LABORATORY AIDS IN THE DIAGNOSIS AND PROGRESS OF TUBERCULOSIS

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This paper is based on the experience gained in laboratory work done for the Pukeroa Sanatorium during the past 2½ years. This Institution is functioning as a Sanatorium, and not as a chest hospital. Cases are admitted from the whole of the North Island area, and consist of people who have been recommended as suitable for sanatorium treatment. Most of them have passed through a general hospital prior to admission.

This paper sets out the routine laboratory procedure adopted in the primary investigation and follow-up of these cases.

Sputum:

1. The Direct Examination: On admission, each patient has a direct examination of the sputum on six successive days. The report consists of:

   1. The microscopic appearance of the sputum.
   2. A report on the number of pus cells present as determined by microscopic examination of the stained slide.
   3. The presence or absence of tubercle bacilli with a rough classification of the numbers present. Slides are reported as Positive or Negative, and the number of bacilli are classified as:

      - Rare: 1 or less per 10 H.P. fields.
      - Few: 1 - 10 per H.P. field.
      - Numerous: 10 or more per H.P field

   The microscopic appearance and report on the number of pus cells is of great value to the clinician. Specimens are classified as:

      1. Purulent.
      2. Muco-purulent.
      3. Mucus.
      4. Saliva.

   A series of Negative reports on specimens consisting solely of mucus or saliva is obviously not as valuable as negatives obtained on specimens of purulent or muco-purulent nature. Of all direct examination positives, about three-quarters were obtained in purulent or muco-purulent material.

   Cases producing purulent or muco-purulent sputum, and Z.N. Negative by direct and more sensitive methods, are worthy of further investigation to determine the presence of secondary infection, especially if the case is clinically and radiologically not typical of tuberculosis.

   The Z.N. stained film is prepared in the usual way, great importance being attached to the selection of a satisfactory portion of the sputum. Variations of the original Z.N. method have been tried, such as counterstaining with picric acid, but our experience has been that there is no great advantage in adopting another method. This is probably a matter of personal preference, as we found that a picric acid counterstain was more trying on the eyes, when long periods have to be spent at the microscope.
Direct films are searched for a minimum of five minutes. During this time 500 microscopic fields can be thoroughly searched, and it is emphasised that the Direct Examination is purely a "screening out" test for fairly obvious Positives, the main purpose being to classify the cases into Direct Examination "Positives" and "Negatives". It is then necessary to further investigate the Negatives, and unnecessary concentration work is avoided on the Direct Examination Positives.

In addition to the six direct examinations on admission every patient has one monthly direct examination of the sputum for TB. This is of value for two reasons:

First, the cases which have been consistently positive, if responding to treatment, will eventually become negative on the monthly direct examination. This is the first laboratory indication of progress, and these patients then have six direct examinations, on successive days. If these all prove negative then the investigation proceeds to more sensitive methods for the detection of the tubercle bacilli. Second, it is of value in cases previously classified as Negative by direct examination, to determine if they remain negative by this method. It can happen that a case which has been negative may regress and produce a positive sputum, and this can sometimes be picked up in the monthly examinations.

At the discretion of the Medical Officer, any case may be investigated by means of six direct examinations on successive days, at a time when the doctor notices that the patient is producing an increasing amount of sputum. This increase may be due to any one of a number of reasons, e.g., the common cold if prevalent throughout the Institution will often result in a marked increase in the number of cases found positive by direct examination.

Concentration and Culture Methods: (three-day collection of Sputum). Cases which have been Negative by direct examination on admission, or have become negative after being positive, are investigated by means of Concentration and Culture Methods. It is not proposed to enter into details of the technique of such methods, beyond stating that we have used the Petroff and Hank’s Alum precipitation methods for concentration, with satisfactory results.

Culture Media used and found satisfactory are Lowenstein, Yolk enriched (Edson), modified yolk enriched, and Petragnani. For simplicity combined with satisfactory growth, modified yolk enriched is an excellent medium. From the description of the direct examination procedure it will be seen that cases proceeding to concentration methods are those producing either very few tubercle bacilli or else none at all. Therefore it is obvious that the concentration picture, as we find it, will not be so spectacular as that shown in a laboratory where all sputa are submitted to concentration without prior “screening out” of obvious positives. The point of importance here is that concentration films must be searched very thoroughly, and our procedure is to search films for 15 minutes before reporting as “Negative.” Films are frequently found “POSITIVE” after 10 minutes or so of concentrated searching, and after the long time involved in the concentrating and preparation of the film it is obviously important to give really adequate time to the microscopic examination. Quite apart from the importance of the result to the patient and clinician, a hurried “Negative” report will be followed by further concentration involving a considerable amount of work, which possibly could have been avoided by adequate searching of the original film.

A case giving a positive concentration result will not have further concentrations for some little while - it will depend on his clinical and radiological progress. Those cases giving negative concentration results will be followed up with further concentrations at the discretion of the Medical Officer concerned. The concentration method is of definite value in two ways:

1. It does determine a considerable number of positives which, although suspected on other grounds, would be bacteriologically negative.

2. Repeated Negative concentrations are much more reliable results than those obtained by direct examination only.

CULTURAL METHODS: All concentration deposits are cultured. Culturing is a more sensitive method again than examination of concentration films. We culture all positive concentration deposits, for the purpose of typing the tubercle bacilli. The negative deposits are cultured to attempt to obtain positive results, and those that are positive are typed.

Cultures have yielded positive results in numerous cases when concentration films have been negative, and we have not yet failed to grow tubercle from a deposit which has been positive in the concentration film. Practically all the tubercle cultivated has been of “human” type. This can be explained by pointing out that most of the cultural work is done on cases of pulmonary tuberculosis. Where there are lesions other than in the lungs, they are usually secondary to a lung condition.
The only “bovine” types encountered have been in hospital cases and not from the Sanatorium.

**GASTRIC CONTENTS:** The examination of the Gastric contents is an important point in the detection of the tubercle bacilli. This method is employed in cases which are producing minimal amounts of sputum, or none at all. When very small amounts of sputum are produced there is a tendency for this sputum to be swallowed instead of expectorated. Children are particularly likely to do this, and some adults will also swallow considerable amounts of sputum. The resting stomach contents are collected by means of a stomach tube on two mornings. The resulting fluid is concentrated and cultured in a similar manner to that employed for three-day sputa.

This is a particular useful line of investigation for children and non-co-operative patients. We have found it an advantage to prolong the period of digestion with NaOH and use exclusively a medium containing malachite green for culturing gastric contents. This seems to obviate difficulties encountered due to contamination of gastric contents cultures with organisms resistant to the NaOH treatment.

**Animal Inoculation:**

This examination is run parallel with cultures, but cannot be done routinely on all concentrations, as this would involve an extremely large amount of work and a very large supply of animals. Inoculations are, therefore, restricted to selected cases: first, cases that have some clinical evidence of T.B. and have not yielded a positive by other methods; and, secondly, in cases that are nearing the point of discharge from the Sanatorium. As a point of interest, and bearing in mind that the animal inoculations only follow after repeated attempts at obtaining positives by other means, we find that animal and culture results are running fairly even.

**Faeces:**

The examination of faeces for tubercle is undertaken:

a) As an alternative to examination of the gastric contents.

b) To aid in the diagnosis of tuberculosis of the bowel.

It is a more tedious procedure than examination of the gastric contents, and our experience has been that it yields no more information than gastric concentration.

With regard to the diagnosis of T.B. of the bowel, it is difficult to come to a conclusion as to the significance of T.B. in the faeces. In open cases of pulmonary T.B., tubercle bacilli can be demonstrated in the faeces without there being any clinical evidence of bowel involvement, and have presumably been from swallowed sputum. In a case where Gastric contents are repeatedly negative, and the faeces positive, then possibly some deductions can be made. Material obtained directly from a lesion in the bowel will give a more reliable result than examination of the faeces. However, in young children, where there is difficulty in obtaining sputum or gastric contents, the examination of the faeces is of value.

**Pleural Effusions:**

You are all quite familiar with the usual methods of examining pleural fluid for T.B. The only point to be made here is the extremely satisfactory results obtained from the culture of pleural fluids, but it is necessary to examine large amounts of the fluid, up to 100 ml. being necessary for satisfactory examination. The few c.c. of fluid so often submitted for examination is very often inadequate, and over the last 11/2 years, since we have introduced the method of repeatedly examining large quantities of fluid, we have not failed to establish the bacteriological aetiology in any Sanatorium case with pleural effusion.

A point of interest is that in the “Lancet,” February 9, 1946, an article points out that it is necessary to aspirate large amounts of fluid to obtain good results, and this article also emphasises the superiority of cultural methods over animal inoculation. The cytology of pleural fluids is to large a question to be dealt with here, and possibly will be the subject of a later article, but the cytology of the pleural fluid is by no means predominantly lymphocytic at every stage, as is often stated. There seems to be a definite connection between the polymorph lymphocyte ratio and the later demonstration of tubercle bacilli, but much more work remains to be done on this subject.
An easy and fairly accurate estimation of the increasing or decreasing cellularity of a pleural fluid can be obtained by centrifuging a specimen in a graduated centrifuge tube for a fixed time at a fixed speed. The deposit is reported in terms of “percentage sediment.” It gives more definite information when combined with the differential count of the cells than does the differential count alone, and the Sanatorium medical officers have found this simple test to be of some value.

The Mantoux Test:

Again, this test is quite familiar to you all. The only point of interest is that it is sometimes overlooked in the investigation of possible cases of T.B. A negative result is of definite value. In Sanatorium work old tuberculin is preferred to P.P.D. as the great difference in 1st and 2nd strength P.P.D. (2nd strength 250 times stronger) can result in too severe reactions in dealing with Sanatorium cases. Old tuberculin in dilutions 1 in 100,000, 1 in 10,000, 1 in 1,000 and sometimes 1 in 100 is used.

The Blood:

The Sedimentation Rate (S.R.): The most widely used test is the S.R. We use the Wintrobe method. Each patient has a monthly S.R. and it is used chiefly as an indication of progress rather than as a diagnostic test. Cases similar clinically will often yield very varying S.R.’s, and most importance is attached to comparative studies of the S.R. Correction for anaemia is done on cases showing a S.R. of over 20 mm. in 1 hour. Both observed and corrected rates are reported and the clinician makes his own interpretation. It is still regarded as a useful laboratory procedure.

The Examination of the Peripheral Blood:

This is done only in selected cases. During 1944 all Sanatorium cases had the blood examined as follows:

a) Differential white count.

b) Arneth count.

c) von Bonsdorff count.

d) Houghton’s index.

As far as we know this was the first extensive work done in N.Z. on the blood changes in active or recently active tuberculosis. The work was done on the lines set out by Houghton in his original article in “Tubercle,” November, 1935, and we refer you to this article for full details.

The results of a year’s work, entailing many thousands of blood examinations, was that the blood picture is of definite value in prognosis.

Conclusions: Progress can be accurately assessed and clinical breakdown anticipated by serial blood examinations. The blood picture is also of assistance in selecting cases for special treatment.

The Urine:

All patients have a routine laboratory examination of the urine on admission. Early cases of renal tuberculosis have been picked up by this means. Any pathological feature reported in the original examination ensures that a more extensive investigation will follow.

The 24-hour specimen is not recommended as a means of demonstrating tubercle. We prefer to examine the last specimen at night and the first morning specimen. This is more satisfactory for cultural work, and from the point of convenience. Usual concentration methods are adopted. T.B. in the urine is now regarded as always being pathological, even in the absence of clinical features. The theory of “excretory bacilluria” is no longer tenable, and serial sections have demonstrated kidney lesions in cases of “symptomless tuberculous bacilluria.”

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