivating most patients to seek medical attention (5). The principal disorders which occur are inflammations of the pelvic organs and tissues and their sequelae, or rheumatism and arthritis.

If asked about the type of microorganisms associated with venereal disease, probably many would refer immediately to syphilis and gonorrhea and state that chancroid and lymphogranuloma venereum are not commonly found in medical practice. As a matter of fact, even recent textbooks on the subject of microorganisms associated with venereal disease refer to agents such as *Chlamydiae* (trachoma-inclusion conjunctivitis (TRIC) agents and *lymphogranuloma venereum,* mycoplasma, *Candida albicans* and *Trichomonas vaginalis,* but fail to add herpesvirus to the list. I wish to emphasize, however, that TRIC agents and *lymphogranuloma venereum* are not viruses and are infrequent etiological agents of venereal disease. On the other hand, during the last several years, herpes simplex (also called *herpes hominis*), type 2, has been shown here and elsewhere to be sexually transmitted and of high incidence in the general population. More importantly, it has been incriminated in uterine cervical carcinoma in connection with coitus, showing an increased frequency where there is an earlier onset of sexual intercourse, a large number of sex partners and when the individual remarries in later life.

Because of the limited amount of time available for a comprehensive discussion on the laboratory aspects of all venereal diseases, I will comment only briefly on those infections of minor clinical importance or on those which occur only rarely in this country. The sequence in which these will be presented, however, does not necessarily correlate with their frequency of occurrence. *Trichomonas vaginalis* is a common cosmopolitan parasite of both males and females. Infection rates vary greatly but may be high in some areas especially where female hygiene is poor. Coitus is the common mode of transmission, but contaminated towels, douche equipment, examination instruments and other objects may be responsible for some new infections. Infants may be infected at birth. Most infections in both sexes are asymptomatic or cause minor symptoms. Control of this parasite requires detection and treatment of the infected male sexual partner at the same time that the infected female is treated. Laboratory diagnosis consists of microscopic examination of vaginal or urethral secretions or discharge for characteristic motile trichomonads. Dried smears may be stained with hematoxylin or one of the Romanowsky stains for later study. Culture of vaginal or urethral discharge, of prostatic secretion or of a semen specimen may be indicated when direct examination is negative.

Candida albicans is probably one of the most common causes of vulval and vaginal symptoms, flourishing particularly in pregnancy, following antibiotic therapy and when glycosuria is present. A low-grade urethritis and balanoposthitis in men not infrequently accompanies a genital candida infection in their consorts. Fresh wet preparations of the vaginal fluid mixed with 10% potassium hydroxide or Gram-stained vaginal smears may lead to the detection of yeasts or blastospores or, less commonly, filaments or hyphae can be seen.

Mycoplasma hominis and T strains are yet to be conclusively demonstrated as the primary etiological agents of genitourinary infections. Some mycoplasmas are inhabitants of the normal genitourinary tract, especially in females. Moderately high serum antibody titers to *Mycoplasma hominis* and T strains (tiny colonies, requiring 10% urea for growth), however, can accompany their presence and they have been cultured occasionally from focal genital abscesses. Special enriched media are required for growth. These organisms are inhibited by tetracycline but not by penicillin in clinical dosages. This is consistent with the clinical response to therapy of a significant proportion of patients with a nonbacterial inflammation.

Chancroid, granuloma inguinale and *lymphogranuloma venereum* are sexually transmitted in a large proportion of cases. They are usually described together since they are usually associated with genital "sores." They are relatively rare and are most prevalent in warmer climates or seaboard cities.

Haemophilus ducreyi, the cause of chancroid, can be detected by smears or cultures from a suppurating ulcer which usually show a mixed bacterial flora, including Gram-negative rods in chains. Serologic tests are rarely done. The Ducrey skin test (*Haemophilus ducreyi* suspension) usually is positive within three-to-five weeks after infection. A positive skin test, however, cannot differentiate between old and recent infection.

Donovania granulomatis, the cause of granuloma inguinale, can be cultivated in complex bacteriologic media but this is rarely attempted in practice. Histologic demonstration of intracellular "Donovan bodies" in biopsied material most frequently supports the clinical diagnosis. Serologic tests are not useful. *Lymphogranuloma venereum* can be recovered by inoculating pus from suppurating lymph nodes into embryonated eggs but the procedure is not practical. Serologic tests, usually complement-fixation, can demonstrate a fourfold antibody titer rise between paired sera collected two weeks apart. The Frei skin test is frequently used to support the clinical diagnosis and it usually becomes positive two-to-three weeks after infection and remains positive for life, but recently the test has been negative in a significant proportion of proved infections. This skin test reflects reactivity to a group-specific antigen which is shared by all members of the group. Past infection with psittacosis or trachoma, therefore, may give rise to a positive Frei test as readily as past infection with *lymphogranuloma venereum.*

Inclusion conjunctivitis usually manifests itself as an acute conjunctivitis of the newborn, or as a venereal disease and eye infection of adults. Immunofluorescence of Giemsa-stained smears of scrapings from the eye, the cervix, or the male urethra may show typical crescent-shaped inclusion bodies in epithelial cells. Culture in irradiated cells may permit isolation of chlamydiae.

From 1947 to 1955, one of the most dramatic and precipitous declines in the history of our country in the reported morbidity of a chronic, social, communicable disease was recorded. This disease was syphilis. Since 1955, a pronounced upward trend has been observed for syphilis, occupying together with gonorrhea a very prominent place among the ten chief causes of morbidity as illustrated in figure 1. There is also growing divergence between the curves representing the incidence of syphilis and of gonorrhea, with a greater rise in the latter. The majority of undiscovered syphilis in the past, today and for the foreseeable future is latent syphilis which presents no signs, symptoms nor findings in the patient other than those demonstrated by appropriate serologic testing. The value of a serological test depends on its sensitivity (ability to react in the presence of syphilis), specificity (inability to react in the absence of syphilis), and reproducibility. The types of serologic tests for syphilis are listed in Table 2. The fluorescent treponemal antibody-absorbed (FTA-ABS) is the most sensitive test available. It becomes reactive early in primary syphilis, and it remains positive almost always indefinitely even after apparently adequate therapy. Occasional false positive results have been reported, but rarely in more than one percent of cases. Venereal Disease Research Laboratory (VDRL), Kolmer complement-fixation (KCF), and Kolmer Reiter protein (KRP) or Reiter protein complement-fixation (RPCF) are less sensitive and less specific. In numerous conditions, both acute and chronic, the reagin or non-treponemal tests react nonspecifically in 30–40% of late or inadequately treated cases of syphilis; these tests are nonreactive. Our own clinicolaboratory studies at the Medical College of Virginia have confirmed these findings. Figure 2 illustrates graphically the humoral immune responses, as detected by the FTA-ABS and the VDRL tests, respectively. Observe that, unless one is dealing with a biological false positive result, a reactive VDRL is always

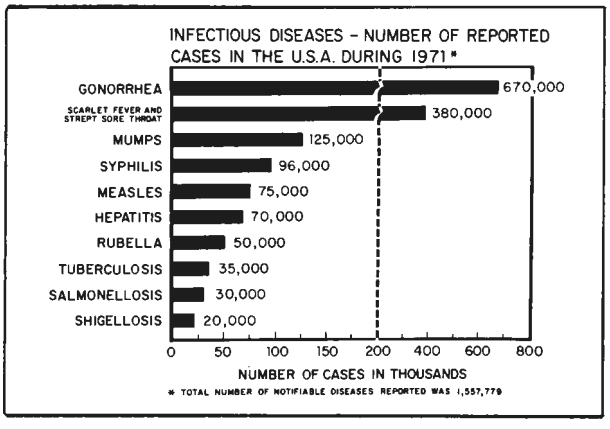

Fig. 1

TABLE 2

TYPES OF SEROLOGIC TESTS FOR SYPHILIS

I. NONTREPONEMAL (nonspecific)		II. TREPONEMAL (specific)	
Flocculation	Complement-Fixation	T. Pallidum	Reiter Strain
VDRL Rapid Reagin	Kolmer	FTA FTA-ABS TPI	KRP or RPCF

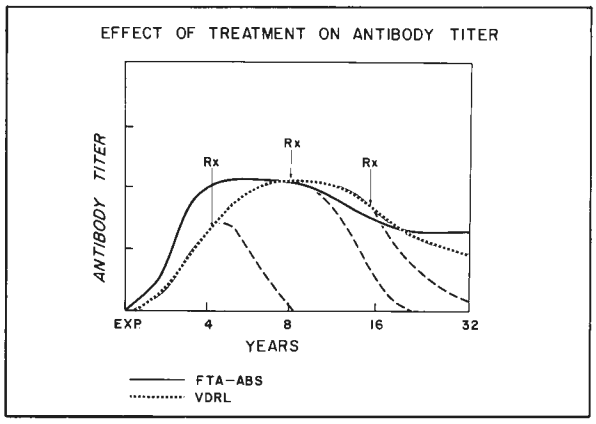

Fig. 3

accompanied by a reactive FTA-ABS. A nonreactive VDRL together with a reactive FTA-ABS, however, may occur in early primary or late syphilis. No reagins can be found in about 40% of untreated patients with tertiary syphilis whose FTA-ABS antibodies are present for life.

Among the complications of late syphilis, neurosyphilis has been particularly difficult to diagnose, due to the lack of sensitivity of certain laboratory tests such as the VDRL and treponema pallidum immobilization (TPI), the obscurity of the clinical symptoms, the rising incidence of its atypical forms and the well-known occurrence of nontreponemal seronegative neurosyphilis. The FTA and FTA-ABS tests were first adapted for use with cerebrospinal fluid (CSF) at the Medical College of Virginia, demonstrating considerably higher specificity of the FTA for the CSF than for blood with a degree of sensitivity comparable to that of the blood FTA-ABS. For the sake of brevity, I would like to refer the reader to three major publications resulting from clinicolaboratory studies done in our institution (1, 2, 4). Recommendations for the use of these tests can be found there and in some more recent work presented at the American Society of Microbiology Annual Meeting held in Philadelphia in April 1972 (3). The effect of antibiotic therapy on antibody titer can be seen in figure 3. No posttherapeutic change can be observed for the FTA-ABS, but the VDRL reactivity gradually disappears after treatment during a period of time proportional to the length of active syphilis infection; that is, the longer the active infection, the longer the time required for serological reversion from reactive to nonreactive.

The number of reported cases of gonorrhea from 1950 to 1972 is shown in figure 4, which

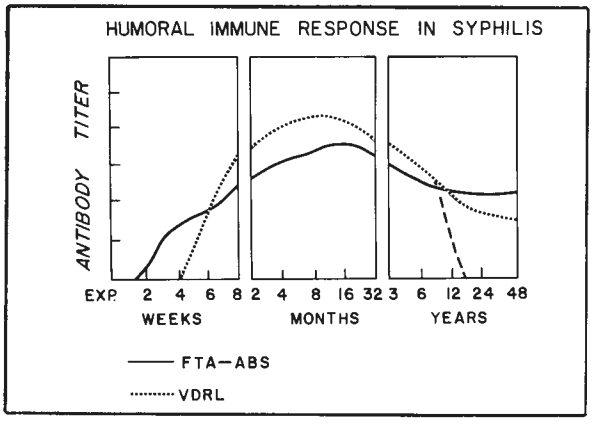

Fig. 2

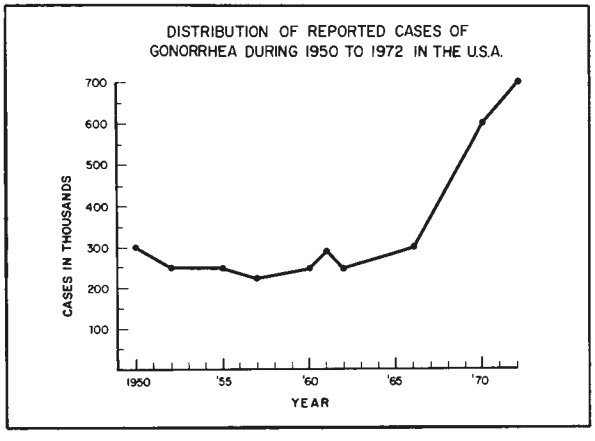

Fig. 4

indicates an upward trend in the incidence of gonorrhea in the United States, as in other parts of the world; thus, gonorrhea has become the leading reportable disease in this country. For example, an incidence of 137 cases of gonorrhea per 100,000 in 1957 had risen to more than 307 per 100,000 in 1971. Also, it is well known that the actual number of cases of gonorrhea is almost four times the total number reported to health authorities by all sources. The age-specific case rates by sex in the United States during 1971 are illustrated in figure 5. One of the major problems in the control of this disease is the existence of a large number of unidentified asymptomatic females estimated to range between 640,000 and 1,000,000 cases. The comparative sensitivity of the Thayer-Martin medium versus smear techniques using cervical specimens is 88% and 54%, respectively. A list of cultural diagnostic techniques is presented in Table 3. Presumptive and definitive tests are included. Although serological procedures are still under evaluation, complement-fixation tests are sometimes performed on individuals with negative cultures who are suspected of harboring chronic gonococcal infection. These tests, however, lack specificity and reliability. Antibodies detected by immunofluorescence are promising in gonococcal arthritis.

Intensified research is required on the biochemical and immunological aspects of venereal diseases. This research might soon lead us not only to a simplified serologic screening test for gonorrhea (the absence of which in a large part accounts for the rise in its incidence as compared to syphilis) but might also lead to an immunizing procedure against treponemal diseases such as syphilis. For years attempts at controlling gonorrhea were abandoned in favor of "treating it out" of existence with increasingly larger doses of penicillin. A decade ago the less sensitive strains requiring 0.1 unit of penicillin per ml for inhibition *in vitro* were extremely rare in clinical practice, but strains requiring 0.5 unit per ml are now common. Today many strains of gonorrhea are so resistant to penicillin that a dose so large as to approach the outer limits of safety is needed to effect a cure.

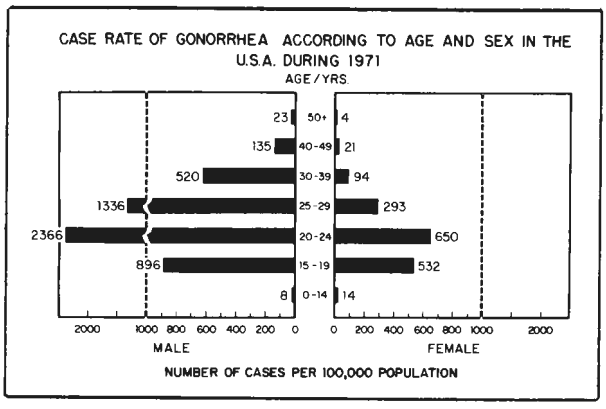

Fig. 5

TABLE 3

PRESUMPTIVELY POSITIVE CULTURAL DIAGNOSIS

Typical Colonial Morphology
Oxidase-Positive Colonies
Gram-Negative Diplococci

DEFINITELY POSITIVE CULTURAL DIAGNOSIS

Sugar Fermentation Reaction
Fluorescent Antibody Staining

The recent recognition of the relatively frequent sexual transmission of herpes simplex and its association with cervical carcinoma following the improvement in virological techniques suggests the important role of viral agents in venereology. The tests, specimens required and time needed for the laboratory diagnosis of herpes simplex infection are listed in Table 4. Most of these procedures are performed routinely in our laboratory, including those for the differentiation of types 1 (oral) and 2 (genital).

Other viruses may with time be shown to be sexually transmitted under certain circumstances, such as the hepatitis virus. Progress cannot be made against any of the venereal diseases without interdisciplinary cooperation between the venereologist and other medical specialists such as obstetricians, gynecologists and general practitioners. At the same time, cooperation of these individuals with public health workers, epidemiologists and researchers as well as with the teacher, health educator and social worker must be forthcoming not only at the patient and institutional level, but also on an interstate, national and international basis. The paramount importance of transfrontier cooperation is illustrated by a recent report of a California prostitute with secondary syphilis who kept a diary. It was found that among some 310 males who were involved as contacts, 168 (all long distance truck drivers) were traced. This threat of spreading disease extended over 34 states in this country and into Canada and Mexico. This epic of epidemiology was written by a

TABLE 4

LABORATORY METHODS FOR HERPES SIMPLEX VIRUS (HSV)

Method	Specimens	Time Required
Light microscopy for multinucleated giant cells and inclusion bodies	Scrapings from base of lesions on glass slides	½–1 hr.
Immunofluorescence for antigen	Vesicle fluid, scrapings, biopsy of brain or other tissue	2 hrs.
Electronmicroscopy for viral particles	Vesicle fluid, scrapings, biopsy of brain, liver and other organs	1–2 hrs.
Tissue culture* for virus isolation	Vesicle fluid, scrapings, CSF, biopsy or postmortem tissues	24–96 hrs.
Serology for antibodies	Acute and convalescent sera (at least a week apart) CSF	24 hrs.
Histology	Biopsy or postmortem tissues in fixative	24 hrs.

* Special procedures (plaquing, type of CPE, etc.) available for differentiation of HSV-1 from HSV-2.

staff reporter and appeared in a periodical infrequently seen by "highly dedicated physicians"—namely the Wall Street Journal (1970).

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