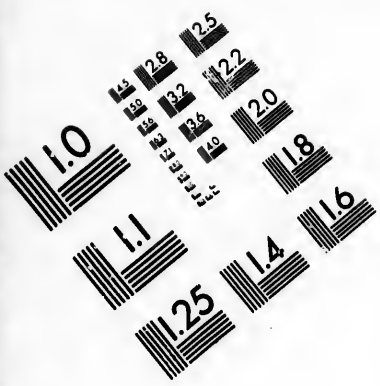

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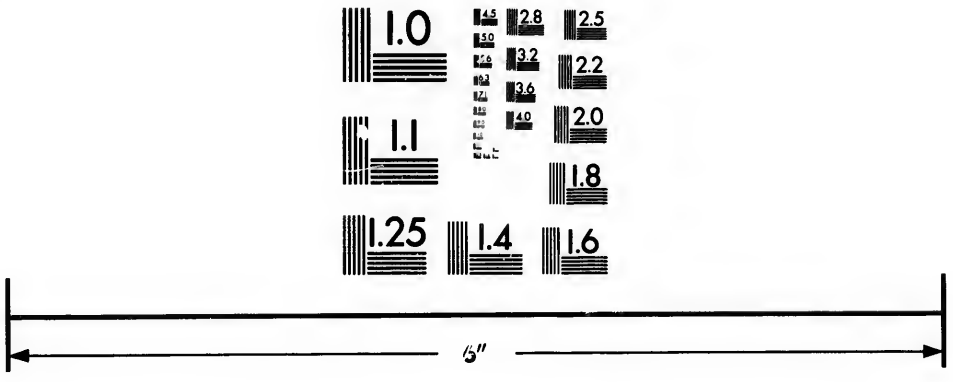

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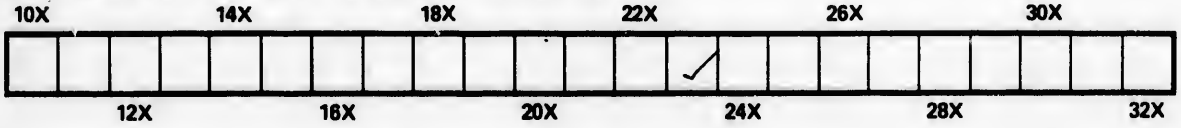

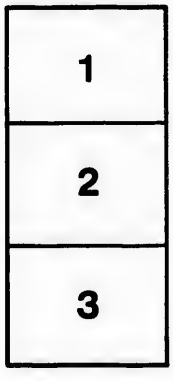

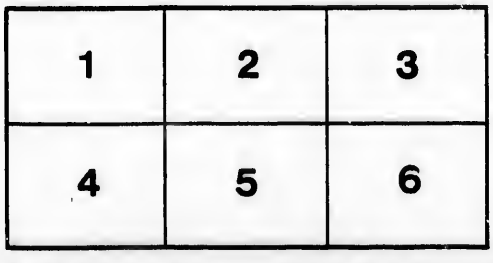

ON THE APPLICATION OF THE SERUM DIAGNOSIS OF TYPHOID FEVER TO THE REQUIREMENTS OF PUBLIC HEALTH LABORATORIES.

By WYATT JOHNSTON, M. D., Montreal,

Bacteriologist to the Board of Health for the Province of Quebec; Assistant Professor of Hygiene, McGill University; Pathologist to the Montreal General Hospital.

Reprinted from Transactions of the American Public Health Association.

CONCORD, N. H.:
PRINTED BY THE REPUBLICAN PRESS ASSOCIATION.
1897.

ON THE APPLICATION OF THE SERUM DIAGNOSIS OF TYPHOID FEVER TO THE REQUIREMENTS OF PUBLIC HEALTH LABORATORIES.[1]

Great interest attaches to Widal's important communication to the effect that the serum of persons suffering from typhoid fever, even in the early stages, is capable, when mixed with a pure culture of the typhoid bacillus in bouillon, of arresting the active movement so characteristic of this organism and causing the bacilli to agglutinate into clumps resembling zoogloœa. The serum of typhoid convalescents and immunized animals had been shown by Pfeiffer, Durham, and Gruber to possess this property. But Widal has certainly been the one to demonstrate its great clinical value. With the serum of an undoubted case of typhoid fever we are able to apply what appears to be the most conclusive of the tests at our disposal in deciding whether a given organism is really the typhoid bacillus or not. On the other hand, with a culture of the genuine typhoid bacillus, we are able to decide whether a doubtful case is or is not typhoid fever.

Although the test is so recent in origin, those who have tried it appear practically unanimous as to its being of great delicacy, and, in particular, the negative results which it furnishes are of nearly as much practical value, something which can scarcely be said of the routine bacterial tests for tuberculosis.

Widal's original method was to obtain the serum from the vein of a patient's arm by means of a sterilized syringe, descanting the serum after it had separated and adding it to bouillon culture of typhoid bacilli. This was then placed in the incubator, and showed, after several hours, a flocculent precipitate composed of the immobilized and agglutinated bacilli and a clearing of the upper part of the fluid. This was found by Widal to be characteristic of typhoid blood. The blood in other febrile disorders, such as malaria, typhus, tuberculosis, pyæmia, etc., as well as the serum of healthy persons, was found to have no power of producing this phenomena when mixed with typhoid cultures. Those who have repeated Widal's experiments have also been able to confirm his statements that the colon bacillus does not give this reaction with typhoid blood.

Widal was fortunately led to simplify the method materially by taking

[1] Read before the American Public Health Association, at Buffalo, N. Y., September 17, 1896.

a few drops of blood from the finger tip, and as soon as the serum was separated from the edge of this, mixing it with a drop of actively mobile typhoid culture, whereupon the reaction could be satisfactorily observed under the microscope and was usually complete in a few minutes.[1] Dieulafoy testifies to the remarkable accuracy of the test and its value in diagnosticating obscure cases.

My attention was first directed to the test through having been consulted by physicians as to the nature of suspected cases of typhoid, and my experience has been thoroughly in accord with that of Widal and others as to its great value as an aid to clinical diagnosis.

As the reaction appeared to depend probably upon the presence of some substance analogous to the ordinary toxines, and as many of these preserve their characteristics in a dry state, it naturally occurred to me that this might be true of the substance producing the serum reaction. The advantage of being able to operate with a dried substance was obvious, especially with reference to the possible application of the method to the rapid bacteriological diagnosis of typhoid fever in municipal laboratories, just as is now done in the case of diphtheria, and my observations have been made with this end in view.

Instead of taking the serum as soon as it exuded, I allowed the drop to dry, and found that upon moistening it subsequently the solution obtained was just as efficacious as the pure serum for the diagnostic purposes of the test.[2]

This power appears to remain practically unimpaired even after the blood has been allowed to dry for many days. My experiments upon how long the blood will continue to react when in this dry state are not yet finished, but blood drops dried for from two to four weeks still give the reaction.

In this manner I have tested the blood of ten patients suffering from undoubted and typical attacks of typhoid. The reaction was obtained conclusively in every instance. In eight cases the loss of mobility and the agglutination was complete in from two to fifteen minutes. Of the two others, one, in a very early stage of the disease, required thirty minutes for the completion of the reaction, while the other in a very late stage, following a relapse, required one hour.

The blood of ten other hospital patients, as well as a number of healthy individuals, was next tested, and in no single instance was the reaction obtained. Occasionally a pseudo-reaction with some agglutination was observed within a few minutes of the mixture of blood solution and culture, but some movements of translation (wandering through the field)

[1] This plan of observing the reaction directly under the microscope had been published by Gruber and Durham some months previously.

[2] Since writing the foregoing, I have been able to obtain fuller accounts of Widal's work than were at first available, and find it stated by him that dried serum, and to a lesser extent dried blood, are capable of furnishing the reaction. This circumstance does not appear to have been hitherto utilized practically.

always persisted on the part of isolated bacilli, and these gradually increased in number and activity till, in an hour or two, lively motion was resumed, and was found to be still present on the following day and, in some instances, where it was followed up, at the end of a week. With the typhoid bloods nothing but the oscillating or "Brownian" movements were seen, as a rule, though where the proportion of serum added was very small peculiar revolving and tugging movements, apparently due to the action of the flagella, could be made out, movements from one part to another of the microscopic field being, however, completely abolished.

In two doubtful cases examined for diagnosis the results were negative. In one of these the malaria plasmodium was subsequently detected. The other left the hospital before the diagnosis was cleared up, but her temperature had remained normal for two weeks, and her only symptoms were persistent headache and giddiness. One of the control cases, examined with negative results, had a history of typhoid two years previously.

In making a communication upon this subject before the American Public Health Association, at Buffalo, N. Y., on September 17, 1896, I subjected the method to what I considered to be a fair practical test as to its applicability to public health purposes. I left instructions for Dr. D. D. McTaggart, resident pathologist, to forward by post to my destination, after I had left Montreal, a letter containing dried blood drops from several cases of undoubted typhoid fever and also dried blood drops for control from other hospital cases, preferably patients suffering from febrile conditions, but making sure that they had not had typhoid recently. All these blood drops were to be numbered and a key giving the clinical diagnosis in each case placed within a separate sealed envelope.

I left Montreal September 13th. Samples of blood from six patients were collected, and forwarded as directed, on September 14th. On September 16th, the letter was delivered unopened at Buffalo, N. Y., to Dr. Bissell, the city bacteriologist for Buffalo, who kindly took charge of the key. At the end of an hour spent in examining the specimens, I wrote my diagnosis upon the outside of the sealed envelope. It will be seen from the subjoined signed statement, which Dr. Bissell kindly made at my request, that the results were perfectly in accord with the clinical diagnosis in each case, while the specimens, which were then examined by a number of competent bacteriologists, showed that good objective grounds existed for arriving at the conclusions given.

STATEMENT BY DR. MCTAGGART, RESIDENT PATHOLOGIST, MONTREAL GENERAL HOSPITAL.

The samples of blood were mailed to Dr. Johnston one day after he had left Montreal. Dr. Johnston had no knowledge of the contents of the "key," and no private means of knowing which of the numbers referred to typhoid and which to non-typhoid blood.

(Signed) D. D. McTaggart.

STATEMENT BY DR. BISSELL, CITY BACTERIOLOGIST, BUFFALO, N. Y.

Buffalo, September 16, 1896.

Received to-day from Dr. Wyatt Johnston a sealed letter, mailed in Canada, with postmark, "Montreal, September 14, 1896." This was opened by me and found to contain (*a*) six glass cover slips, numbered from 1 to 6, with a drop of dried blood on each, and (*b*) also a sealed envelope marked "key." Received from Dr. Johnston, after examining the blood by the (Widal) serum diagnostic test, the following report: No. 1, typhoid; No. 2, typhoid; No. 3, typhoid; No. 4, not typhoid; No. 5, not typhoid; No. 6, doubtful,

probably not typhoid. The key was then opened by me, and the clinical diagnosis from all cases found as follows: No. 1, typhoid; No. 2, typhoid; No. 3, typhoid; No. 4, malaria; No. 5, enlarged glands of neck; No. 6, heart disease.

(Signed) WILLIAM E. BISSELL.

It will be noticed that a qualified though correct opinion was given at the time of my making the report in one of the negative cases (No. 6). This doubt was owing to the fact that it was the last specimen examined, and that a partial agglutination appeared to take place at first, though motion was not abolished. Subsequent examination some hours later showed such lively motion that I should have had no hesitation in declaring it not to be typhoid, had the circumstances permitted that much delay before an opinion was given.

A ready means of diagnosis in typhoid fever is something which has long been desired by sanitary officials. The medical profession is proverbially lax with regard to the notification of typhoid cases, and we may assume that this neglect is in part due to the want of any adequate *quid pro quo* in return for such notification. Probably the assistance derived from a prompt bacteriological diagnosis, or even corroboration of diagnosis in the early stages of typhoid, will lead to the more uniform reporting of cases. Besides distinguishing typhoid from such well-characterized diseases as tuberculosis and malarial disease, this test may also be expected to clear up the mystery which surrounds those doubtful cases of so-called bilious fever, remittent fever, gastric fever, typho-malarial fever, etc., which are so common in times and places where typhoid is prevalent, and rare in the absence of typhoid, at least in temperate regions which are free from malaria.

Those who are called upon to investigate epidemics of typhoid are much perplexed by the large number of cases of ill-defined and transitory fever occurring among those personally exposed to the infection, and the impossibility of coming to anything like a definite conclusion upon the evidence hitherto obtainable as to whether these are to be regarded as cases of abortive typhoid or not. In my own experience, such cases have usually equaled or outnumbered the cases where the symptoms justified a definite diagnosis.

I may add a few words with regard to technic. I use a dry lens of about one-fourth inch focal distance. The dry blood drop is partly dissolved with germ-free water, and a drop of the solution obtained is placed upon a cover glass which has just been passed through a flame and mixed with a drop of a typhoid bouillon (a watery suspension of an agar culture also answers very well). This is placed over a hollow cell sealed by vaseline. I control the examination by comparing it with a blood drop from an undoubtedly typhoid case, and also with normal blood. It is also advantageous to place a minute drop of the blood solution upon the cover slip alongside the mixture of culture and serum, so as to satisfy one's self in negative cases that the blood contains no motile bacteria. Uniformity of temperature is the chief detail to be attended to, as the agglutination does not take place so well if the movements are sluggish.

A hot-water dish filled with warm water forms a cheap and convenient substitute for an incubator, and a simple warm stage made of a sheet of copper is also useful. In a well-warmed laboratory, however, the use of these adjuncts is unnecessary. Hollow cells are convenient, but not indispensable. For collecting the blood drop, any smooth surface suffices; cover glasses or slides have the advantage of being clean and sterile, but I have found ordinary writing paper or smooth cardboard most convenient, as it could be more easily labeled or forwarded. The swabs used for diphtheria outfits will answer, but the presence of extraneous substances, such as fibres, was found annoying. The presence of blood pigment is rather an advantage, as it enables the drop to be more easily focused. The small fibrin particles of clot sometimes bear a superficial resemblance to the islets of agglutinated typhoid bacilli, but are readily distinguished from them by the presence of leucocytes in their meshes.

One advantage of having the blood dried is that it insures it against contaminating growth occurring during shipment. In case any doubt as to the reaction exists at first, it will usually be dispelled by watching the preparations for some hours, or, if necessary, for a day or two. This permits a decided and progressive increase of motion in non-typhoid cases and allows the more perfect agglutination in the genuine ones.

The one indispensable factor is perfect purity of the culture. The one which I use was kindly forwarded me by Mr. J. J. Mackenzie, bacteriologist to the Ontario Provincial Board of Health, and was stated to have come originally from the Berlin Hygienic Institute. It grows typically on gelatin, potato, bouillon, agar, and milk; reacts typically with litmus agar, produces no indol or gas, and shows the motility and staining reactions characteristic of the Eberth bacillus.

I have made this communication because the method here suggested seems better adapted than those hitherto employed for bringing this test within the range of ordinary public-health laboratory work, and enabling it to be dealt with, if I may so express it, in a wholesale manner.[1]

This article was published in the "New York Medical Journal" of October 31, 1896. Further articles on the same subject were published by me in the "New York Medical Journal," November 28, 1896 (with Dr. D. D. McTaggart) in the "British Medical Journal," December 5, 1896; Circular of the Board of Health of the Province of Quebec, January 7, 1897 (with Dr. D. D. McTaggart) in the "Montreal Medical Journal," March, 1897; "Centralblatt für Bakteriologie," Baud XXI, 1897.

[1] Drying the blood as a preliminary step has enabled the Board of Health of the Province of Quebec to offer to the medical profession here a free public service of typhoid diagnosis by the serum method similar to that which is followed in diphtheria. Outfits consisting of a folded and sterilized piece of paper, in which the blood drop is sent inclosed in a suitable envelope, are placed in convenient depots. In case of negative results, an additional sample, taken by collecting a few drops of blood in a small glass tube, is examined, but this extra precaution is seldom necessary. As to the degree of accuracy which this application of the test may afford, it is too early to speak positively. From my experience hitherto, I am inclined to believe that it will compare not unfavorably with those obtained in the cases of diphtheria and tuberculosis. In one case the reaction was present on the third day.

LABORATORY OF THE BOARD OF HEALTH OF THE PROVINCE OF QUEBEC.

CIRCULAR ON ATTENUATED TEST CULTURES AS A SAFEGUARD AGAINST PSEUDO-REACTIONS IN SERUM DIAGNOSIS OF TYPHOID BY THE DRIED BLOOD METHOD.

MONTREAL, 7th January, 1897.

To the President of the Board of Health of the Province of Quebec:

SIR: In my work in serum diagnosis done jointly with Dr. D. D. McTaggart, we recently met with a series of peculiar partial reactions in which the dried blood solution from many perfectly healthy persons gave a very decided agglutination. The blood serum from the same persons was found much less liable to give these pseudo-reactions. This made it less easy to exclude other febrile diseases, and as with this test accuracy in the negative diagnosis is of great practical importance, others who may meet with similar pseudo-reactions will be interested in learning how they may be avoided.

These pseudo-reactions were not encountered in our earlier cases when attenuated cultures were used. They began to appear when we employed a short time virulent cultures, and disappeared again on resuming the use of attenuated ones. Active, virulent cultures, intensified by daily transplantation and growth, at the body temperature, are therefore not suitable for the dried blood test. Where only active cultures are employed, we do not think that the dried blood method can be considered to have had a fair trial.

The explanation of this difference appears to be that the serum contains relatively less of the substances causing agglutination than solution of the entire blood. Hence solutions of the entire blood react more intensely to the test than solutions of the blood serum alone. This was the reverse of what we had anticipated.

It is found that old laboratory stock cultures kept at room temperature, and transplanted at intervals of about one month, give us the best results. Bouillon test cultures grown from this stock for twelve to twenty-four hours at body temperature are found to react decisively with solutions of typhoid blood or typhoid serum, the reaction being, as a rule, well marked within fifteen minutes. With non-typhoid blood or serum solutions, the same test cultures give no reaction even after twenty-four or forty-eight hours' contact. Intraperitoneal injection of one c. c. of such living bouillon culture produces in guinea pigs a marked blood reaction and immunity without much disturbance of health. We find that the best results in cases of dried blood are obtained with cultures where the motion, as seen under the microscope, is of a rapid, gliding character, but free from darting movements. If the movement is sluggish, owing to too great attenuation of the culture, a few daily transplantations at body temperature will make it more active. Exact estimation of the degree of dilution has not been found necessary for ordinary diagnostic work when attenuated cultures are used. A very faint tint in the drop examined usually indicated sufficient strength. The solution should not be thick and viscid.

All the results which I have reported ("N. Y. Medical Journal," Oct. 3, 1896, and "British Medical Journal," Dec. 5, 1896,) were obtained with attenuated cultures. A report, giving some additional technical details, has been prepared, and can be sent to any who desire further information.

I remain, yours respectfully,

WYATT JOHNSTON,
Bacteriologist to the Board of Health, Province of Quebec.

NOTE.—Subsequent experience has confirmed the above statements as to the best method of technique with the cultures which I have used. From the recent literature of the subject it seems, however, established that the cultures used by others give the best results under different conditions, so that it is safer not to generalize.