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# JOURNAL

of the

# NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS



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## **NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS**

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#### **EDITORIAL**

The commencement of a Journal is never a step to be undertaken lightly, especially when subjects of a scientific nature are to be dealt with. However, it was the unanimous opinion of those present at the first Annual General Meeting of the Association, held in Wellington, that a Journal was a necessity as a means of keeping all the members of the Association acquainted with the progress of their fellow members and the dissemination of all knowledge thought to be of interest and use.

The progress of the Journal and its value will, however, depend on the active support of all members, senior and junior, for material to publish, for constructive criticisms and suggestions, and in the initial stages for a generous allowance for difficulties in publication.

At the present it is intended to make this a quarterly journal and to print it in Auckland with the assistance of members of that Laboratory. Should this venture prove a success, the problem of providing a suitable press will have to be faced and a discussion on the Journal and its future should be a subject for consideration at the next Annual General Meeting. In the meantime the Editor would be grateful for suggestions, notes, articles and references for the next issue, these to be to hand by June 1st for the July issue.

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# A CULTIVATION METHOD FOR THE DIFFERENTIATION OF THE OVA OF HOOKWORM AND STRONGYLOIDES

#### (D. F. CREED)

Theoretically, only the ova of hookworm and the rhabditiform larvae of *Strongyloides stercoralis* are found in freshly passed facees. In laboratory practice, this seldom applies because of two factors: (1) The age of specimens reaching the lab from outpatients, and (2) the use of a purging technique by the hospital to assist in the demonstration of parasites.

The effect of (1) would be best demonstrated in a stool from a double infection by both worms, when the rhabditiform larvae of both Hookworm and Strongyloides would be found, the former having had ample time in which to mature and hatch if the specimen had been passed a day or more before examination. With (2) in use as a routine ward procedure, the general result would be, in the same instance, the demonstration of ova of both species.

The identification of the larvae is relatively simple, of the ova almost impossible. Why not, then, leave the faeces for some hours till the larvae can hatch out? Here the chief consideration is the uncertainty of attaining favourable conditions for the maturation of the eggs.

In New Caledonia, the natives examined were found to be infested with numerous parasitic worms, but almost invariably with both *Necator americanus* and *Strongyloides stercoralis*. The faeces sometimes contained the larvae of both, but more generally the ova of both.

At first, methods of identification by measurement with a micrometer eyepiece were attempted, but the variance in diameter of each ovum was so extreme, that no mean average could be determined as a standard. The method was soon abandoned in favour of the following, for which I am indebted to Capt. M. W. A. Gatman.

About five grams of faeces containing the typical unidenti fiable ova is placed in a petri dish, covered with slightly damped earth, and incubated overnight. (This resulted in an almost 100 per cent. yield of rhabditiform larvae.) The faeces should not be incubated for more than two days, as the change from the rhabditiform stage to the filariform commences under such ideal conditions at the end of the second day.

The faeces-earth mixture is then placed into a shallow bowllikepiece of wire gauze, shaped to fit into the top of a conical test-glass with a pointed base.

The glass is filled with warm water, so that the base of the gauze just touches the surface. Chipped ice is then heaped around and over the upper part of the faeces; filling the gauze container.

To escape the cold from above, the worms begin an almost immediate migration through the strainer to the warm water, and sink into the pointed bottom of the glass. They are pipetted off and examined in wet preparation.

RHABDITIFORM LARVAL IDENTIFICATION depends on one characteristic difference: The depth of the imouth cavity (i.e., the distance from the mouth opening in the head of the worm to its junction with the oesophagus).

In Strongyloides it is short, approximately one-third of the width of the worm.

In Hookworm, the depth approximates the width of the larvae, nearly three times as long as that of Strongyloides.

FILARIFORM LARVAL IDENTIFICATION is dependent on the total length of the oesophagus.

In Strongyloides, the oesophagus occupies one-half of the length of the worm.

In the Hookworm, it extends down only one-third or less of the body-length.

N.B.—Throughout I have classed Ankylostoma duodenale and Necator americanus as one, since clinically their differentiation is unnecessary.

# PRIMARY ISOLATION OF BR. ABORTUS FROM MILK

J. J. G. PEDDIE

The following procedure has been used very successfully for many years in the isolation of Br. abortus from infected milk in cases of contagious abortion in cattle.

The medium used may be potato-broth agar, or liver-infusion agar instead of the serum-agar described (all pH 6.6-6.8)

The milk to be tested should be thoroughly shaken on receipt and then allowed to stand at room temperature for about 6-8 hours, or even overnight.

The gravity cream is used for culture purposes.

The medium preferred is serum agar — ordinary nutrient agar (pH 6.6-6.8, agar  $2\frac{1}{2}\%$ ), to which is added 10% sterile serum immediately before pouring. Ox serum is used which can be kept sterile by the addition of 5% ether, which is driven off by heating at 45°C. in a water bath when required.

To this serum-agar, methyl violet is added in the propertion of 1 in 40,000 immediately before pouring. In addition to largely suppressing the micrococci, etc., usually present, the colonies of *Brucella* take up the stain and appear as clear translucent colonies of a deep lavender colour, easily picked out from other colonies on the petri-dish.

The petri-dishes are poured and dried in the incubator for 30 minutes before use.

About 0.1 c.c. (2-3 drops) of the gravity cream is well rubbed in to the surface of the dried plate, using an "L" shaped piece of sterile glass for this purpose.

The plates are then incubated in an anaerobic jar or other suitable container, in an atmosphere of approximately 10% CO2.

Incubation is carried out for 5 days, when the *Brucella* colonies if not too numerous may measure up to  $\frac{1}{8}$  in diameter.

In the absence of a CO2 cylinder, the CO2 can be generated in a pressure flask with marble chips and dilute HC1.

The volume of the container for holding the petri-dishes during incubation is found, and the 10% volume of C02 calculated, and is run in and measured by displacement of air, ignoring the volume of glass etc. of the petri-dishes. The volume of air displaced may be measured by running a tube from the outlet cock of the petri-dish container under a measuring cylinder full of water inverted in a water trough in the usual manner of displacement experiments. When the volume of displaced air amounts to 10% of the volume of the petri-dish container both cocks on this latter are closed, the rubber tubes disconnected and the container transferred to the incubator.

# BACTERIOLOGICAL ASSISTANTS' EXAMINATION FOR CERTIFICATE OF PROFICIENCY

The 1946 Examination was held in the Medical School, Dunedin, on March 1st, 2nd, and 4th; and the examiners were Drs. Hercus, D'Ath and Mercer.

#### Practical Examination, March 1st

1. Identify specimens a-j (1 minute allowed for each. Identify and if possible write short notes on each.)

The specimens were the following: — Louse, Scabies, Ascaris worm; Avian tuberculosis in a bird; Ascaris and Trichiuris ova; Trichinella spiralis; Schistosoma mansoni; large spore ringworm in stained preparation; M. bancrofti in thick blood film; Scolices of T. echinococcus.

- 2. Obtain and prepare a sample of complement for the Wasserman reaction.
- 3. Report on the faeces provided. (Specimen completely negative.)
- 4. Serum "W" is from a convalescent case of suspected salmonella food poisoning. Investigate it as far as you are able. (The specimen agglutinated *aertrycke* serum 1-10.000.)
- 5. Make a full blood examination of the specimen of blood provided.
- 6. Report on the specimen of spinal fluid submitted. (Bacteriological examination not required.) Estimate the chloride content.

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#### Theoretical Examination, March 4th

- 1. Enumerate the reagents required to perform the Wasserman reaction and discuss their preparation.
- 2. The Medical Officer of Health requires a investigation into an outbreak of food poisoning. What specimens would you require and how would you deal with these? What foodstuffs are associated with food poisoning?
- 3. Write notes on : (a) The use of the haematocrit.
  - (b) The estimation of acidity in gastric contents.
  - (c) The prothrombin time of blood.
  - (d) Conglutination.
- 4. Discuss the preparations of penicillin which are used for local application with special reference to how they are made and the methods of control.
- 5. Discuss the laboratory procedure involved in the standard biological test for pregnancy.

#### **Oral Examinations**

#### Drs. Hercus and D'Ath

The subjects dealt with were :—Puerperal sepsis, throat swabs, food poisoning with rats and mice as carriers, guinea pig complement, C.S.F. with particular reference to cells and spiderweb clot, the composition of blood film stains, the icterus index and the Van den Bergh reaction, blood counts with particular reference to haematocrit tubes and oxalate tubes, agglutination and absorption, types of *C. diphtheriae*, types of streptococci.

#### Dr. Mercer

The subjects dealt with were :--Rh, the estimation of haemoglobin, the estimation of blood sugar, food and food poisoning,

*E. histolytica,* intravenous solutions, examinees' experience in laboratory work.

# HOSPITAL BOARDS' ASSOCIATION OF NEW ZEALAND CLASSIFIED SALARY SCALES FOR LABORATORIES OF PUBLIC HOSPITALS

#### Cadets

1st year—£130. 2nd year—£170.

#### Trainees

1st year—£270. 2nd year—£295. 3rd year—£320.

A trainee is one who (a) on commencement is the holder of a science degree, or (b) has completed two years' service in a laboratory which is a training school, and who has been recommended as a trainee by the Pathologist.

#### **Qualified Technicians**

- (a) Holder of Diploma in Bacteriology only. Qualifying salary £400, maximum £550, maximum increment £25 p.a.
- (b) Holder of Science Degree and Diploma in Bacteriology. Qualifying salary £450, maximum £550, maximum increment £25 p.a.
- (c) Sole Bacteriologist, being officer in sole charge of an approved laboratory, and who does not hold a medical degree. Appointment to be at a rate not exceeding  $\pm 50$  above the rate appropriate under (a) and (b) above, and being not more than  $\pm 550$  p.a., maximum  $\pm 600$ , maximum increment  $\pm 25$  p.a.

#### Laboratory Assistants (non-technical)

- 1st year—£220. 2nd year—£270. 3rd year—£320. 4th year—£345.
- 5th year—£390.

(Editor's Note.—These scales came into effect from April 1st, 1945. In the case of the Auckland Hospital Laboratory, most of the increases paid in February 1946 dated back to this.)

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