

SHORT COMMUNICATION

Serodiagnosis of Syphilis — Current Status

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THE clinical and public health problem posed by syphilis is still present and cannot be regarded with complacency. During the last 20 years, penicillin has reduced the period of infectiousness of the individual patient, but reinfection is common. The number of known cases of syphilis in Canada in 1961 is less than one-tenth that of peak war years. In Ontario, for instance, a rate of 175 per 100,000 population in 1943, which had been reduced to 25 in 1950, reached a low of 7 in 1958. However, the best venereal disease control program can merely reduce incidence and not eradicate the disease. Over-optimism is a mistake, because, on this continent as a whole, each of the last six years has seen an upward trend recorded in syphilis. Medical practitioners are seeing most of the fresh infection among teenagers. Since treatment is ambulatory and no longer centred in the public clinic, reporting by individual physicians may be neglected and search for contacts by public health workers handicapped. Any relaxation of effort in control measures would be premature.

Every control program depends principally upon early diagnosis of the infectious case and the subsequent seeking out of the source and contacts. The investigation of the "facilitation"³ of venereal disease is dealt with in some places by specially trained interviewers. Requirements for international travel and for premarital and prenatal blood testing vary greatly, and mass testing during pre-employment examinations in industry, on hospital admission, and in blood donor clinics, is a case-finding mechanism of considerable cost but is sufficiently rewarding to justify its continuation. With the open chancre being seen again more commonly, it is practical to revive use of the dark-field microscopic examination of exudate from a suspect sore in which the *Treponema pallidum* may be recognized. Mail transport to the laboratory of a sealed capillary tube of exudate is practical and efficient.

Laboratory Procedures

The serodiagnosis of syphilis is more precise today than ever before. Throughout the first half of this century, many laboratory methods identified the presence of "reagin", an antibody-like response of the blood to syphilitic infection. During the last 10 years, use of the spirochete itself has made

possible a more specific approach. During late stages of infection both reagin and treponemal antibodies may be present in spinal fluid. Quantitative tests of serum and spinal fluid may provide a rough measure of the favourable response of the patient to treatment in the form of reduction of the titre (concentration) of antibody present. In reporting a quantitative test, the figure named is the greatest serum dilution which shows reaction. The nonspecific colloidal gold test is not done by many routine laboratories today. In Canada, the public health laboratory services of all provinces cooperate closely with the Federal Laboratory of Hygiene in surveys to evaluate the efficiency of serodiagnostic methods and attempt to standardize laboratory practice in the use of the common antigens. For many years, the Kahn tube-flocculation test was the method of choice throughout the world: it employed an extract of beef heart. A Canadian chemist, Baer, was concerned with synthesis of certain fats, lecithin and cardiolipin. This work assisted in the development of cardiolipin antigen as a substitute for crude beef-heart extract. With development of cardiolipin antigens, the Venereal Disease Research Laboratory of the United States Public Health Service developed what is known as the VDRL slide-flocculation test as a sensitive "screening test" for routine use. This now has become the basis of a triple test plan¹ outlined elsewhere in this communication. Present methods of complement fixation are modifications of the previous tests of Wassermann, Kolmer and Harrison.

Many treponemal procedures have been devised, but the one which has become a criterion is the microscopic *Treponema pallidum* immobilization test (TPI) which makes use of living, motile *Treponema* which has been maintained in rabbit testes. An extract of a similar spirochete, which can be cultivated on artificial media, is used in the tube Reiter-protein complement-fixation test (RPCF). More recently, the use of fluorescent staining of organisms makes possible the microscopic observation of antigen-antibody union under ultraviolet light. Another method examines the plasma which has been obtained rapidly from fresh blood and treated with an anticoagulant.

DISCUSSION

The VDRL slide flocculation test, which is done initially and is a sensitive screening procedure, if "non-reactive" (negative) can be reported immediately as almost ruling out syphilis, in 96%

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of blood specimens received in a routine public health laboratory. For confirmation of a "reactive" (positive) screening test, the RPCF tube complement-fixation test is used and if reactive is considered to indicate syphilis, present or past. Serum which is reactive with the screening slide-flocculation test but non-reactive in the tube complement-fixation procedure, requires recourse to the most specific TPI microscopic test; reaction here indicates syphilis, present or past. The clinical problem of the "biologic false-positive" is represented by a serum which, although showing reaction in the VDRL, is non-reactive in the TPI and RPCF, and this may occur occasionally in a variety of conditions other than syphilis, such as pregnancy, leprosy, lymphogranuloma venereum and even transient respiratory infections. Although more than one million screening tests for syphilis are performed by Canadian public health laboratories each year, less than 6000 TPI tests are done in problem diagnoses.

Reporting of serologic tests for syphilis today avoids the use of the vague terms "doubtful" and "inconclusive". The VDRL screening test does employ the term "weakly reactive," but such a serum may not be proved syphilitic in confirmatory and specific tests. Since the VDRL is a single proportion test with whole serum, it is true that it may fail to show reaction in a rare syphilitic specimen which contains a blocking substance which prevents reaction and which might be demonstrated only in a quantitative test. This represents the type of suspected case in which the interested physician should seek closer co-operation with the laboratory rather than condemn the procedures for failing to confirm a clinical diagnosis.

An interesting historical note recalls that Ehrlich's Nobel Prize of 1909, for work in immunobiology that introduced the chemotherapy of syphilis with arsenicals, followed closely the recognition of the spirochete by Schaudinn in 1905 and the demonstration of reagin in a serologic complement-fixation by Wassermann in 1906. Forty years later, Fleming and Florey's Nobel Prize of 1945 for penicillin, an agent that became the most satisfactory antibiotic treatment of this infection, was followed by successful use of the live organism in specific serodiagnosis by Nelson and Mayer in 1949.

Fig. 1 presents a practical "triple test plan" which has been adopted in Ontario where the TPI test has been available since 1951. Fig. 2 shows three specimen reports. Fig 3 indicates comparative sensitivity of the three types of test. Early treatment can be expected to remove reagin and complement-fixing antibody as reflected in non-reactive VDRL flocculation and RPCF complement-fixation tests. Treatment later in the course of infection delays such favourable response, and reduction in titre of reagin shown by repeated quantitative flocculation tests may be a measure of response. Olansky states⁴ that "During the primary

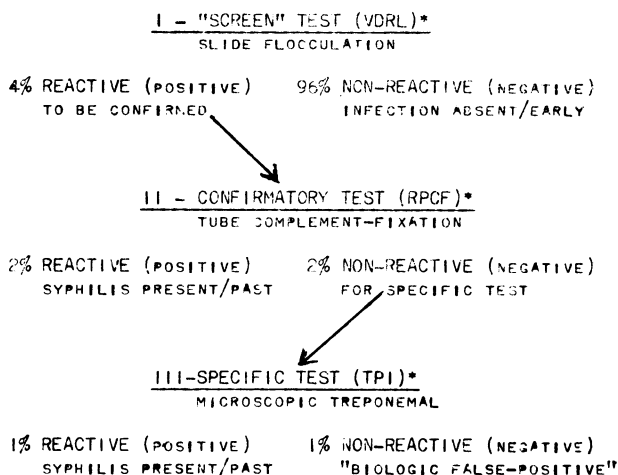


FIG. 1. TRIPLE TEST PLAN.

*VDRL VENEREAL DISEASE RESEARCH LABORATORY
RPCF REITER PROTEIN COMPLEMENT FIXATION
TPI TREPONEMA PALLIDUM IMMOBILIZATION

stage, seronegativity is usually obtained within six months to a year of adequate treatment and in secondary disease within eighteen months to two years." Fixed reagin, unresponsive to treatment, can present difficulties in interpretation and may lead to overtreatment. Immobilizing antibody, demonstrated by the microscopic TPI test, may remain more or less permanently in the serum of

READING OF TEST	I SCREEN TEST VDRL	II CONFIRMATORY RPCF	III SPECIFIC TEST TPI
"NEGATIVE" REPORT			
NON-REACTIVE	✓		
WEAKLY REACTIVE			
REACTIVE			
QUANTITATIVE			
"POSITIVE" REPORT			
NON-REACTIVE			
WEAKLY REACTIVE			
REACTIVE	✓	✓	
QUANTITATIVE	8		
"BIOLOGIC FALSE-POSITIVE"			
NON-REACTIVE		✓	✓
WEAKLY REACTIVE	✓		
REACTIVE			
QUANTITATIVE			

FIG. 2. REPRESENTATIVE REPORTS: TRIPLE TEST PLAN.

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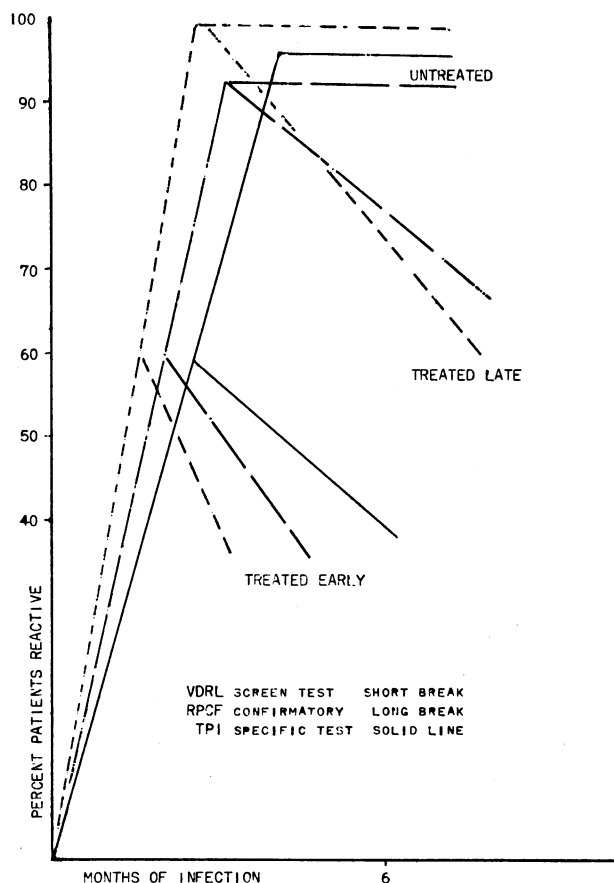


FIG. 3. RESPONSE TO TREATMENT: VDRL/RPCF/TPI TESTS.

a patient once infected, but does not necessarily indicate activity of infection.

The term serodiagnosis may be misleading, for simple routine demonstration of antibodies is merely one indication of response of the body to injury reflected in changing serum protein fractions. With relation to syphilis, this response can be compared with the response of the body to other infection. For example, the serologic method of complement fixation can be adapted to any suitable type of antigen derived from infectious agents, bacterial, viral, fungal and parasitic. Serologic response to treatment varies in accordance with duration and stage of the disease.² The laboratory reports merely what it observes, according to limitations of methods of blood examination currently in use, but it cannot make a clinical diagnosis. This remains necessarily and desirably the prerogative and responsibility of the physician in charge of the patient.

SUMMARY

Although the incidence of syphilis has been reduced markedly by antibiotic treatment, the infection continues to be a social problem, and control measures cannot be relaxed. Laboratory aids to diagnosis are reviewed. Darkfield microscopic examination for the treponema of syphilis, in exudate from a suspect sore, is still a practical procedure. In serodiagnosis, a triple

test plan is described in which a slide flocculation "screening" test (VDRL), if non-reactive (negative), may exclude most (in Canada, 96%) of routine blood specimens as non-syphilitic. Specimens of blood which are reactive in the screening test are further examined by a confirmatory tube complement-fixation test (RPCF) which employs, as antigen, a treponemal extract and, if reactive (positive), is supporting evidence of syphilis, present or past. A quantitative flocculation test provides a measure of the concentration of antibody in syphilitic blood which may disappear with treatment. Specimens which are reactive in the screening test but non-reactive in the confirmatory test are further examined by a specific treponemal microscopic test (TPI) which makes use of the living motile organism. This is a final criterion and, if reactive (positive), is taken to indicate syphilis, present or past. A non-reactive (negative) specific test, on a blood which has shown previous non-specific reaction in screen test, can be regarded as excluding syphilis and clarifying a "biologic false-positive" screen test.

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PAGES OUT OF THE PAST: FROM THE JOURNAL OF FIFTY YEARS AGO

LOW PERCENTAGES IN INFANT FEEDING

In the Edinburgh Hospital for Sick Children they recently tried, with fear and doubting, the feeding of infants upon undiluted cow's milk, in the way introduced by the late Professor Budin, and since employed so largely in France. The method consists in giving undiluted cow's milk, which has been heated to 212° Fahr. and kept at that temperature for forty minutes. "The results were excellent," says Dr. Fowler, "and unexcelled by any other method short of breast feeding." F. S. Langmead read a paper before the Harveian Society of London lately upon the use of undiluted cow's milk in the feeding of infants. He added citrate of soda in the proportion of two grains to the ounce of milk. He had treated seventy infants thus at the Paddington Green Children's Hospital in the past three years, all of whom were not progressing by other methods. All had done well and gained weight. No rickets were observed. It was not found suitable in acute diarrhoea. Again, F. G. Haworth writes in the *British Medical Journal* of 1907: "I have used undiluted cow's milk sterilized now for ten years, with five per cent. addition of lime water and sugar. I begin the feeding at birth, feeding for the first week every hour and a half, with ten feeds in the twenty-four hours; after that for a month every two hours, and eight feeds in the twenty-four hours. It has not disagreed in spite of the larger amount of protein in the form of casein. . . . I hope that some men will be induced to try the undiluted sterilized pure cow's milk as a food for infants, and let us hear less of barley water and other patent foods." Fowler says in this relation: "I have certainly observed the substitution of undiluted for diluted milk followed by cessation of colic and curdy stools. The prolonged heating, which is an essential feature of the method, precipitates the lime salts and thus materially softens the curd produced in the stomach."—Robert Dawson Rudolf, *Canad. Med. Ass. J.*, 2: 174, 1912.