ol. 4.—No. 2. APRIL, 1949.

# JOURNAL OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

EDITED, PRINTED AND PUBLISHED FOR THE ASSOCIATION

BY

DOUGLAS WHILLANS

## CONTENTS



Communications regarding this JOURNAL should be sent to the Editor at the Department of Pathology, Public Hospital, Auckland, C.3.

Communications primarily affecting the Association should be addressed to the Secretary, Mr. S. O. Jarratt, Pathology Department, Public Hospital, Palmerston North.

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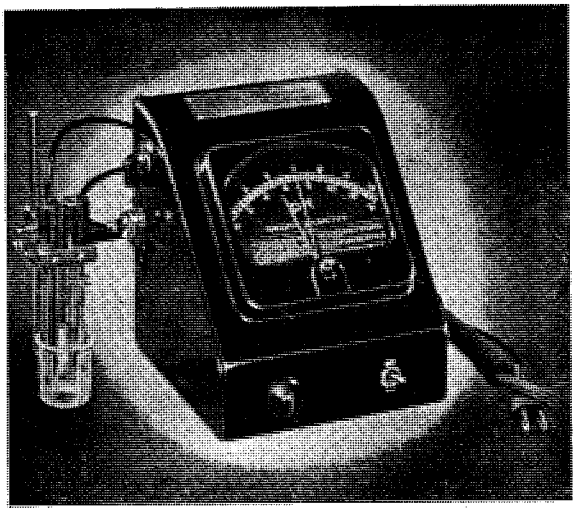

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# JOURNAL

## of the

# NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

Editors: D. Whillans, Auckland,

H. T. G. Olive, Wellington and M. O. Ekdahl, New Plymouth.

Vol. 4.—No. 2. APRIL, 1949.


## EDITORIAL

It is said that in a certain research laboratory there hangs the following inscription, "The answer, when known, will be found to be simple." Now that the attention of all senior bacteriologists is turning to the teaching of their juniors, this truism should be remembered and it is their duty to break down the almost terrifying mysteries which loom before the learner, so that he perceives that even as the enormous edifice is but a collection of bricks placed there one after another, so the acquiring of knowledge is but the building of small items of information upon a sound base. It should be remembered, too, that information in too large portions is liable to cause mental indigestion and that a garnish in the form of relevant anecdote will make the lesson more palatable.

# SALIVARY ANALYSIS AND THIOCYANATE ESTIMATION

A. FISCHMAN

*AUCKLAND*

Studies of saliva chemistry offer little to those engaged in the practice of diagnostic procedures. With few exceptions results in this field are of purely theoretical interest as yet. There is a great wealth of unsolved problems, one of these being the search for the factors which in spite of many decades of work, prevented the utilisation of salivary analysis as a practical laboratory procedure.

*Thiocyanate in Saliva*

It was with a practical aim in mind, that experiments on salivary analysis were started in our laboratory. As well known, thiocyanate treatment in hypertension is controlled by regular SCN determinations in the serum, the dosage being regulated in such a manner, that the serum level is not allowed to exceed 12-15 mgms. per cent. The remarkable fact, that saliva normally contains SCN in excess of the quantity found in other body fluids, also, that ingested SCN appears in the saliva, suggested the possibility of using salivary as a substitute for serum SCN estimations. This work yielded no useful result. It was impossible to demonstrate a constant ratio of serum and saliva values, permitting the substitution of the latter for the former.

It became evident, that technical problems different from those encountered in serum analysis, have to be taken into consideration, also that normal concentrations have to be determined prior to estimation of SCN after ingestion of the drug. Perusal of published work to explain the great variability of SCN concentration showed lack of uniformity. Disagreement in results may be found regarding percentage composition of many chemical constituents of saliva.

*Method of Collection*

One relevant finding emerged from recent studies: it is hopeless to use samples of saliva without a standardised collection procedure and expect comparable results. It seems that one of the main reasons of discrepancy between most workers in the past was, that they either used different methods of collection, or paid

no attention to method, allowing the patient to produce the specimen as he chose. It has been shown that saliva produced by spontaneous flow has a different composition from that obtained by activation. It is usual now to collect either after chewing one gram of paraffin for a specified period (activated saliva), or by attempting to exclude all stimulation as far as practicable (resting saliva; empty stomach, no brushing of teeth, no smoking, allowing the saliva to flow from mouth without movement of jaw, etc.) Outside noises and other psychological factors are reduced to a minimum. Unless a standardised method is used, and this stated, results are not comparable. Some workers as recently as 1948 make no statement regarding the method of collection. While adherence to one method tends to lessen discrepancies, even so there is no complete unanimity between different authors. Pathological changes in the calcium and phosphorus content of saliva in caries were claimed by some, and denied by others.

Treatment of the collected sample before the chemical procedure is also of some importance. To obtain a clear sample, filtration was applied in some early works. This, however, means a considerable loss of material. When collecting resting saliva, the amount is frequently small, therefore precious. Alternate methods are centrifuging or allowing to stand for a few hours. While SCN is considered to remain stable on standing, some other constituents (carbohydrates, etc.) might be affected by putrefaction. For uniform results, immediate centrifuging followed by deproteinisation is advisable. If this is not practicable, the sample should be kept in the ice-box.

*Estimation of Thiocyanate*

In most body fluids, concentration of thiocyanate is determined from the depth of colour developed with ferric nitrate reagent in a trichloracetic acid filtrate. Most early workers have omitted deproteinisation of saliva. Lately it is usual to remove proteins prior to estimation of any chemical constituent. Originally colour was developed with a ferric chloride hydro-chloric acid reagent. Schreiber replaced this by a ferric nitrate-nitric acid reagent. A slight modification by Crandall and Anderson (1934) is now commonly employed, consisting of 25 grams Fe $(NO_3)_3.9H_2O$ and 12.5 cc of $HNO_3$ made up to 500 c.c. with water. Several modifications, including the routine estimation of SCN in serum by Barker, adaption to spectrophotometer by Gregersen and Stewart (1939), and to photoelectric colorimeter by Elkinton and Taffel (1942) differ only in the proportion of the reagents added. Some precautions have to be observed. The colour developed fades in daylight. Use of artificial light is suggested, the sample to be kept in the dark, until the colorimeter is read. Reading within 10-15 minutes is also advocated. This seems un-

necessary if the sample is kept in a dark place, as under these conditions no appreciable fading occurs for many hours. Trichloracetic acid reduces the intensity of the colour, therefore adding trichloracetic acid to the standard solution is essential. Bowler suggested a modification of the reagent (1944) which eliminates the effect of trichloracetic acid on colour intensity. Most workers find the original reagent satisfactory, provided the concentration of trichloracetic acid is kept constant in standard and unknown.

Lang (1947) considered the above method non-specific and unsatisfactory for body fluids with very low concentration of SCN, such as serum of subjects, who did not ingest SCN. Nevertheless, most workers employ the ferric nitrate method even for such fluids. This objection only rarely applies to saliva. Lang's method consists essentially of forming a precipitate with silver nitrate from the trichloracetic acid filtrate, and after addition of pyridin and copper sulphate isolating a complex copper-pyridin-thiocyanate compound, giving a green colour. Another method considered accurate and specific is that of Hartner. A cadmium hydroxide filtrate is used, and from the precipitate formed by addition of silver nitrate SCN is dissolved with sodium bromide and determined by the iodometric titration of Treadwell and Mayr.

*Resting Saliva Experiments*

In our experiments fair uniformity was found in the SCN content of resting saliva in the same subject. This was shown in different ways. (1) All patients if practicable, initially had 2-4 samples tested, collected on different days. In 90 per cent of cases fluctuation in concentration was from 0 to 9 per cent. Only in 10 per cent of cases was this exceeded, showing differences up to 40 per cent. For the purpose of judging the increase in concentration after ingestion of KSCN fluctuations were negligible, as the increase in mg. per cent was much higher (50 to 120 per cent). In one typical case after initial concentrations of 13.0, 13.7 and 12.5 mg. per cent, a rise of 23.7 was found after ingestion. In another case initial values of 9.4 and 9.5 were followed by a rise of 22.5 mg per cent. (2) In a few subjects in whom estimations were made at the beginning of treatment, and many months later, a remarkable uniformity in values was shown. (3) On the whole, the response followed the change in dose. This could not have been so, had the natural fluctuation of the original value been considerable.

*Factors Influencing Composition*

The range of values indicating concentrations of salivary con-

stituents was found to be wide and there is an overlapping between values in normals and under pathological conditions. Attempts were made to eliminate factors causing overlapping. The resting saliva method tends to eliminate some disturbing influences. Others as yet to be eliminated are dietary factors, metabolic changes, drugs, climate, smoking, etc. The effect of variations in the rate of flow of saliva on composition of resting saliva has been studied. An inverse relationship of calcium and phosphorus concentration to the rate of flow has been reported by Becks and Wainwright, and of SCN from our laboratory (1948). The relationship, however, is more clear-cut when very low rates are compared with high ones. If the high rate of flow group is further subdivided, a plateau concentration is shown with increasing rate of flow.

It remains to be seen, whether or not further research will enable us to recognise abnormal values, characteristic of certain pathological states.

*Summary*

Some problems of salivary analysis, with special reference to thiocyanate estimation are discussed. Difficulties in utilising these procedures for practical diagnostic purposes, are pointed out.

*References*

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## A SIMPLE COUNTING BOX FOR MILKS AND WATERS

P. H. Curtis

*(From the Department of Pathology, Public Hospital, Auckland)*

A simple but effective box for the counting of milk and water plates has been constructed and has been thoroughly tested in this laboratory, and it is felt, may be of interest to other workers in

this country as it is easily and inexpensively constructed and saves an immense amount of time and trouble.

The general construction of the box will be evident from the accompanying diagrams. The principle of illumination is one of indirect reflected lighting on to the base of the agar plate. The petri dish rests, in a circular hole of 4 ins. diameter cut in the lid of the box, on a plain sheet of glass, 5 x 5 ins. ruled into squares with a blunt diamond cutter. (If a sharp one is used, the glass will merely fall apart). This sheet of glass is set back from the back of the lid by 1/16in. so that base of the petri dish protrudes by this amount into the box.

Seven-eighths inch behind the ruled glass sheet is a baffle for the direct light consisting of two sheets of frosted glass, 5 x 5ins. in between which has been placed a filter paper soaked in methylene blue and dried. The baffle and the ruled glass sheet are held in place by four square pegs grooved to take the glass and secured to the lid by four small carriage bolts which pass through the pegs and the lid. This enables the glass to be replaced easily if necessary.

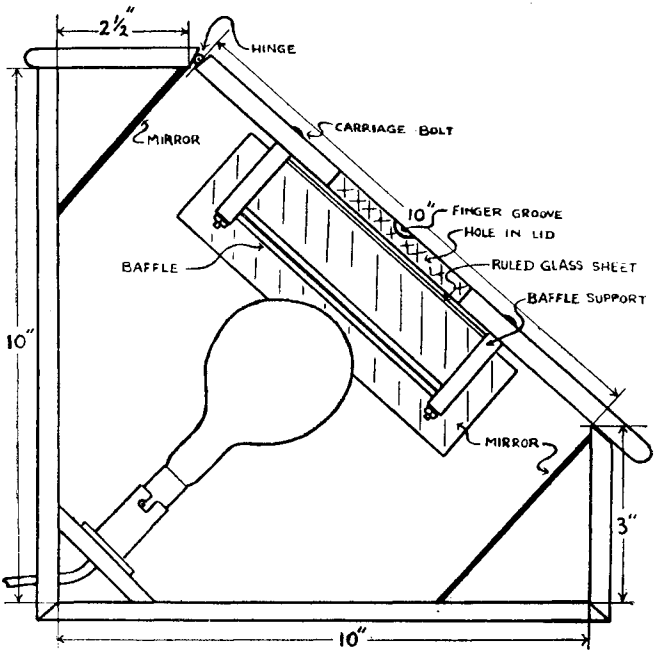
For the reflected lighting, four mirrors, 5 x 2ins. are fastened to the sides of the box at right angles to the base of the agar plate, so that the top of each mirror almost touches the lid when closed. This allows sufficient light to be reflected between the baffle and the ruled glass sheet.

Holes are drilled in the base and back of the box to allow sufficient escape of heat from the 40 watt frosted glass bulb.

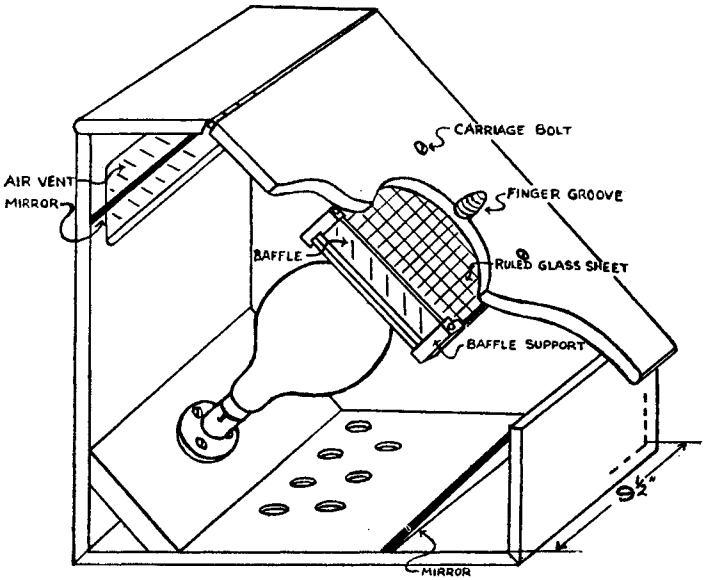
In use, the petri dish containing the colonies to be counted is placed base down with its cover off, the light is switched on, and the colonies in each square counted with the aid of a 4in. hand lens of magnification X4. By slight movement it is particularly easy to make sure that colonies one above the other are not missed and a very satisfactory stereoscopic image is obtained.

*Summary*: An effective and inexpensive illuminated box for simplifying the counting of colonies on milk and water plates is described. As well as saving time, it provides for stereoscopic visualisation of the colonies.

*Acknowledgment*: I wish to thank Dr. Lindsay Brown, Pathologist, Cornwall Hospital, for his assistance in the design and construction of this apparatus.




SIDE ELEVATION (WIDTH 9½")




A CUTAWAY VIEW.

## DEPARTMENT OF HEALTH
## NEW ZEALAND

## CERTIFICATE OF PROFICIENCY IN BACTERIOLOGY AND CLINICAL PATHOLOGY

Otago Medical School, Dunedin,

February, 1949.

*Examiners*: Professor E. F. D'Ath, Dr. J. O. Mercer and Dr. M. H. Watt.

### PAPER

(3 *hours*)

1. Describe and discuss the Bacteriological examination of water supplies.
2. Discuss the sources, transmission and laboratory investigation of plague.
3. Describe the various methods of sterilisation which may be used in a Hospital Laboratory. Discuss the principles which underlie the use of each method.
4. How are the following tests carried out:—
   (1) Quantitative estimation of the protein content of cerebrospinal fluid?
   (2) An examination of the sensitivity of a given organism to penicillin?
   (3) A reticulocyte count?

### PRACTICAL A

(3 *hours*)

1. Examine and report on the blood films A, B and C.
   (*Infectious mononucleosis, erythroblastosis and chronic myelogenous leukaemia*).
2. How would you sterilise these preparations and pieces of apparatus.
   (*Two per cent procaine, sulphathiazole powder, Seitz filter, 3 times normal saline*).

3. What is this piece of apparatus. Suggest possible uses for it in a Hospital Laboratory.

   (*Sintered glass filter*).

4. Briefly outline the mode of preparation of:—(1) Nessler's reagent; (2) Carbol fuchsin (Z.N.); (3) Pandy's fluid.

5. Examine the centrifuged urinary deposit provided.

   (*Pus and blood and amorphous phosphate*).

6. Stain by Leishman's stain the blood film provided.

7. How would you prepare a normal solution of the chemical substance provided?

   (*Potassium hydroxide*).

8. How would you obtain and prepare complement and a patient's serum for the Wassermann test?

Read under Practical B, 2, below.

(a) How would you have gone about isolating the organism in the first place?

(b) Report on the culture.

(c) What further procedure is necessary in order to identify it?

(d) What steps would you take to determine if it was the cause of the outbreak?

(*Organism was a gram negative bacillus.*)

## PRACTICAL B

(*3 hours*)

1. Tube A contains typing serum A (anti-B); tube B contains standard typing serum B (anti-A). Tubes 1, 2, 3, 4 contain cells in saline and serum of four different people. Find the group to which each belongs. If No. 1 is a patient in need of a transfusion, and Nos. 2, 3 and 4 are volunteer donors, which of them would you choose? State your technique and your reasons for your selection. Given compatibility of bloods is there anything else which would influence your choice?

(*Groups were in order A, A, O and A.*).

2. On June 4th, there was a supper attended by 39 persons. From June 5th to June 6th, 29 of these became ill, the remainder developed symptoms on June 7th. The only food stuff involved in all the cases was some cream mixture, and none of this was available for investigation. It was possible for the cream to have been contaminated in the mixing machine with some egg powder that had previously been used in a sponge cake mixture. Culture A

is a sub-culture of an organism isolated from the same sample of egg powder.

3. Examine the specimen of faeces B and C for cysts. Report on the specimen and describe your technique.

(*Giardia lamblia and E. Coli.*)

4. The hairs provided (D) are from a case suspected of ringworm. Report.

5. Appropriately stain the following fixed smears and report your findings, and state what further procedure (if any) you would carry out to confirm your diagnosis.

(a) Urethral smear; (b) Sputum; (c) Urinary deposit; (d) C.S.F.; (e) Pleural fluid; (f) Cooked meat culture from a sample of Talcum powder; (g) Peritoneal exudate from a mouse infected with material from a shipment of shaving brushes.

(*Gm-diplococcus, acid-fast bacillus, acid-fast bacillus, H. influenzae, Pneumococcus, C. tetani, B. anthracis*).

## ORALS

(*The following are stated by the candidates to have been asked in the orals. They do not necessarily cover the complete scope of questions asked and were written down from memory afterwards*).

*Dr. D'Ath*

Haemoglobin and methods of estimation, analysis of gastric contents, blood and urine sugar, types of microtomes, pH, deep freeze, Rh. Van Gieson stain, sterilisation of planocaine and of sulphathiazole powder, standard for Folin-Wu sugar method, total protein in particular, using trichloracetic acid or sulphosalicylic acid, standardisation of Beckman, photoelectric colorimeter, sterilisation of 0.85 per cent NaCl and 5 per cent glucose, colorimeters, urine urea estimation, composition of sodium hypobromite and its preparation, theory of pH meter, care of colorimeter, colorimetric pH, Folin-Wu filtrate red cell counting pipette, sintered glass filters, pyrogens, preservation of parenteral solutions, stills, histological stains, buffer solutions, vaccines, sterilisation of procaine, spectrophotometers.

*Dr. Mercer*

The staining of blood films, platelet counts, agar media, Casoni test with reading of reactions and preparation of testing fluid,

preparation of acid digestion mixture, end product of digestion of N.P.N., microscope, resolution and definition, sedimentation rate, skin tests, gastric analysis, meaning of pH, pH meters, indicators, Loeffler and preparation, Folin-Wu filtrates, Paul-Bunnell extended test, Rh and preparation of antisera, Fisher-Race nomenclature, sections and methods, urine—testing, various techniques of blood sugar, animal inoculations.

*Dr. Watt*

Preparation of calf lymph, vaccines and skin test controls, preparation of haemolysin, Rh factor, spot tests of various parasites, Buchner anaerobic cylinder, N.I.H. swabs, staphylococcus toxins, autoclaves, steam sterilisers, hot air ovens, filters, Edson's medium for culture of M. tuberculosis, animal gestation periods, and feeding of animals, K.L.B. culture and virulence, T.B. types and differentiation.

## COUNCIL MEETING

This was held in the X-ray Department of the Palmerston North Hospital on February 26th, 1949, at 10.30 a.m.

There were present Mr. N. J. Ellison (President and Chairman of the meeting), Messrs. E. L. F. Buxton and D. Whillans (vice-presidents), Messrs. G. W. McKinley, H. T. G. Olive, and M. O. Ekdahl (ordinary members of Council), and Mr. S. O. Jarratt (Hon Secretary). Apologies were received from Messrs. J. A. Samuel and D. H. Adamson.

The following resignations were received: Mr. G. B. Kiddle (Wellington), Mr. C. Felmingham (Palmerston North), and Mr. R. B. Stockwell (Auckland).

The following junior members were admitted:—Mr. F. E. Kershaw (Dunedin), Mr. B. W. Main (Oamaru), Miss Wyllie (Waikato), Miss S. Perl (Auckland).

During the discussion on the accounts, it was made clear that now the finances of the Association were on a firm footing, Council members could not be expected to continue to pay their own expenses to Council Meetings. In a certain number of cases, the Hospital Board concerned had been assisting in travel fares, but this has now been barred by the Department of Health's auditors, and that a considerable sum would need to be put aside to meet these payments.

Donations for both Junior Essay Competition and Junior Technical Competition have now been received and these competitions will close with the Secretary on July 1st, 1949. Competitors are referred to the amended Rule 27. (See last Journal).

No further official word of the Intermediate Examination has been received. While it had been hoped that the first examination would be held in Auckland in May, 1949, it now seems too late to hope for this. The Department of Health, however, will send circulars to all Laboratories when the details are settled.

Further word on the Diploma question is awaited and will be circulated. The amended syllabus will also be decided upon at the same time.

The Salaries and Conditions of Employment Regulations, 1948, as applying to Bacteriologists brought forward a deal of helpful criticism which will enable the members of the Advisory Council to bring forward other views later in the year on the termination of the present agreement. In general the regulations were satisfactory and points which were not satisfactory were to be brought before the Minister. In particular, it was agreed that 3. Laboratory Assistant (c) should read "After the eighth year of service or earlier an amount not exceeding £500 per annum on the recommendation of the Pathologist as approved by the Minister in the special circumstances."

## HERE AND THERE

*Auckland.*—Dr. E. F. Fowler has very generously added to the list of donations to the Publishing Fund with a further cheque for five guineas. This is his second such donation, and is most acceptable.

Miss S. Perl, formerly of the Otago Medical School, has joined the staff of the Auckland Hospital Board's Laboratories.

*Wellington.*—Members of the Wellington Hospital Board's Laboratory are co-operating with the Wallaceville workers in organising the next conference to be held on July 29th and 30th next. They would be grateful for any promises of material for demonstrations and lectures.

*Marriages:*

Miss M. R. Harrow, of the Wanganui Laboratory, to Mr. J. Brock.

Miss Isobel Munro, of the Waipukurau Laboratory, to Mr. Roderick Chisholm.

Miss Kathleen Riley, of the Waikato Hospital Laboratory, to Mr. Leonard Gilbert.

Copies of the Hospital Employment Regulations, 1948, Amendment No. 2 (1948/192) may be obtained from the Government Printer, Wellington, at a price of 6d each. It is recommended that every member of the Association purchase a copy. In the case of certain Laboratories, one member of the staff arranged to purchase a number of copies for distribution, thus saving postage.

A few complete sets of the JOURNAL are now available for sale, at the following prices:—Vol. 1 (4 numbers), 5/-; Vol. 2 (3 numbers), 3/6; Vol. 3 (4 numbers), 5/-, post free. Single copies, if available, 1/6, post free. Preference will be given to those desiring full sets, or numbers to complete full sets. Orders will be filled in rotation of posting date and should not be accompanied by money. If supplied, sets or numbers will contain an account which must be paid to the Treasurer.

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