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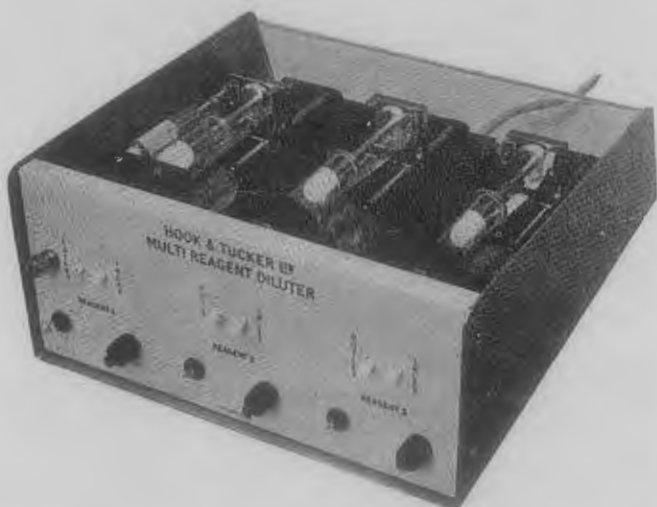
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A Modified Procedure for the Estimation of Serum Haptoglobins Using Sephadex G-100

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Central Laboratory, Auckland Hospital.

Received for Publication, July 1968.

Introduction:

Haptoglobins are a group of closely related glycoproteins found in normal human serum. They can bind haemoglobin *in vivo* and *in vitro*. By the conventional electrophoretic techniques for serum protein separation, haptoglobins migrate with the α_2 globulin fraction (Javid ⁴).

In vivo, the binding of any plasma haemoglobin by haptoglobin is the normal mechanism by which plasma haemoglobin is removed from the circulation and taken up by the reticulo-endothelial system. In the liver, the enzyme heme alpha-methenyl oxygenase degrades the haemoglobin from haptoglobin combination. This occurs more rapidly for type 2-2 haptoglobins (Javid ⁴). Haemoglobinuria may occur when the plasma haemoglobin concentration exceeds the combining capacity of the haptoglobin. On the occasions that haptoglobin is not available, a beta globulin called haemopexin will combine with and remove haemoglobin and hematin. When neither haptoglobin nor haemopexin are present, haemoglobin breaks down to haematin and is bound by albumin to form methaemalbumin.

In vitro, the haemoglobin binding capacity of haptoglobin is used to express the amount of haptoglobin present in serum, by measurement of the haemoglobin present in combination (Owen *et al.* ⁷).

When acute or chronic intravascular haemolysis causes increased turnover of serum haptoglobin (Hp), levels often become temporarily low or even absent. This may also happen in liver cell disease, in toxoplasmosis and in infectious mononucleosis (Nyman ⁶). Nevertheless, with careful interpretation the Hp level may still be regarded as a useful sign of increased haemolysis in the diagnosis of haemolytic anaemias (Nyman ⁶) or of incompatible blood transfusion (Fink *et al.* ²). Inflammation, infection and steroid therapy often cause raised Hp levels which mask the effects of increased haemolysis (Nyman ⁶).

Principle of Estimation:

When excess haemoglobin (Hb) is added to a serum aliquot, Hb-Hp complex is formed. If this is allowed to filter down through a column of the dextran gel Sephadex G-100 (Pharmacia) washed with 2g./100ml. sodium chloride solution, the bigger mole-

cules of Hp-Hb complex travel faster down the column and are collected ahead of, and separated from, the small molecules of the excess free Hb which follow. See Figure 1. (The smaller molecules have a longer route in the gel pores.)

Collection of the appropriate aliquot from the column and estimation of Hb therein gives the amount of Hp present expressed in terms of Hb binding capacity (Hodgson & Sewell³; Ratcliff & Hardwicke⁹; Lionetti *et al.*⁵). Modified use of the Parker Lewis⁸ method for estimation of plasma Hb results in more accurate measurement of very low levels of Hb than is possible by direct reading techniques, where turbidity not apparent to visual inspection may cause inaccurately high levels of Hb to be read on the spectrophotometer.

The Parker Lewis⁸ method relies on haemoglobin acting with hydrogen peroxide as a peroxidase system oxidising orthotolidine to give a coloured product which is measured at 630 m μ (pH 2.2).

Reagents:

Eluant: 100 g. sodium chloride is made up to 5 litres with deionised water. Eluant is boiled in litre volumes prior to use, to drive off any dissolved gases.

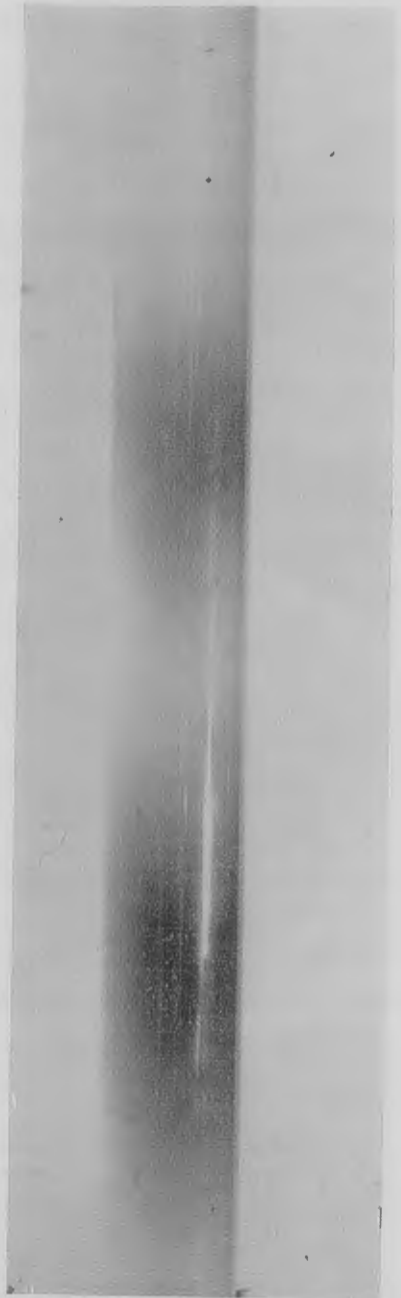


Figure 1: Showing the two haemoglobin bands in the Sephadex G-100 column. The lower band contains haemoglobin bound to serum haptoglobin; the upper band is excess haemoglobin.

Haemolysate: With a fresh blood of Hb near 13.5 g.%, wash red cells three times with isotonic saline. Add de-ionised water to make haemolysate with a haemoglobin level between 12.5 g.% and 13.5 g.%. Shake for 5 minutes and centrifuge at 3000 r.p.m./10 minutes (*i.e.* 1200 G). Dispense into sterile bijou bottles in 0.5 ml. aliquots and store at -20°C .

Ortho-tolidine Reagent: Dissolve 0.25 g. ortho-tolidine (A.R.) in 80 ml. glacial acetic acid and add 10 ml. de-ionised water. Mix and make up to 100 ml. with glacial acetic acid. Store at 4°C . and re-make every 8 weeks. (Tends to darken normally). Care in handling ortho-tolidine is essential as it has been reported as a carcinogen.

Hydrogen peroxide: Take 1.2 ml. of a 100 volumes solution (30% w/v) of hydrogen peroxide and make up to 100 ml. with de-ionised water. Stable for 3 days at 4°C .

Stock Haemoglobin Standard: 2 ml packed washed red cells are haemolysed with 2 ml. de-ionised water and 0.2 ml. of ether by shaking for 3 minutes. Centrifuge 10 minutes/3000 r.p.m. Estimate total haemoglobin of haemolysate supernatant (0.1 ml. into 25 ml. Drabkins or modified Drabkins solution and comparison with a conventional cyanmethaemoglobin standard.) Dilute to exactly 10 g.%. Again dilute 0.2 ml. of that solution with a "to contain" pipette into a volumetric flask and fill to 100 ml. with de-ionised water. Filter through a cellulose acetate membrane ($0.45\ \mu$). Ascertain the exact value of this standard by taking about 15 ml. and adding 1 drop 10% sodium cyanide and one drop of 5% potassium ferricyanide and reading after 20 minutes. (It should be near 20 mg.%) Store stock at 4°C . (This standard is also used for the plasma haemoglobin method.)

Diluted Haemoglobin Standard: For use the above stock is diluted 1 in 10 with de-ionised water.

Acetic acid diluent: 50 ml. glacial acetic acid is diluted to 500 ml. with de-ionised water.

Glassware should be specially clean (10% Decon 75) and all distilled or de-ionised water used should be free from contaminating "peroxidase" activity.

Preparation of the Sephadex G-100 column:

Weigh approximately 6-7 g. Sephadex G-100 and soak in excess eluant for two days to allow swelling and formation of a gel slurry.

Clamp vertically a glass tube 40 x 1.5 cm., closed at the bottom by a rubber bung with glass stop-cock on an outlet tube of 4 mm. internal diameter. Above the bung place layers of graded glass beads over a thin layer of medium glass chips. (The total depth of all this is 13 mm.) Align the tube vertically with a plumbline³. See Figure 2 for apparatus assembly.

Fill column with eluant to quarter depth and evenly run the gel slurry into this until the column gel bed is finally within 3 cm. of top. After $1\frac{1}{2}$ hours, open stop-cock.

Wash through column with eluant for total of 12 hours, to pack gel. Connect to a reservoir 500 ml. Quickfit flask fitted for continuous flow and constant level. The hydrostatic head should not be very great but *do not ever let the column run dry*.

Into the 2.5 cm. head of eluant in column, float a filter paper disc carefully on to the top of the gel bed which should be even and absolutely flat. Renew the filter paper daily.

Calibration of Sephadex Column Effluent:

The haemoglobin/haptoglobin complex is most conveniently collected as a 9 ml. aliquot of effluent. In order to determine the point at which this aliquot is to be collected it is necessary first to establish the "void volume." (See Figure 3).

This is done by collecting 2 ml. aliquots from the moment the serum with haemolysate is applied at the column head, and estimating the haemoglobin per sample. (See "Method" below). If a normal serum is used the first 14 or 15 ml. of effluent may be discarded, but exact

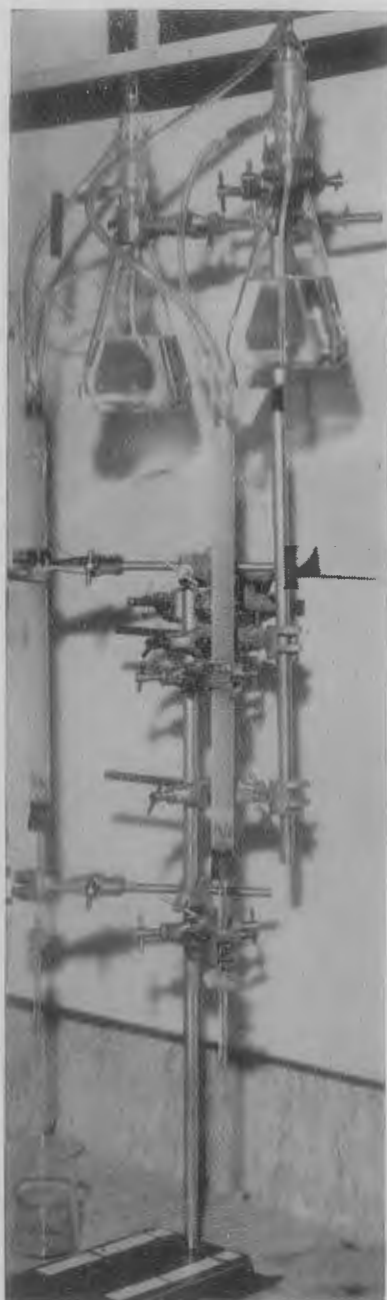


Figure 2: Showing two Sephadex G-100 columns clamped in position with reservoir flasks connected. At the top of the left hand column, serum with haemolysate has been applied.

measurement of the discarded aliquot should be made.

The position of the Hp-Hb band makes it obvious when the 2 ml. collections for haemoglobin estimations should begin.

If column and packing are kept of constant dimensions it is not necessary to calibrate the column effluent for every newly-established Sephadex column.

As will be seen in Figure 3, the trough between peaks of Hb does not drop to zero Absorbance as do the initial effluent aliquots. Even if 0.4 ml. aliquots are collected instead of 2 ml. aliquots for the "trough area," it is not possible to lower the curve in the trough. Although it is of no great practical value in the haptoglobin estimation, the cause of the peroxidase activity of this portion of effluent has still not been traced in spite of attempts to solve its nature.

Method:

Place 5 mg. solid sodium chloride in a $3 \times \frac{3}{8}$ inch test tube, and add exactly 0.5 ml. test serum and 0.02 ml. haemolysate; (2.5 mg.Hb approx.) Mix gently, avoiding bubbles.

Turn off the reservoir and let the filter paper run just dry before turning off bottom stop-cock.

Carefully, with a very fine Pasteur pipette, place all the test mixture on to the filter paper and drain into gel. Close the stop-cock and pipette a little eluant on to the top of the filter paper.

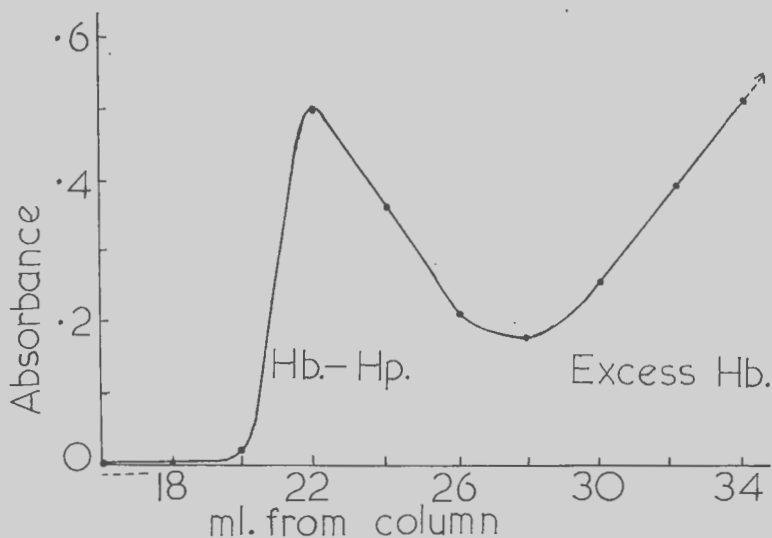


Figure 3: Showing calibration of a Sephadex G-100 column by collecting 2 ml. aliquots after the first 14 ml. of effluent was discarded. Colour proportional to the haemoglobin was developed from samples of each 2ml. aliquot and Absorbances at $630 m\mu$ were plotted from a Unicam SP 600 spectrophotometer.

Place a 25 ml. cylinder beneath the column; let the column run and open the reservoir of eluant.

Collect aliquots of 15 ml. (for example) and then 2 ml. and then the 9 ml. which contains the haemoglobin-haptoglobin band (lower red) and 1.0 ml. which should appear free of Hb. (see "Calibration" above).

Then let the column run to elute all free haemoglobin in the upper band and discard this.

The column is then ready for another test; the whole procedure takes 1 to 1½ hours.

To estimate the haemoglobin bound to haptoglobin, proceed as follows:—Pipette 2.5 ml. ortho-tolidine reagent into each of three 5 x 5/8 inch test-tubes.

To the first tube add 0.2 ml. of the effluent aliquot containing the Hb-Hp complex (test).

To the second tube, add 0.2 ml. dilute haemoglobin standard (Standard).

To the third tube, add 0.2 ml. de-ionised water (Reagent blank).

After 2 minutes pipette from each tube a 1 ml. aliquot into appropriately labelled test-tubes.

Add 1 ml. dilute hydrogen peroxide to each tube, mix, and leave for exactly 10 minutes.

Add 2.5 ml. dilute acetic acid to each tube.

Mix and read Absorbance immediately at 630 mμ (Unicam SP 600 is a suitable spectrophotometer to use).

Calculation:

$$\frac{\text{Absorbance of test} \times \text{Dilute standard Value mg\%}}{\text{Absorbance of standard}} = \frac{Z \text{ mg Hb/100 ml. Aliquot}}{100} = Z \text{ mg. Hb/ml.}$$

Serum Haptoglobin = Hb in mg./ml. x aliquot vol. Hb/Hp in eluant x volume adjusted for 0.5 ml. serum

$$= \frac{Z}{100} \times 9 \times \frac{100}{0.5} \text{ mg. per 100 ml.}$$

(expressed as "haemoglobin binding capacity").

Normal Range:

95% of normal people have a serum haptoglobin in the 28-200 mg./100ml. range. The common genetic types of haptoglobin are 1-1, 1-2, and 2-2. The normal range of haptoglobins has been shown to vary with the genetic types (Nyman⁶) but there is a great overlap of values between types.

Nyman⁶ gives values as follows:—

Type 1 — 1	77 — 194 mg./100 ml. serum
Type 2 — 1	32 — 184 mg./100 ml. serum
Type 2 — 2	15 — 149 mg./100 ml. serum

The mean for females is slightly lower than the mean for males.

To know the type of the haptoglobin only becomes of practical value in the interpretation of borderline values in the 25—35 mg.% range; these may be normal for type 2 — 2 but greatly lowered values for 1 — 1. The newborn have no haptoglobin, but a level of 30 mg./% is found within 6 months. (Bergstrand *et al.*)¹ Haptoglobins are typed by starch gel electrophoresis (Smithies)¹⁰.

Direct Quantitation of Haemoglobin in Aliquot: (Ratcliff & Hardwicke)⁹

It is possible to obtain the haemoglobin value by reading, at 415 m μ , the absorbance of the aliquot on a very good spectrophotometer. This is, however, not recommended for routine use.

After doing 170 haptoglobins by both direct and peroxidase colour development methods, it was found that very faint cloudiness was frequently interfering with the direct readings and giving falsely high haptoglobin values.

The faint cloudiness was very difficult to detect visually and probably originated in chylous or pseudo-chylous sera. It is possible that if fasting sera were available routinely, the problem would be overcome; however, this is not practical in day-to-day work.

Another possible way to overcome faint turbidity is the use of detergents such as Nonidet P40 (Shell) or Lissapol NDB (ICI). In fact, encouraging results were obtained by adding 1 drop of Nonidet P40 to the 9 ml. aliquot collected from the column and shaking very vigorously before reading at 415 m μ after froth had completely cleared; but occasionally even this technique was unreliable. A calibration graph for a Unicam SP 600 spectrophotometer with Hb values from 0 to 0.1 mg./ml. and the calibration line extrapolated to cover values up to 0.13 mg./ml. permitted immediate conversion of Absorbance to haemoglobin.

Correlation with other Methods of Estimation:

(a) *Starch gel electrophoresis (Smithies)*¹⁰

A series of 300 serum haptoglobins were done in parallel by starch gel electrophoresis at the Auckland Blood Transfusion Service laboratory and by this Sephadex column method at Auckland Hospital Laboratory. The starch gel bands were visually evaluated and reported as decreased, normal or increased levels. Correlation of values was excellent in 80% of tests done. On the occasions when correlation was poor, it was usually a type 2-1 haptoglobin. The significance of this is a further field for investigation.

(b) *Colorimetric Guaiacol Method (Owen et al.)*⁷

The Sephadex method is preferable in many ways to the guaiacol method for routine clinical laboratory use. It is less time-consuming and gives more reliable results. In a series of 80 serum Hp estimations done by both the Sephadex and guaiacol methods, correlation was not good.

Discussion:

When the serum and haemoglobin are allowed to run through a Sephadex G-100 column, only two bands separate: namely, the Hp-Hb band and the free haemoglobin which follows it. If the serum bilirubin level is elevated above normal, bilirubin is seen as a band in contact with the lower edge of the free haemoglobin zone. Its molecular size in combination with albumin is slightly larger than the free haemoglobin molecule.

During many months, when some hundreds of sera were tested for haptoglobin levels, only once did a third band appear. Visible by its faint haemoglobin tinge it was slow-moving and obviously had a much smaller molecule than free haemoglobin. It was still near the top of the gel column when the Hp-Hb complex was at the bottom. The serum came from a patient who had a mild haemolytic condition due to excessive ingestion of phenacetin. It was not possible to establish the identity or significance of this third band on the column.

Haemolysed sera from patients do not pose any problem in the main method for Hp estimation described in this paper, and this is an advantage in daily laboratory routine. Gross haemolysis provides excess haemoglobin needed in the test. However, it is advisable to add extra haemolysate as a precaution for binding to possible high levels of serum Hp.

An excess of haemoglobin is present when a haemolysate of 13g.% is used. Then, theoretically, 2.6 m.g Hb are available for combination with the haptoglobin in the 0.5 ml. serum. This allows for enough Hb to combine with Hp of a 520 mg./100ml. serum. However, this does not seem to be verified in practice. In the few very high serum haptoglobins found, visible haemoglobin has all been bound by Hp of about 350 mg./100 ml.

Serum protein dyscrasias can occasionally cause conflicting haptoglobin results between the starch gel electrophoresis method and the Sephadex column method. One patient's serum, with an increased IgA protein, persistently showed no haptoglobin on starch gel electrophoresis and yet equally often gave a Sephadex Hp value of 40 mg./100 ml. serum. This was probably due to the filtration effect of starch gel on the IgA polymer.

Dextran gel such as the Sephadex G-100 used in the column for Hps is nutritional material for bacteria and moulds, but in practice only the top exposed surface of the gel seems to grow occasional small colonies within the 4 week period for which each column is used. This is partially overcome by discarding the top filter paper disc each day. Preservatives such as sodium azide are often used, but this was found to interfere with the peroxidase ortho-tolidine colour development. In practice it was found unnecessary to use a preservative. Buffer salts dissolved in the 2% sodium chloride solution (Hodgson and Sewell)³ were also found to be unnecessary.

Flow rate of these Sephadex columns is variable; they slow down with age and use, but this is partially overcome by daily washing with sodium chloride eluant for about 15 minutes.

Sera for Hp estimation should be separated from the red cell clot as soon as possible and can be stored deep-frozen (-20°C) for at least 2 weeks before Hp estimation. In fact control sera have been quite stable for six months or more as long as they are only thawed once.

Summary:

1. A modified procedure is described for the estimation of haemoglobin in combination with serum haptoglobin separated from excess free haemoglobin by means of filtration through a Sephadex G-100 column.
2. This peroxidase ortho-tolidine method is evaluated by comparison with:
 - (a) A sephadex G-100 method with direct spectrophotometric reading at $415\text{ m}\mu$.
 - (b) A starch gel electrophoresis method for Hp estimation.
 - (c) A guaiacol colorimetric method for Hp estimation.
3. Haptoglobin function in the body is reviewed briefly.
4. Aspects of the Sephadex G-100 column method are discussed, including
 - (a) The finding of a third unidentified band tinged with Hb, or its derivatives;
 - (b) The finding of a serum with IgA which could be estimated for Hp on a Sephadex column but not by starch gel electrophoresis.

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The Staining Reaction of Neutrophils

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Received for publication, September, 1968.

Introduction

The strongly positive periodic acid-Schiff (PAS) reaction in the cytoplasm of human polymorphonuclear leucocytes has been attributed to the presence of glycogen (Wislocki, Rheingold and Dempsey, 1949⁷; Gibb and Stowell, 1949³). However, Pearse (1960⁵) found that a considerable amount of PAS positive material remained in human neutrophils in blood smears and in formalin-fixed paraffin sections after the prolonged action of saliva or diastase. The PAS reaction was applied to milk smears to demonstrate the neutrophils, as an aid in the detection of mastitis (Young, 1968⁸). It became suspected, while staining neutrophils in milk samples, that because of the positive reaction in fragmented cells from which glycogen would be expected to have dissolved before fixation, further substances were involved.

The PAS reaction is widely used in the study of leukaemia, and several recent publications refer to neutrophil staining as probably due to the presence of glycogen (Dacie and Lewis, 1963²; Sewel, 1967⁶). As a result of the conflicting evidence concerning the nature of the material in the neutrophils, some of its properties were investigated; particularly the effects of digestion and of various fixatives and solubility.

Materials and Methods

- (1) *Neutrophils* in fresh human peripheral blood smears.
- (2) *Glycogen-rich liver cells* in smears and paraffin wax sections. These were obtained from a guinea pig and a mouse, both freshly-killed, healthy animals.
- (3) *PAS technique* (Young, 1968⁸).
- (4) *Best's carmine method* (Carleton and Drury, 1957¹). This stain is considered to be highly specific for glycogen, but will stain some other substances such as mucin and fibrin. These are coloured so faintly compared with the staining of glycogen that confusion is unlikely.
- (5) *Digestion techniques*: 1% malt diastase in pH 7.0 buffer solution for 1 hour at 37°C and saliva for 30 minutes at 20°C. Glycogen can be depolymerised by digestion with these enzymes and thereafter will give negative staining results.

Note: Unless otherwise stated, the smears were fixed immediately after being air-dried and the blocks of tissue for the sections were fixed within a few minutes of the death of the animal.

Experimental

Digestion

Several blood and liver cell smears were fixed in formalin vapour for 15 to 30 minutes and stained by the PAS technique and Best's carmine method. Some smears from each source were digested with saliva and others with diastase before being stained. The results are shown in Table I.

Table I: Comparison of the Staining Reactions of the Cells in Liver and Blood Smears.

Staining Methods	Liver Cells		Blood Neutrophils	
	Before digestion	After digestion	Before digestion	After digestion
PAS	++	—	++	++
Best's Carmine	++	—	+	—

Code: ++ The majority of cell cytoplasm dark red.
 + Occasional cells showing some small red granules.
 — No appreciable staining.

Note: Digestion with either saliva or diastase gave the same results.

These results appeared to show that the neutrophils contained relatively small amounts of glycogen and that PAS positive substances in addition to glycogen were present.

Further tests were conducted comparing salivary digestion of formalin vapour-fixed fresh peripheral blood smears with formol saline-fixed wax sections of guinea pig and mouse livers containing large amounts of glycogen. It was found that the glycogen was completely removed from the liver sections in 20 minutes by saliva and that prolonged digestion of the blood smears from 1 to 2 hours did not appreciably affect the intensity of the PAS staining.

Fixation

Several different fixatives, whose glycogen - preserving qualities in histological sections are known, were used on fresh peripheral blood smears in the following experiment. The fixatives used and the results of the experiment are shown in Table II.

Table II: Comparison of Various Fixatives on Blood Smears.

Fixatives	Glycogen fixing quality (Manns, 1958)	PAS staining of neutrophil cytoplasm
Carnoy's fluid	Excellent	Poor
Rossmann's fluid	Excellent	Strong
Acetic formol alcohol	Excellent	Moderate
Formol alcohol	Good	Strong
Formol Sublimate	Adequate	Strong

The most notable results were that the neutrophils stained strongly with PAS after formol sublimate fixation (the poorest glycogen fixative used), whereas they stained weakly after fixation with Carnoy's fluid (an excellent glycogen fixative).

Methyl alcohol is a commonly used haematological fixative and is recommended as a fixative for blood smears prior to PAS staining (Dacie and Lewis, 1963²). The qualities of methyl alcohol as a fixative for glycogen and for the PAS positive material in neutrophils were investigated by using it to fix some blocks of fresh guinea pig and mouse livers and, for comparison, some blood smears. The blocks of liver were processed to wax and sectioned. Salivary digestion was used to remove the glycogen from control sections. Subsequent staining showed that, while methanol was quite a good fixative for the glycogen in liver cells, it gave variable preservation of the material in neutrophil cytoplasm as the staining intensity varied from smear to smear.

The period of fixation of the smears in methyl alcohol was varied from 2 minutes to 2 hours, and the interval between obtaining and smearing the blood and fixing the smear was varied from a few minutes up to several days. The results obtained were again variable and showed no definite pattern.

Some fresh blood smears were fixed in Rossman's fluid, this fluid having been used by Wislocki *et al.* (1949)⁷, in their experiments. Half of these smears were treated with diastase and subsequently all the smears were stained with PAS, together with formalin vapour fixed smears as controls. The Rossman's smears showed dark red neutrophil cytoplasm in the untreated smears and pink cytoplasm in the digested smears. The formalin vapour control smears showed dark red cytoplasm in both digested and untreated smears.

Formol saline (10%) was compared with formalin vapour as a fixative for blood smears. Although the saline gave a weaker staining reaction with PAS than the vapour the intensity of staining of the neutrophils was not diminished by digestion. The fixation period in formol saline was varied from a few minutes up to several days using a series of smears; however, these variations of the fixation period did not intensify subsequent staining.

Solubility

All the smears had been air dried after fixing and before staining. To test the water solubility of the PAS positive material, this procedure was altered and some were washed in water immediately after fixing and were then stained by the PAS technique. Three fixatives were used: formalin vapour, 5% formol alcohol and ethanol. The results are shown in Table III.

Washing made no appreciable difference with the formalin vapour smears, but reduced the PAS positive material in the

Table III: The Water Solubility of the PAS Positive Material after Fixation.

Fixative	PAS staining of neutrophil cytoplasm	
	No wash	After washing
Formalin vapour	+++	+++
5% Formol alcohol	+++	++
Ethanol	++	+

neutrophils of the formol alcohol smears, and left only small amounts of red granular material in the neutrophils of the ethanol smears.

The solubility of the material in chloroform was tested by comparing smears fixed in formalin vapour. It was found that smears rinsed in chloroform prior to formalin vapour fixation contained less PAS positive material than those not treated with chloroform.

Summary of findings

Most of the PAS positive material in the cytoplasm of neutrophils is:

- (a) Not well fixed by methyl alcohol.
- (b) Not well fixed by ethanol and can be partially removed by water after being treated by it.
- (c) Fixed well by formalin vapour, after which it resists removal by water.
- (d) Not well fixed by Carnoy's fluid or acetic formol alcohol, which are both excellent glycogen fixatives.
- (e) Highly resistant to digestion by diastase or saliva after formalin vapour fixation.
- (f) Slightly soluble in chloroform prior to fixation.

Discussion

The findings reported here showed that the bulk of the PAS positive material in the cytoplasm of neutrophils does not behave like glycogen, particularly after formalin fixation. This appears to confirm the findings of Pearse (1961)⁵, who stated that his findings could be due to:—

- (a) the presence of carbohydrate-containing substances other than glycogen, or
- (b) the presence of lipid substances, or
- (c) incomplete removal of glycogen by diastase.

He concluded that the material demonstrated was mainly a carbohydrate-protein complex, as incomplete removal of glycogen by diastase has not been recorded and lipids could not be responsible for the reaction in paraffin sections. However, as reported by Wislocki *et al.* (1949)⁷, the material did digest in the same manner as glycogen when fixed by Rossman's fluid.

Formalin vapour would appear to be the most suitable fixative for the demonstration of cell types by the PAS reaction.

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C. S. Curtis,
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Use of the Kelsey Capacity Test for Disinfectant Evaluation

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Received for publication, July, 1968.

Introduction

Traditionally bacteriological evaluations of disinfectant materials have been based on the Rideal-Walker (1903) and Chick-Martin (1908) test techniques. These have been modified in various ways by different workers, but even now they are still used widely throughout the world as a basis for:—

- (a) registration with Health Departments or other regulatory authorities,
- (b) attempting to determine effective use-dilution figures,
- (c) comparing the relative efficiency of different antiseptics and
- (d) routine quality-control during production.

These two tests and their variations have tended to be used, in many cases, on the mistaken premise that all disinfectants will have properties directly comparable with phenol, and in the early years of this century, when phenolic germicides were the only types in use, this argument held good. However, in latter years, with the range of available materials growing ever wider, it has become obvious that the reliance placed on the Rideal-Walker and Chick-Martin tests by so many people is no longer justified.

When considering test methods, Sykes (1962)⁴ drew attention to the need for clarity about the purpose of the determination: "whether it is simply to check the quality of routine production material, or whether it is to assess the performance characteristics of a preparation, for these two approaches are quite different. The former is concerned only with finding out whether the preparation conforms to a given set of conditions — conditions which, as in the Rideal-Walker and similar coefficient tests, are quite artificial in many respects — whereas evaluation implies determining whether it is efficacious in practice for the purposes required; that is, whether it remains active under a variety of natural conditions, against a varied range of organisms with possible differences in temperature, in periods of contact, in the concentrations and types of organic matter present, and so on . . ." This is not to say that any given test method may not be applicable to both purposes, but it is necessary to see the pitfalls in applying the results of any determination, and it is evident that the phenol-coefficient, in the light of present-day circumstances, has very limited value other than for in-factory control purposes.

Government regulations concerning disinfectant materials in New Zealand are not concerned with tests other than the Rideal-Walker test, and there is no rigidly controlled "official" system of standard methods as is the case in the U.S.A.⁵ When writing

about the testing of disinfectants in the United Kingdom, Walters (1963)⁶ suggested the adoption in the U.K. of some system of evaluation tests such as the U.S. one, and the same suggestion could well be made in New Zealand. Whether the U.S. series of official tests should be adopted in its entirety is open to question, and suggestions have been made that not all these tests give easily reproducible results, and that the Use-Dilution Test does not distinguish adequately between disinfection and detergency.

If this is the case, and alternative tests are desired, then the enquiring reader is faced with a bewildering array of suggestions. There is a multiplicity of published "new" test techniques, many of them evolutionary stages of previously published methods, and many of them evolved to show a particular new product in a more favourable light than tests at that time available. However, one method which has been found of value by the author is the Capacity Use-Dilution Test of Kelsey, Beeby and Whitehouse (1965).¹ This technique was evolved at the Disinfection Reference Laboratory of the Public Health Laboratory Service in London, in an attempt to provide a test which was simple, realistic and reproducible, which could take into account organic soil, and which could use the more troublesome pathogens as test organisms. In this technique the test organism (*Pseudomonas aeruginosa* or *Staphylococcus aureus*) is suspended in either nutrient broth or yeast suspension and added to the disinfectant under test in a series of increments at fixed intervals, the mixture being tested for sterility by subculture immediately before each new addition. In practice a use-dilution is considered satisfactory if 3 or more increments can be added before a positive culture is obtained, and the results can be expressed either in terms of the number of increments giving no growth or in terms of the dilution at which the disinfectant will pass the test.

An advantage of this test is that it requires no special apparatus in the way of stainless steel cylinders etc. (as in the A.O.A.C. Use-Dilution Test) and can thus be performed in any laboratory at minimal expense. However, it must be emphasised that any bacteriological testing of this nature will be reliable only when performed by a worker with experience in this field. Most test techniques lay down specific conditions which must be adhered to rigidly if the results are to be credible or reproducible, and the manual dexterity required for these techniques is not necessarily acquired at the first attempt. Furthermore, results obtained from a single test should be treated with caution until confirmed by repeat testing.

While this technique was originally intended for testing phenolic disinfectants, and as such was specifically recommended only for use with phenolics, experience has shown that it gives comparable results for other types of disinfectants and is therefore of value for comparative purposes. This has been confirmed by Dr Kelsey (personal communication). However, when testing materials other than phenolics it is advisable to include an appropriate neutraliser in the nutrient broth used for sterility subcultures.

Test Organisms

Cultures of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were obtained from the New Zealand National Health Institute in Wellington, each culture being of an antibiotic-resistant strain which had proved troublesome in the hospital environment. The required organism is inoculated into 20 ml. of nutrient broth and incubated 18 hours at 37°C. When a yeast suspension of organisms is required the broth cultures are centrifuged and the supernatant replaced with 20 ml. of yeast suspension. This has been found to give a concentration of approximately 10^9 organisms per ml. for the pseudomonads and 2×10^8 organisms per ml. for the staphylococci.

Yeast Suspension

This was made as specified in BS808:1938 (NZSS 585) for the Chick Martin test to contain 5 per cent. dry weight yeast at pH 6.9 to 7.1, except that "TONYC" brand compressed yeast (manufactured by The Dominion Compressed Yeast Company Ltd.) was used.

Recovery Medium

Commercial dried media (*Oxoid* Nutrient Broth No. 2, Code No. CM67) is used both for providing the initial organism suspension and as a recovery medium. Rubbo and Gardner (1965)³ quote a list of appropriate neutralisers which may be made up with this broth as required.

Dilutions

Disinfectant dilutions should be made in sterile glass measuring cylinders using sterile distilled water as diluent. Stock dilutions may be kept for 1 week.

Method

12 ml. of the disinfectant dilution under test are placed in a sterile 4 oz. screw-capped jar. At 10 minute intervals 2 ml. of the organism suspension are added and the contents mixed by swirling. Eight minutes after each addition the pots are sampled by withdrawing some of the mixture in a sterile 50 dropper pasteur pipette and adding one drop (0.02 ml. approx.) to each of two 1 oz bottles containing 20 ml. of nutrient broth.

The test is complete after one hour by which time there have been six additions of organisms and the concentration of the disinfectant dilution has been reduced by half. The broth bottles are incubated at 37°C and the results recorded after 48 hours. In practice one person can perform four tests in just over an hour by spacing the addition of organisms.

End Point

The end point is taken as the highest increment number giving less than two positive broths out of two. (*i.e.* showing no growth in either one or both bottles).

Results

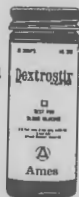
Table I shows some results obtained in this laboratory with a number of disinfectant materials available in New Zealand. The

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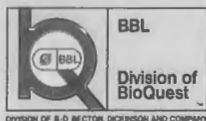
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TABLE I

Showing results of Kelsey Capacity Tests on 11 products (Test Organism: *Ps. aeruginosa*), together with Rideal-Walker Coefficients and manufacturers' recommended use-dilutions.

PRODUCT	TYPE	R.W. COEFF.	MANUFACTURERS' RECOMMENDED USE-DILUTION	HIGHEST DILN. TO PASS KELSEY CAPACITY TEST	
				WITHOUT YEAST	WITH YEAST
General Disinfectant	Chloroxylenol	5.0	1/20 — 1/30	1/7.5	NT*
"	"	3.0	1/20 — 1/60	1/30	1/15
"	"	1.2	1/20 — 1/80	1/10	NT*
"	Q.A.C.	30.0	1/300 — 1/500	1/300	1/30
"	"	25.0	1/30 — 1/200	1/400	1/30
General Sanitiser/Cleaner	Q.A.C.	NT*	1/20 — 1/60	1/80	NT*
"	Phenolic	0.2	1/15 — 1/30	1/30	NT*
Farm Dairy Sanitiser/Cleaner	Iodophor	NT*	1/640	1/150	1/60
"	"	NT*	1/640	1/100	1/50
"	"	NT*	1/640	1/80	1/50
Farm Dairy Teat Dip	"	NT*	Use undiluted	1/10	NT*

NT* = not tested

figures quoted for the Kelsey Capacity Test were obtained from tests against *Pseudomonas aeruginosa*, which has proved to be a more resistant organism than the *Staphylococcus aureus*. Some of the less powerful products have not been tested in the presence of yeast. In addition, Rideal-Walker coefficients are given, where available, and the manufacturers' recommended use-dilution rates as stated on the labels.

Discussion

One of the most interesting observations on these results is the marked difference between the dilutions required to pass the "Kelsey test" with and without yeast. The quaternary ammonium compounds in particular are very sensitive to the presence of organic matter, whereas the one chlorinated phenolic tested against yeast ("B" in the Table) and the iodophors are much less affected. Criticism has been made of the high concentration of yeast used, and Kelsey and Maurer (1966)² report modifying the test and using 1 per cent. yeast with apparently satisfactory results. However, as they point out, it is possible, within limits, to obtain any desired result by varying time of exposure, number of organisms and concentration of organic matter, and therefore it may be preferable to retain the 5 per cent. yeast despite the severity of this test, rather than to perpetuate the previously mentioned series of modified test techniques.

The other interesting feature of these results is the poor degree of correlation between manufacturers' recommendations and "Kelsey test" results, and it is obvious that some manufacturers base their figures on little real evidence. However in the case of iodophors the 1:640 ($\frac{1}{4}$ oz. per gallon) use-dilution has been reasonably well proved in service, and by work performed at the Ruakura Animal Research Centre⁷.

Summary

The Kelsey Capacity Use-Dilution test is offered as an informative and reliable technique, being simple and cheap to perform. It has been used in our laboratory with satisfactory and reproducible results, and it appears from our work and from the literature to be a useful means of evaluating disinfectants.

Acknowledgements

I wish to thank the management of Tasman Vaccine Laboratory Limited for permission to publish this paper, also Mr L. Reynolds and Mr C. L. H. Stonyer for their advice and encouragement during its preparation.

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Technical Communication

Embedding Moulds

In order to reduce the time and effort involved in trimming and mounting paraffin blocks, several methods of embedding were tried. Plastic ice cube trays (Young, 1958), obtainable locally, were either too large or too small. Other systems were found to be fairly time consuming and difficult to fit into our existing filing system.

The embedding moulds illustrated were designed and proved to be quite versatile. By placing the open ends of some to the closed backs of others one is provided with a number of small and medium-sized spaces and by placing the open ends together a number of medium to large sized spaces are available.

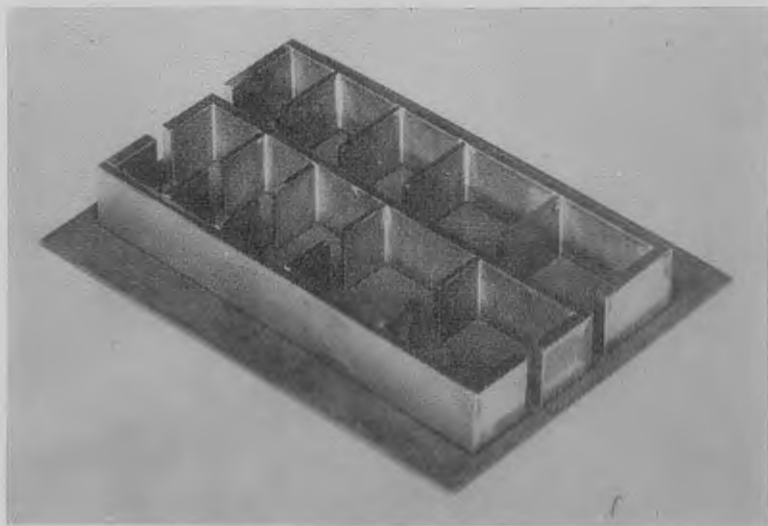


Fig. 1. Embedding moulds on stainless steel plates. The moulds are closed together before filling with molten wax.

The moulds were made from flat brass strip measuring $5/8''$ x $1/8''$ thick, inside arms $5/8''$ x $1/6''$. The arms were fixed to the backs using hard solder (*Easy-flo*). The distance between the arms of these moulds are $1/2''$ to $1''$ but these can be varied to suit individual requirements.

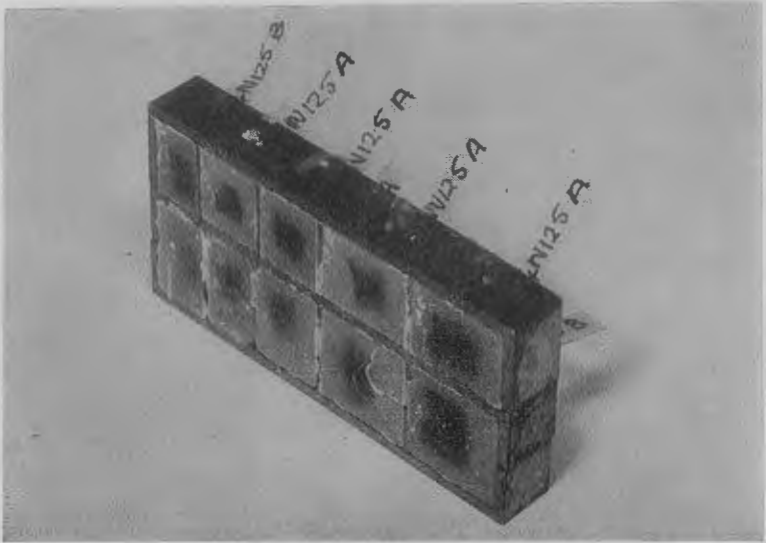


Fig. 2. Showing pieces of tissue embedded in wax after cooling and removing from baseplate.

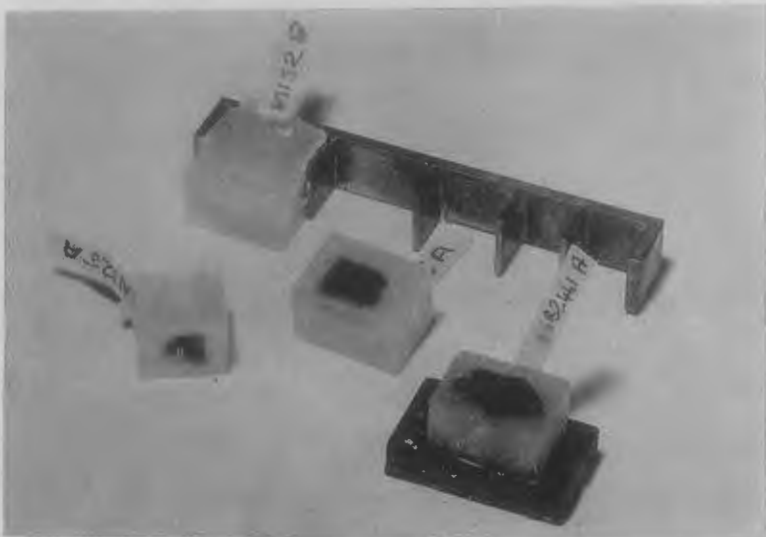


Fig. 3. Moulds broken apart to show blocks and demonstrate ease of removal.

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uncomplicated
accuracy

The Urastrat® chromatography strip below contains, in precise amounts, all the reagents required for one fully quantitative Urastrat urea nitrogen assay.

Serum volume: 0.2 ml.

Time required: 1 minute working time, 30 minutes incubation at room temperature.

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250-test boxes	225/-

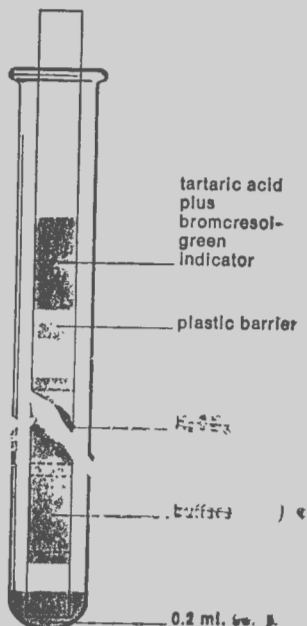
Outwardly simple, the Urastrat assay is actually a precisely controlled sequence of chemical reactions closely paralleling those of the Conway microdiffusion method.

As the serum rises up the Urastrat strip by capillary action, a zone of buffered, high-potency urease (specially purified by dialysis) splits the urea present, yielding ammonia in quantity proportional to the urea nitrogen concentration.

Next, K_2CO_3 releases the ammonia as a free gas. Ascent of the serum stops at the plastic barrier, but the gaseous ammonia migrates upward to be trapped by the tartaric acid in the indicator band, causing a pH change which turns the bromocresol-green indicator from yellow to blue.

The more urea nitrogen originally present, the more ammonia is trapped and the higher the blue frontier rises on the indicator band.

After 30 minutes incubation at room temperature you measure the height of the color change in millimeters, translate into μ g. urea nitrogen/100 ml. serum by a simple calculation.



For full information write to

①

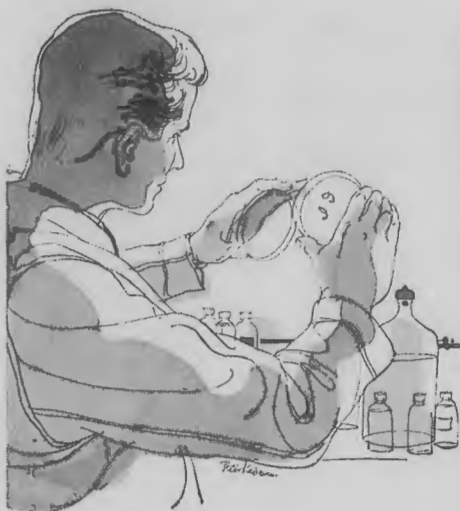
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This unique diagnostic sensitivity test agar permits simultaneous determination of antibiotic and sulfonamide susceptibility, with the additional advantage of permitting direct observation of hemolytic activity. The performance of the medium is consistent with the criteria laid down by an international committee on sensitivity testing.*

*WHO Technical Report No. 210.



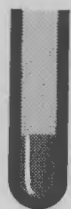
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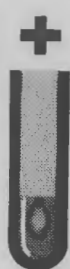
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in the coagulase test

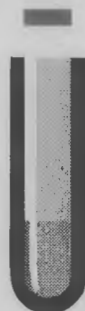


?



NO
false
negatives

NO
false
positives



**IF your substrate is
coagulase-standardized human plasma***

With nonhuman plasma you may get false positive results because of "species differences in coagulase activators and strain differences in coagulase production."¹ Tompsett² used human plasma in differentiating between 'Staphylococci' with negative and positive clumping factor — because rabbit plasma gave coagulase positive reactions in all of them.

With human plasma over four hours old (as blood bank plasma is very likely to be) you may get false negative results. In a comparison study,³ lyophilized human plasma* detected 122 strains of proven pathogenic 'Staphylococci' by positive coagulase reactions; pooled plasma over 4 hours old detected only 91. With plasmas of dubious age it is possible that, even within a 24-hour period, no clot will form. On the other hand, the coagulase-producing organism may also produce staphylokinase^{4,5} which, within a 24-hour period, may cause a formed clot to lyse.

Diagnostic Plasma/Warner-Chilcott gives results identical to those obtained with freshly drawn human plasma.^{6,7} It is standardized against strongly positive, weakly positive and negative coagulase-producing 'Staphylococci'. From vial to vial, lot to lot, Diagnostic Plasma/Warner-Chilcott contains optimal concentrations of clotting factors. Results are usually visible within one hour,⁷ always within three hours.

When ordering,

specify *DIAGNOSTIC PLASMA/WARNER-CHILCOTT

1. Rammelkamp, C.H., Jr., and Lebovitz, J.L.: *Ann. New York Acad. Sc.* 65:144, 1956.
2. Tompsett, R., in Finland, M., and Savage, G. M.: *Antimicrobial Agents and Chemotherapy*, Ann Arbor, Braun-Brumfield, 1961, pp. 67-73.
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7. Boyd, H.: *Am. J. Med. Tech.* 22:232, 1956.

Diagnostic Plasma / Warner-Chilcott is available in boxes of:
10 vials, 2.5 ml. size
(15 coagulase tests per vial)
10 vials, 0.5 ml. size
(3 coagulase tests per vial)

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accuracy

accuracy is the closeness of an observed value to a true value



accuracy + precision

precision is the degree to which observed values can be repeated



accuracy + precision = reliability

reliability is the degree to which a test system will yield accuracy and precision

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- for prothrombin-time testing of patient plasmas over four hours old
- for prothrombin-proconvertin testing without preparation of prothrombin-free plasma

Simplastin[®]-A

lyophilized thromboplastin - calcium extract with Factor V and fibrinogen added.

Simplastin-A is freeze-dried thromboplastin-calcium containing optimum amounts of Factor V and fibrinogen. It is designed for one-stage prothrombin-time testing of plasmas *over four hours old* and for the P & P test. It is controlled against normal human whole and dilute plasma and against plasma from patients on anticoagulant therapy—both fresh plasmas and plasmas that have been allowed to age for 72 hours at room temperature. Simplastin-A is stable after reconstitution for 1 working day.

Prepare Simplastin-A for use by addition of chemically pure distilled or deionized water with a pH not lower than 6.0 at a temperature not over 37°C.

Use Simplastin-A for control of anticoagulant therapy in modifications of these procedures: Quick one-stage assay for prothrombin • Owren prothrombin-proconvertin test • Prothrombin time test (Link-Shapiro modification of the Quick procedure)* • Ware and Stragnell modification of the prothrombin-proconvertin test.

Simplastin-A is designed for control of anticoagulant therapy. Because Factor V and fibrinogen are added, it **MUST NOT BE USED** in screening tests for coagulation defects. There are only two exceptions to this rule: Simplastin-A may be used in the prothrombin consumption test. Simplastin-A may be used in a suspected deficiency of Factor V, run in comparison with test results with Simplastin.

Simplastin-A is the thromboplastin of choice for prothrombin times

on patient plasmas over four hours old because:

It is reproducible in both the normal and the therapeutic range—from vial to vial, from lot to lot. **It is precontrolled.** Simplastin-A is made to exacting specifications for tissue source and conditions of extraction; for particle size and number; for ionic strength and pH; for optimum concentrations of Factor V and fibrinogen.

It is standardized for physical appearance, pH, moisture content, cake weight, heat stability, sodium and calcium content, Factor V and fibrinogen levels. After reconstitution it is stable, if refrigerated, for 1 working day.

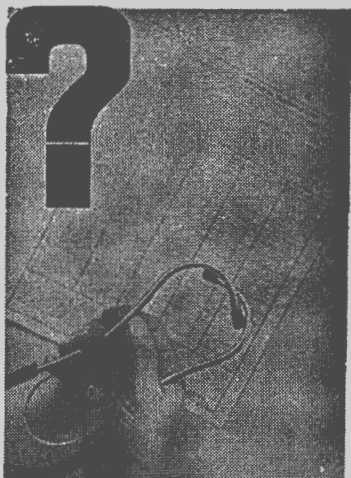
It is convenient and economical. Simplastin-A permits the laboratory to hold samples arriving during off hours so all prothrombin time tests can be run at the same time, by the same technologist, under the same conditions. In the pro-thrombin-proconvertin test the availability of Simplastin-A eliminates the need for special preparation of prothrombin-free plasma.

Simplastin-A is available in boxes of 10 vials, 20 determinations.

**Factor V and fibrinogen are added to Simplastin-A specifically for the prothrombin time testing of patient plasmas over four hours old. For plasmas less than four hours old, Simplastin, the standard thromboplastin-calcium, is recommended.*

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what he doesn't know can hurt

Are you testing **gram-negative organisms** routinely for sensitivity to

GRAM-NEGATIVE SPECIFIC ANTIBIOTIC

COLY-MYCIN INJECTABLE colistimethate sodium

for urinary, respiratory, surgical, wound, burn and blood stream infections

- primarily bactericidal against most gram-negative pathogens — especially *Pseudomonas* and *E. coli* (not recommended for *Proteus*)
- exceptionally safe when used as recommended (exercise caution in presence of renal impairment)
- rarely induces bacterial resistance
- therapeutic blood and urine levels rapidly attained

Please remember: Since Coly-Mycin (colistin) is a polypeptide antibiotic, a clear zone of inhibition, regardless of size, indicates sensitivity — usually high sensitivity.

Sensitivity discs are available from your regular suppliers, from this office— free of charge, or your Warner-Chilcott representative. Also, have you seen the 4½ minute bacteriology film on Coly-Mycin (colistin)? Ask your Warner-Chilcott representative about it the next time he calls.

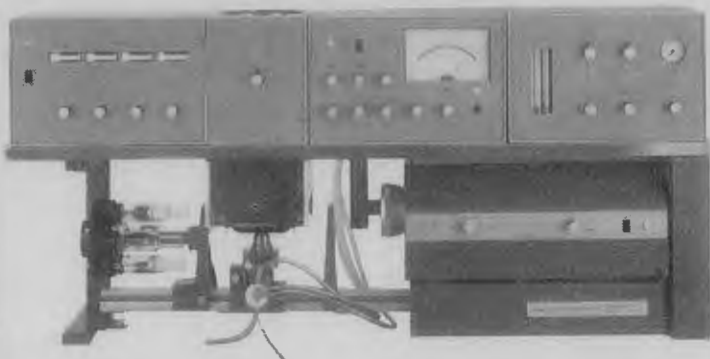
Side Effects: Occasional reactions such as circumoral paresthesias, nausea, dermatitis, drug fever, transient vertigo, and dizziness have been reported and usually disappear upon discontinuance of drug or reduction of dosage.

Precautions: Exercise caution in renal impairment. Transient elevations of BUN have been reported. As a routine precaution blood studies should be made during prolonged therapy.

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The Techtron AA-5 is a modular instrument offering the versatility to perform every type of atomic absorption task. In addition, while it retains the flexibility and convenience provided by its modular construction, this new instrument features an integrated design and greatly improved appearance.

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 - * **Completely solid-state electronics**, for greater reliability.
 - * **Improved hollow cathode lamp power supply**, with single-knob control and current meter for each lamp. All four lamps warm up with regulated, modulated power at normal operating current.
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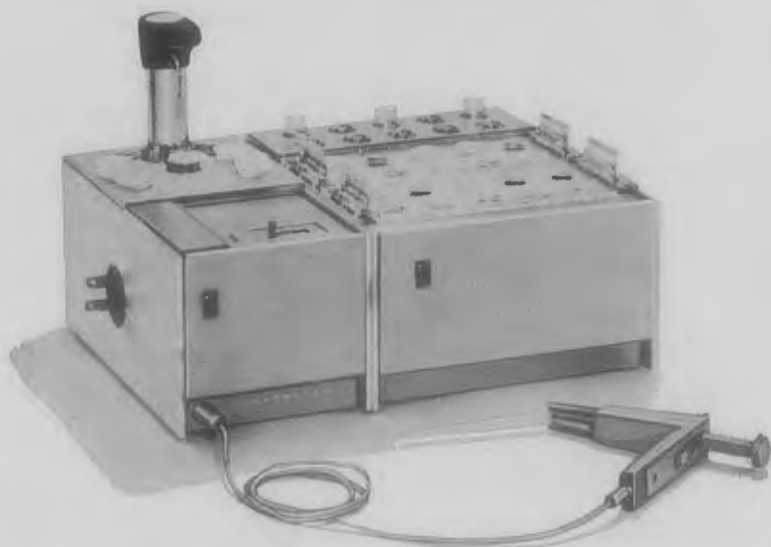
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the world's first
precision
coagulation timer**



The moulds were constructed by Mr J. P. Uitermark in the M.R.C. Workshop, Medical School, Dunedin, and photographed by Mr D. V. Weston.

D. TINGLE,
Pathology Department,
Medical School,
Dunedin.

REFERENCES:

Young, B. J. (1958), *J.med.Lab.Tech.*, 15, 33.

Correction

Iso-Enzyme Terminology

There is presently much confusion in the literature devoted to iso-enzymes because of the completely reversed system of nomenclature in the American and European journals. To avoid confusion in the *Journal* the following system is recommended.

Iso-enzyme	1	equivalent	to	mobility	of	γ	globulin
"	2	"	"	"	"	β	globulin
"	3	"	"	"	"	α_2	globulin
"	4	"	"	"	"	α_1	globulin
"	5	"	"	"	"		Albumin

These mobilities are only approximate. They are based on the definition that the most negative enzyme is iso-enzyme 1. (The alternative system defines the "first iso-enzyme" as that having the least mobility to the cathode.)

G. R. McLAREN,
Dunedin Hospital.

Examination Papers

J. L. BRAIDWOOD,
Dunedin Hospital.

Received for publication, September 1968.

Recent correspondence to the *Journal* has focussed attention on the preparation and marking of examination papers. Most people concerned with teaching and examining would, I think, agree with the following statements:—

1. The examiner should have a preconceived idea of how his questions are to be answered. Only then can his marking be seen to be objective.
2. The way to do this is to prepare a marking key.
3. The results of such examinations should be produced in block form, e.g. Less than 50% = D (fail) 50-60% = C (pass), 60-75% = B (high pass), over 75% = A (distinction).
4. The multiple-choice type of question should be a regular and important feature of the written examination.

This is of course merely paraphrasing what has already been said. However, the last item warrants closer examination. There are two principal problems associated with multiple choice examinations. The most important of these is to ensure that there is a sufficiently large pool of questions. This will be required if continuity is to be maintained. The other problem is of course the need to prepare the candidates for this type of paper. The candidates should for instance know in advance what multiple choice does not (or should not) mean: *e.g.* It does not mean, for instance, asking the candidate to answer 6 out of 10 questions. Nor does it mean simple Yes/No questions. The diversity of ways in which multiple-choice questions can be presented may well cause a nervous candidate to fail to understand the question. Some of the examples given below may illustrate this.

There are basically seven different ways of phrasing multiple-choice questions Stafford (1965).¹ With some variation the total may be raised threefold. Some examples of the basic forms will be given.

1. Five Choice Completion

Instructions to Candidates:

The question is accompanied by 5 suggested answers.

Select the one which is correct.

Bilirubin is derived from the metabolic degradation of haemoglobin, in where does this occur?

- A The erythrocyte.
- B The liver parenchyma.
- C The reticulo-endothelial system.
- D The kidney tubule.
- E All of these.

2. Five Choice Association

Instructions to candidates:

A — E below, are five different diseases of the cerebrospinal system.

1 — 5 are five different sets of laboratory results.

Put the appropriate number against the letter that best fits the results.

A T.B. Meningitis

B Acute bacterial meningitis.

C Cerebrovascular accident.

D Poliomyelitis.

E Disseminated sclerosis.

1. Appearance opalescent, cells increased lymphocytes, protein moderate increase, glucose reduced.

2. Appearance turbid, cells increased polymorphs, protein marked increase, glucose greatly reduced.

3. Appearance clear, cells increased lymphocytes, protein slightly increased, glucose normal.

4. Appearance blood stained. cells predominantly red blood cells, protein increased, glucose normal.

5. Appearance normal, cells no increase, protein upper limit of normal, glucose normal.

3. Four Choice Association

Instructions to candidates:

1 — 4 below are four different statements. A — E indicate how many of the four statements are correct.

Circle the letter that you think gives the most correct answer.

1. The addition of sodium thioglycollate to liquid media will prolong the maintenance of anaerobic conditions.

2. p-Amino benzoic acid may be added to blood culture media to overcome the presence of chloramphenicol in the patient's blood.

3. Tellurite malt agar is a low pH, high sugar content medium, rich in Vitamin B complex. The addition of tellurite is to inhibit bacteria.

4. Lowenstein-Jensen medium contains, potassium dihydrogen phosphate, manganese sulphate, manganese citrate, asparagine, glycerol, malachite green, eggs and water.

A If 1, 2 and 3 are correct, but not 4.

B If 1 and 3 are correct, but not 2 and 4.

C If 2 and 4 are correct, but not 1 and 3.

D If only 4 is correct, but not 1, 2 and 3.

E If all four are correct.

4. Excluded Term

Instructions to candidates:

A 4 out of the 5 numbered constituents of urine will give one of the three reactions labelled A, B or C.

Indicate the REACTION by circling either A, B or C.

B One of the urine constituents will give a negative result with all three reactions. Indicate the CONSTITUENT by circling the number.

A Positive Schlesinger's test.

B Positive Benedict's test.

C Positive Rothera's test.

1. Creatinine in high concentration.

2. Urates in high concentration.

3. Homogentisic acid.

4. Salicylates.

5. Porphobilinogen.

5. Relationship Analysis

Instructions to candidates:

Each question in this section is comprised of an assertion and a reason.

In the left hand column of the answer sheet circle the letter.

- A If both the assertion and the reason are true statements and the reason is a correct explanation of the assertion.
 B If both the assertion and the reason are true statements, but the reason is NOT a correct explanation of the assertion.
 C If the assertion is true but the reason is a false statement.
 D If the assertion is false but the reason is a true statement.
 E If both the assertion and the reason are false statements.

1. A spectrophotometer is more accurate than a filter colorimeter because a filter colorimeter does not have a sufficiently intense light source.
2. A diffraction grating monochromator may be preferable to a prism monochromator because a prism monochromator tends to cause more dispersion of the light than does a diffraction grating.

6. Quantitative Comparison

Instructions to candidates:

In the following comparisons select one of the following:

response A (circle A) if A is greater than B

response B (circle B) if B is greater than A

response C (circle C) if the two are equal or nearly equal.

Example 1 A Leucocyte alkaline phosphatase activity in chronic infection.

B Leucocyte alkaline phosphatase activity in chronic granulocytic leukaemia.

Example 2 A Red cell lysis with acidified serum and thrombin in paroxysmal nocturnal haemoglobinuria.

B Red cell lysis with acidified serum and thrombin in paroxysmal cold haemoglobinuria.

7. Multiple Completion

Instructions to Candidates:

Each question in this section is followed by four suggested answers — one or more of which are correct.

Select A (circle A) if only Numbers 1, 2 and 3 are correct.

Select B (circle B) if only Numbers 1 and 3 are correct.

Select C (circle C) if only Number 2 and 4 are correct.

Select D (circle D) if only Number 4 is correct.

Select E (circle E) if all four are correct.

Which amongst the following would be suitable for absorbing cells in the preparation of specific anti-D serum. The serum being donated by a group O. Cde mother with anti-D.

1. O, cde, cde cells.
2. O, Cde, cde cells.
3. A₂B, CDe, cde cells.
4. A₁B, cde, cde cells.

In examples 2 (Five choice association) and 4 (Excluded term), the wording of the instructions to candidates have been modified to aid clarity. Also the questions are normally set out in such a manner as to require a letter to be circled, the letters appearing at the left side of the alternative answers.

REFERENCE:

1. Stafford, J. L., (1965), *Gazette. Inst.med.Lab.Technol.* 42, 1.

FURTHER READING:

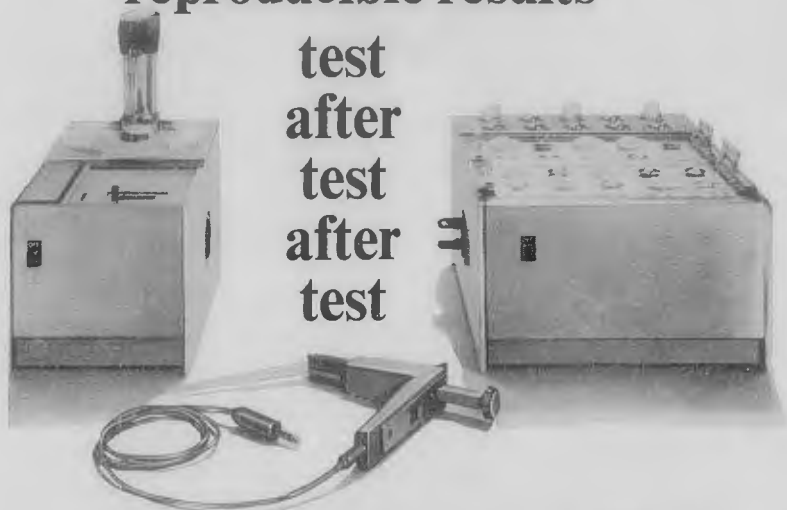
Hubbard, J P. and Cleman, W. V. (1961), *Multiple-Choice Examinations in Medicine*, Lea and Febiger (Henry Kimpton, London).

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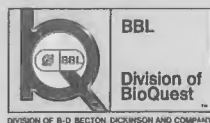


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tiring, not-always-reproducible manual testing

The FIBROMETER System is so reproducible that two or three lab technologists can run hundreds of tests a day and obtain consistently uniform results. Briefly, here's how each part of the system works: The Thermal-Prep Block preheats and maintains plasma and reagents at 37°C, eliminating the need for a water bath and the extra motions involved; the Automatic Pipette dispenses plasma and reagents uniformly through disposable tips into the disposable FIBROTUBE Cups and starts the sensing and timing device; the FIBROMETER Unit electromechanically measures and records the clot formation time to 0.1 of a second. And the entire clot sensing and timing process is automatic. In addition, the FIBROMETER System is of a modular design so you can use any combination, up to 6 units, with a single electrical outlet, for your particular coagulation test work load.

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Colorimetry

D. McIVER,
North Shore Hospital, Takapuna.

Winner of the Essay Section of the Junior Essay Competition, 1968.

Most biochemical analytical methods produce coloured solutions. To measure the concentration of the solution, a relationship between the concentration of a known solution and an unknown solution had to be found. This fundamental law on which visual colorimetry is based is Beer's law, which states that the intensity of colour of a solution when viewed by transmitted light depends on the number of coloured particles present independently of their concentration. From this it follows that the same amount of colour is seen when, for example, a 10% solution is viewed through a depth of 3 cms, as when a 30% solution is viewed through a depth of 1 cm. Therefore, the intensity of colour seen is proportional to the concentration (c) of the substance, multiplied by the depth (t) of the solution. Then it follows that, when two solutions of a substance viewed by transmitted light, have the same intensity of colour

$$c_1 t_1 = c_2 t_2$$

By introducing a suitable standard and by balancing the intensity of colour of the unknown to that of the standard, the concentration of the unknown can be found.

$$\text{Concentration of unknown} = \frac{\text{Reading of standard}}{\text{Reading of unknown}} \times \text{Conc. of Std.}$$

This relationship is used in all visual colorimetry, where the most common types of colorimeters are the Lovibond comparator and the Dubosq colorimeter.

The Lovibond comparator consists of a box containing two tubes, one containing the test solution and the other containing a "blank" solution, and a rotatable disc mounted in front of the two tubes. The central window of the box is in front of the test solution. The other window is in a position so that the rotatable disc superimposes the coloured glass standards in it on the blank solution. When the colour intensity matches that of the test solution, the answer is read off the disc. A standard solution is also put up with the test to ensure that there has been no error in technique and also as a check on the permanent standard. The Lovibond comparator is used for determining pH for a wide range of indicators, and many other purposes including determination of protein by the Biuret method.

The Dubosq-type colorimeter, which has been almost totally replaced by the photo-electric instruments, consists essentially of two glass containers, each of which contains a solid cylindrical

glass plunger, capable of being raised or lowered. The source of light is usually in the base of the colorimeter so that the light passes vertically with equal intensity through the two cups which are opaque at the sides with a transparent base. The plungers are transparent at the top and bottom, but are otherwise opaque. The depth of solution may be varied by raising or lowering the plungers (or in some types, the cups). The plunger carriers are provided with graduated scales so that the depths of solution can be read. The zero of these corresponds to the point at which the plunger just reaches to the bottom of the cup. After passing through the coloured solutions and plungers, the two beams of light are brought by a prism into the eyepiece so that they can be observed together, each forming half of the circular area of colour seen there. When the colour intensity is matched, the depth of one solution multiplied by its concentration is equal to the depth times the concentration of the other. By using a standard solution the unknown concentration can be found.

$$\text{Concentration of unknown} = \frac{\text{Reading of standard}}{\text{Reading of unknown}} \times \text{Conc. of Std.}$$

The difference between the visual colorimeters and those that are classed as photo-electric colorimeters is that they are not colorimeters, but absorptimeters. Photo-electric absorptimeters use photo-electric cells, either of the barrier layer type or the emissive type. Light falling on these cells generates an electric current which is made to turn the galvanometer needle; the deflection being proportional to the light intensity. The more concentrated a solution is, the more light it absorbs; it therefore transmits less light to the photo-electric cell and the current generated is smaller.

For photo-electric colorimeters another law is brought in. This is Lambert's law, which states that the absorption of light is independent of the intensity of radiant energy passed through a solution containing a solute. As the light passes through the solution it is subjected to a logarithmic reduction in its intensity as the concentration of the solution increases.

When light falls on a homogeneous medium, a portion of the incident light is reflected, a portion is absorbed within the medium, and the remainder is transmitted off the intensity of the incident light is I_0 that of the reflected light in I_r that of the light is I_a , and that of the transmitted light is I_t then

$$I_0 = I_a + I_t + I_r$$

For air-glass surfaces, the amount of reflected light is eliminated by the use of the control, (a water blank)

$$I_0 = I_a + I_t \dots \dots \dots [1]$$

Lambert (1760) investigated the relationship between I_0 and I_t and Beer (1852) extended this to experiments with solutions.

We may express Lambert's law, previously stated, by the differential equation.

$$-\frac{dI}{dt} = kI \quad \dots \dots \dots [2]$$

Where I is the intensity of the incident light of wavelength, t is the thickness of the medium, and k is a proportionality factor.

Integrating (2) and putting I = I₀, when t = 0, we obtain

$$L_n \frac{I_0}{I_t} = kt$$

or stated in other terms

$$I = I_0 e^{-kt} \quad \dots \dots \dots [3]$$

where I₀ — is the intensity of the light falling upon an absorbing medium of thickness t, I_t is in the intensity of the transmitted light and k is a constant called the absorption co-efficient for the wavelength and the absorbing medium used by changing from natural to Briggsian logarithms we obtain

$$I_t = I_0 \cdot 10^{-0.4343kt} = I_0 \cdot 10^{-Kt} \quad \dots \dots [4]$$

where K = k/2.3036 and is usually called the extinction co-efficient. The extinction co-efficient is generally defined as the reciprocal of the thickness (tcm) required to reduce the light to 1/10 of its intensity. This follows from equation [4] since:

$$I_t/I_0 = 0.1 = 10^{-Kt} \quad \text{or } Kt = 1 \text{ and } K = 1/t$$

The ratio of I_t/I₀ is the fraction of the incident light transmitted by a thickness t of the medium, and is termed the transmission or transmittance I. Its reciprocal I₀/I_t is the opacity, and the optical density of the medium, sometimes designated the extinction E or absorbance A, is given by

$$D = \log I_0/I_t$$

By combining Lambert's law with the former Beer's law we derive the equation

$$\frac{I_e}{I_0} = e^{-kct}$$

- in which I_e = intensity of the emergent light
- I₀ = intensity of the incident light
- k = a constant
- c = concentration of coloured substance
- t = thickness of the layer of solution
- e = 2.718, the base of natural or Napierian logarithms

This states that when monochromatic light passes through a coloured solution the amount of light transmitted decreases exponentially (a) with increase in thickness of the layer of solution through which the light passes and (b) with increase in concentration of the coloured substance. The first of these follows from Lambert's law and the second from Beer's law.

The ratio I_e/I_o is known as the transmission (T) therefore $T = e^{-kct}$

$$\text{or } \text{Log}_e T = -kct$$

$$\text{or } -\log_e T = kct$$

$$\text{or } -\log_{10} T = Kct$$

where K is another constant.

The quantity $-\log T$ or $\log (1/T)$ is termed the optical density (D) or or the extinction (E).

When using photo-electric instruments we substitute the solvent or blank for the coloured test solution and measure the intensity of the emergent light from either of these. This can be denoted I_B and is substituted for I_o to give

$$\frac{I_E}{I_B} = e^{-kct}$$

where $\frac{I_E}{I_B}$ has been termed the transmittance.

Some photo-electric instruments are provided with two scales, one from 0 to 100 showing percentage transmission (or transmittance), the other from infinity (∞) to 0 showing the optical density. While the transmission scale is linear, the latter is logarithmic. From this it can be seen that the optical density and the percentage transmission are related

$$D = \log \frac{100}{\text{per cent transmission}}$$

$$\text{or } D = 2 - \log \text{ per cent. transmission}$$

In practice the instrument is usually set up for 100 per cent. transmission or optical density 0 with water or a blank in position. The test solution is then introduced and the optical density is again read

$$\begin{aligned} \text{since } D &= -\log T \\ D &= Kct \end{aligned}$$

So, if the thickness remains the same D is proportional to the concentration. As a result, provided Beer's law is obeyed, when optical density is plotted against concentration a straight line is obtained. On the other hand if per cent. transmission is read, as it is in some instruments, and is plotted against concentration, we do not get a straight line, but a curve.

So we see that if a suitable standard is prepared and the optical densities of this and the unknown solution are read, we have

$$\frac{\text{Conc. of unknown}}{\text{Conc. of std.}} = \frac{\text{O.D. of unknown}}{\text{O.D. of std.}}$$

$$\text{or, Concentration of unknown} = \frac{\text{O.D. of unknown}}{\text{O.D. of std.}} \times \text{Conc. of std.}$$

In order to be sure that the optical density is due solely to the substance under test, the reading of the "blank" — an identical solution to test containing all the reagents but without the test substance — solution must be considered with the reading obtained from the "test" and "standard" solutions. This detects colour due to the reagent and not caused by the test. The blank reading must then be subtracted from the test and the standard reading.

Therefore: concentration of unknown =
$$\frac{\text{O.D. of unknown} - \text{blank}}{\text{O.D. of std.} - \text{blank}} \times \text{Conc. of std.}$$

By using this formula for determining the concentration of substances, we can find the concentration of most constituents of blood by using appropriate standards.

When the Beer-Lambert law is obeyed, a straight line graph can be obtained by plotting the optical density against various concentrations of the solution. Also Beer-Lambert law is obeyed by most solutions provided that they are dilute. In a more concentrated solution there may be divergencies from the law caused by the association of molecules and the formation of complexions. The law, also, only applies to monochromatic light, that is, light of a narrow band of wavelengths.

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Selected Abstracts

Contributors to this issue: J. Hannan and D. Tingle.

BLOOD BANKING

The Chronic Problem of Rh Nomenclature. Ranganathan, K. S. (1968), *Antiseptic*, 65, 445.

The purpose of this communication is to stimulate thought among workers in the field of blood grouping and teachers of blood group serology on the imperative need, as in the case of the ABO groups, for a single, simple and precise nomenclature for the Rh-Hr types. Those charged with the duty of teaching have a major responsibility for correctness and they should realise that the use of a dual nomenclature for the Rh-Hr types lacks mathematical precision since two different notations cannot both be correct. J.H.

CHEMICAL PATHOLOGY

An Analysis of 4000 Chemistry Graphs. Reece, R. L. (1968), *Minn. Med.*, 51, 351.

Multiple Channel Autoanalyzers (SMA - 12, SMA - 12/60) simultaneously measure 12 serum substances. Among the 4,000 specimens analysed by a "Survey Model," 37.5% came from hospital patients and the remainder from office patients. Most sera were from individuals with known or suspected disease.

Of the 4,000 graphs, 1,896 (or 47.4%) showed at least one abnormal test. Tests most frequently abnormal included uric acid (11.0%), glucose (10.5%), alkaline phosphatase (10.4%), urea nitrogen (9.2%) and LDH (8.9%). J.H.

Assay of Human Leucoagglutinins by Capillary Migration. Thompson, J. S., Severson, C. D., Lavender, A. R., Forland, M. and Russe, H. P. (1968), *Transplantation*, 6, 728.

There are several recent studies which correlate the survival of human allografts with serological leucocyte typing. The authors have developed a new method for assaying human leucoagglutinins which is 10-100 times more sensitive than microscopic agglutination. Serum-cell mixtures were incubated, then drawn into siliconised microhaematocrit tubes. Following centrifugation, the tubes were held at a 45° angle for 20 minutes with the cell pellet uppermost. Streamer length was considered to be inversely proportional to agglutinin concentration. J.H.

CYTOLOGY

Recent Progress in the Interpretation of Malignancy Associated Changes (MAC). Nieburgs, H. E. (1968), *Acta cytol.*, 12, 445.

Increased interest in MAC during the last decade has led to an accumulation of data on various aspects concerning the interpretation of MAC. Microscopic studies of nuclear MAC by impression, using few objective criteria, failed to render satisfactory results. Sources of error include lack of experience, improper magnification and poor staining.

The author feels that training by tutorial sessions is an essential prerequisite for the adequate utilisation of MAC criteria. J.H.

Spinal Fluid Cytomorphology. Kolar, O. and Zeman, W. (1968), *Archs Neurol.*, Chicago, 18, 44.

Probably the most important result from the routine application of a cell concentration technique to more than 3,600 specimens, which forms the basis of this study, is the finding that spinal fluid with a normal cell count may contain abnormal cells, and often these are of pathognomonic significance. There was an array of cellular responses which reflected the evolution and natural history of many nervous diseases quite accurately. J.H.

Differentiating Epithelial Cells from Leucocytes in Urine. Bradley, G. M. (1968), *Postgrad Med.*, 43, 245.

Determining whether increased "white cell" excretion is predominantly leucocytic or due to renal tubular cells can be important in the diagnosis of renal disease.

Cellular peroxidase activity is represented by discrete dark blue granules in the cytoplasm when the sediment is subjected to a staining procedure based on the benzidine reaction. The cytoplasm of polymorphonuclear leucocytes is usually filled with stained granules. The large "glitter" cells stain as intensely as the smaller compact neutrophils. Renal epithelial cells stain with the safranine counterstain. J.H.

Cancer Cells in the Blood Stream: Review and Personal Findings with the Fluorochrome Method. Turchetti, G., Palagi, R. and Gacci, G. (1968), *Gynaecologia*, 165, 252.

Acridine Orange selectively stains RNA and DNA; there is a linear relationship between the intensity of fluorescence and the nucleic acid content within the cells. Actively proliferating cells, such as cancer cells, have a greater content of both nucleic acids than normal adult cells, so that they become more fluorescent than normal cells.

Seventy-one untreated female patients were examined; all were afflicted with malignant neoplasms of the genital tract. Of these, 19 (27%) showed cells in the peripheral blood "considered as neoplastic."

Red fluorescence of the cytoplasm cannot always be considered a certain sign of malignancy as it can also be found in cells with basophil cytoplasm, mainly in the immature elements of the white and red series and plasma cells. The important aspect for differentiation, besides the volume and shape of the cells, are the characteristics of the nucleus and of its elements, rather than those of the cytoplasm. J.H.

A Guide to Synovial Fluid Analysis. Kra, S. J. (1968), *Conn. Med.*, 32, 109.

Aspects included: gross examination, mucin clot test, examination for rheumatoid factor, and microscopic examination.

The author has performed 250 examinations during a 2½-year period. Fifteen cases had typical urate crystals in the fluid, in spite of the fact that the serum uric acid was normal. One patient had numerous L E cells in the fluid.

A haemosiderin stain can sometimes be made on the fluid for the determination of its iron content as arthropathy has been reported in haemochromatosis. J.H.

HAEMATOLOGY

An Improved Method for the Mass Screening of Haemoglobin Diseases. Breen, Moira and Weinstein, H. G. (1968). *Biochem. Med.*, 2, 35.

In electrophoretic separation of haemoglobins, Tris-glycine buffer, pH 9.3, ionic strength 0.075, was found superior to the usual barbital buffer. Its use results in a procedure which is rapid, has a high degree of resolution and detects haemoglobinopathies which otherwise might be missed. J.H.

Tests of Platelet Adhesiveness and Their Clinical Significance. Hartman, R. C. (1968). *Semin. Hemat.*, 5, 60.

Roller flask, glass wool, and glass bead column tests are briefly described. One hope is that with rigid standardisation a relatively simple technique such as the Salzman procedure can be reliably used to corroborate the diagnosis of von Willebrand's disease and certain other haemorrhagic disorders. In the Salzman method, blood is passed directly from the venipuncture needle through a glass bead column, negative pressure being provided by a Vacutainer. The normal value is 25%-60% adhesive platelets. J.H.

Thrombopathy. Bowie, E. J. W. and Owen, C. A., Jr. (1968), *Semin. Hemat.*, 5, 73.

Thrombopathy has been the subject of a great deal of scepticism largely because the inadequacy of the assay procedures available for platelet Factor 3 has given these diseases a rather intangible and nebulous quality.

The fact remains, however, that patients with bleeding diatheses are seen who have abnormal prothrombin consumption tests which are corrected by Inosithin, in the face of a normal platelet count, and in the authors' experience these patients are common. This is not generally recognised because most laboratories do not screen for platelet thromboplastic function as a routine procedure.

There may be an increase in platelet anisocytosis; round, isolated forms may be seen in a peripheral blood film. In some patients giant "lymphocytoid" platelets occur (more than 4μ and up to 12μ in diameter) and in these patients the platelet count may be reduced or normal.

It is emphasised that congenital functional thrombopathy is perhaps the most common of the minor bleeding diseases. J.H.

HISTOPATHOLOGY

Lipofuscinosis of the Gastrointestinal Tract in Man. Fox, B., (1967), *J.clin.Path.*, 20, 806-813.

This paper describes three cases of lipofuscin pigmentation of the gastrointestinal tract discovered in surgical biopsy material. The staining properties of the pigment and several techniques used to identify the pigment are given in table form. D.T.

Incidence and Significance of Argentaffin and Paneth Cells in Some Tumours of the Large Intestine. Gibbs, N. M. (1967), *J.Clin.Path.*, 20, 826-831.

The incidence of argentaffin cells and Paneth cells in epithelial tumours of the large intestine was investigated. The identification of argentaffin and Paneth cells enable the crypts to be defined and provided a useful practical aid in the differentiation between neoplasms and disorders of epithelial growth. Staining techniques used were phloxine tartrazine, P.T.A.H. and Weigerts modification of Grams stain for Paneth cells; Fontana's silver impregnation, Schmorl's reaction and the diazo method for enterochromaffin granules; and Perl's reaction for haemosiderin. D.T.

MICROBIOLOGY

Listeria monocytogenes: Six Cases Affecting the Central Nervous System. Ford, P. M., Herzberg, L. and Ford, S. E. (1968), *Q. J. Med.*, 37, 281.

Six cases of infection of the central nervous system by *L. monocytogenes* occurring in one hospital in two years are presented, emphasising that the infection is commoner than the reported cases in the authors' country (England) would indicate. The bacteriological diagnosis was in all cases made by the use of routine methods without recourse to special techniques.

In one of the present cases, the organism was isolated only from the blood, although the patient clearly had a central nervous system infection. This underlines the importance of blood cultures in such cases. On no occasion were organisms seen in the Gram-stained smears of the cerebrospinal fluid.

In some cases the cerebrospinal fluid is clear and the cell count usually below 400 and always below 1,000 per cu. mm., these being predominantly lymphocytes.

A positive blood culture of Gram-positive rods must not necessarily be dismissed as being due to contamination. J.H.

Bacteroidaceae: Anaerobic Organisms Encountered in Surgical Infections. Saksena, D. S., Block, M. A., McHenry, M. C. and Truant, J. P. (1968), *Surgery*, 63, 261.

Although the Bacteroidaceae are among the most numerous organisms in the normal intestinal flora, difficulties in isolating these anaerobes from clinical material have hampered assessment of their frequency and significance in human infections.

During the two-year period of 1964 and 1965, approximately 91,000 specimens were examined. Of these, 8,900 were cultured anaerobically as well as aerobically; Bacteroidaceae were isolated from 174 (2.0%).

J.H.



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References: 1. Wilkinson, J. F.; Nour-Eldin, F.; Israels, M. C. G., and Barrett, K. E.: *Lancet* 2:947 (Oct. 28) 1961.
2. Hicks, N. D., and Pitney, W. R.: *Brit. J. Haem.* 3:277, 1957.
3. Langdell, R. D.; Wagner, R. H., and Brinkhouse, K. M.: *J. Lab. & Clin. Med.* 41:637, 1953.

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SEROLOGY

HIM Test (Hepatitis-Infectious Mononucleosis Antibody Test). Hoke, H. F. (1968), *Va med. Mon.*, 95, 701.

The HIM test was designed to differentiate between viral hepatitis-infectious mononucleosis and other liver diseases and hopefully to detect the carrier states of virus hepatitis.

The antigen is a virus isolated in tissue culture from the blood of volunteers with icteric hepatitis. The virus is then absorbed on latex to form a reagent for detecting agglutinins in hepatitis sera.

Other workers performed a clinical evaluation of the HIM test in 1966. Results of this study show that a positive HIM test may prove to be quite useless. However, with the serum of a jaundiced patient, a negative HIM test would tend to exclude hepatitis or infectious mononucleosis as the cause of hepatic disease.

It was obvious that with the high number of positive tests in the controls and the non-hepatic disease group that the test is not practical for the screening of blood donors. J.H.

VIROLOGY

Laboratory Diagnosis of Human Viral Diseases: Recent Developments and Future Trends. Behbehani, A. M. (1968), *J. Kans. med. Soc.*, 69, 127.

Presently viral diseases are the most prevalent infections of man. Extensive improvements in the efficiency, speed and practicability of laboratory procedures for the diagnosis of viral diseases have been introduced during the last two decades. It is now feasible for a hospital with well-equipped clinical laboratories to initiate a virus diagnostic service. The causative viruses of a large percentage of human viral diseases can be isolated within a few days.

Since the procedures are more expensive and time-consuming than other microbiological procedures, it is suggested that various hospitals in a metropolitan area pool their resources and establish a centrally-located virus diagnostic laboratory to serve all clinicians and public health officers of the area. J.H.

The Junior Essay Competition

The Council of the Institute invites entries from unqualified members of the Institute for the 1969 Junior Essay Competition.

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Book Reviews

Blood Groups in Man. Fifth Edition. R. R. Race, Ph.D., F.R.C.P., F.R.S. and Ruth Sanger, Ph.D., B.Sc. 599 pages. Blackwell Scientific Publications, Oxford. (1968). Price in U.K. 110s 0d.

When this book made its first appearance, in 1950, it satisfied a long-felt need for an authoritative and detailed textbook dealing with what was then already an explosively advancing science. In presenting it to the world the authors incurred for themselves the obligation to revise and review their work continuously as further knowledge inevitably came to light, and this obligation they have fulfilled faithfully in a succession of new editions.

The amount and complexity of that new knowledge in the past eighteen years has been very considerable, and it is evident already from the Addenda which Race and Sanger have thoughtfully seen fit to provide that the end is by no means yet in sight. In fact, as they say themselves (in quite another context), the extent to which human knowledge of blood groups might advance in the next decade or so "crushes the imagination."

This is a most comprehensive summary of knowledge, a thorough and meticulous aggregation of facts from the world literature, systematically and intelligibly presented, and richly spiced with humour to aid the digestion.

Most of the chapters have been extended, some new ones have been added, and whole passages have been rewritten to take newly-discovered facts into consideration. No detail is considered too trivial for mention, and no reported exceptions to long-established rules are swept under the rug, all being recorded faithfully with an explanation of their possible significance in relation to existing theories being rendered for the reader's enlightenment.

This is an exceptionally readable book, a veritable goldmine of information presented in an agreeable style. It is quite indispensable in any blood grouping laboratory and deserves to be read by all workers in the field. Manifestly, blood group curiosities abound and are there awaiting discovery by those possessing the percipience to recognise their significance. Every one overlooked represents a lost opportunity to open up new avenues of understanding, and this book is replete with examples of seemingly trivial discoveries which have led to the illumination of profoundly significant new facts. Its earlier editions must already have contributed immeasurably in this way by the insight they have given their readers, and there is so much food for thought in this new one that we can be confident that a sixth edition will become necessary within the next few years.

J.C.

Bray's Clinical Laboratory Methods. J. D. Bauer, M.D., P. G. Ackerman, Ph.D., and G. Toro, Ph.D. 764 pages, many illustrations. C. V. Mosby Co., St. Louis (1968). U.S. price \$14.85.

Drawn from lectures to residents in clinical pathology and to medical laboratory students, and dealing with all disciplines in laboratory medicine, this book sets itself a difficult task. Chapters such as "Methods in Microbiology" and "Clinical Chemistry" have been rewritten, a section on quality control has been added, and other chapters have been expanded. Good glossaries have been added to the chapters on blood bank serology and mycology. The result is a good training and reference volume, except for the sections devoted to microbiology and parasitology, where the individual bacteria and parasites have received comparatively superficial treatment.

The chapter on urinalysis, which includes a good description of abnormal urine pigments and a very good chart entitled "Classification and Manifestations of Kidney Disease," deals well with renal clearance tests, such investigations as V.M.A. and catecholamines and with routine urine testing, except that it ignores the white cell count and the bacterial count is not dealt with until nine chapters later.

The section devoted to haematology impressed me. Nine methods for haemoglobin estimation are dealt with, the cyanmethaemoglobin and Wong's iron in full, and the others sufficient to cover the Part 1 syllabus. Good coverage is given to the factors influencing the E.S.R., red and white cell inclusions, descriptions of all blood cells, the classification of anaemias, leukaemias and myeloproliferative disorders, and to the coagulation mechanism including euglobulin clot lysis. Unfortunately some of the colour plates of cells are drawings, not photographs.

The chapter devoted to blood bank serology is also good for a book of this type, and includes a description and the characteristics of IgM, IgG, IgA, IgD and IgE antibodies, and a workable chart of antibodies encountered in cross-matching, although the blood group systems other than ABO and Rhesus are dealt with sketchily. Wiener notation is used for Rhesus nomenclature, and although Fisher notation is added in brackets in most cases, I found some difficulty, being familiar only with the Fisher and English system.

Clinical chemistry opens with a brief description of atomic absorption spectrophotometry, thin-layer chromatography, gas chromatography and the like, an asset for those who do not have the equipment, but wish to know something about it.

Excellent coverage is given to a wide range of enzymes, and in fact it would be an exotic request that could not be met from this volume, complete with principles and interpretations in brief. Surprisingly though, only the Folin-Wu and Somogyi sugar methods are dealt with.

The section on toxicology, giving methods for estimating poisons and drugs in biological fluids is a very useful one, and ranges from tranquilisers, through drugs such as I.N.H., P.A.S. and the sulphonamides, to poisons such as arsenic and methanol. Little more than staining techniques is dealt with in "Tissue Examinations," and while "Mycology" would serve as a useful introduction to the subject, it is too brief for positive identification of species.

Even for a Part 1 candidate, the chapter devoted to microbiology would be inadequate. All media is apparently obtained only in dehydrated form, and the Shigellae are dismissed in half a page. Staining and cultural techniques are dealt with, but not deeply, and the same comment applies to parasitology, where most parasites are described.

With the exceptions mentioned, I would recommend this book to any laboratory with trainees.

C.E.F.

Clinical Bacteriology. Third Edition. E. Joan Stokes, M.B., F.R.C.P., F.C.Path., 345 pages, illustrated. Edward Arnold, London (1968). U.K. price, 50s 0d.

In recent years there have been many advances in the diagnosis of infectious diseases with the introduction of many new methods and techniques. The author's aim in the first two editions, and in the present one, has been to present well known methods and newer methods which in her experience have been reliable in diagnostic bacteriology.

The first two chapters discuss clinical bacteriology and general procedures in the laboratory, with the purpose of presenting an academic basis for routine diagnostic methods. The next four chapters, much of which have been rewritten, deal with the methods used in the routine culture of all types of specimens and then with the identification of organisms isolated from this clinical material. The nomenclature and classification of Gram-negative bacilli has been changed considerably, the older names being replaced by those now in current use. There have also been timely changes in the section dealing with tuberculosis.

Two excellent chapters on antibacterial drugs and hospital epidemiology provide information that is not always readily available in such a usable and concise form. The chapter on clinical immunology now makes mention of the use of treponemal antigens and the significance of these tests. The final chapter again discusses media testing and control. It

has also included details of media and tests used at the University College Hospital for the isolation and identification of organisms.

Dr Stokes has succeeded in her aim to produce a book on clinical bacteriology based on sound methods, with additional information useful to the technologist employing the techniques and interpreting their results.
G.R.R.

Laboratory Manual of Pediatric Micro-biochemical Techniques. 4th Edition. D. O'Brien, M. D., F.A. Ibbott, Ph.D. and D. O. Rodgerson, M. S. 384 pages, 400 illustrations. Cloth binding, Harper & Row, New York (1968). U.S. price \$11.90.

As the title implies, this work is primarily a collection of methods. Introductory sections deal with normal and therapeutic values, blood collection, pipettes for micromethods, quality control and reproducibility of common micromethods. The methods which make up the great bulk of the book are easily located from the clear alphabetic table of contents. With very few exceptions the arrangement of material follows the same pattern.

In a clinical commentary section, the authors discuss the importance of the test in diagnosis and treatment. While concise the commentaries give useful background information on such things as symptoms, heredity, frequency of occurrence of the disease and also discuss complementary and alternative techniques.

The technical commentary section gives the principle of the method, special precautions necessary in collection or handling of the specimen and discusses the specificity and sensitivity of the method. Any major difficulties encountered in the technique are noted here. In this respect the authors render great service by giving a clear statement that the method is either:—(a) simple and straightforward or (b) technically difficult or requiring specialized or expensive equipment.

A brief statement is given of the normal ranges encountered by the authors in their work.

Reagents, procedure and calculation are then clearly set out.

The format makes the book one which can be conveniently used at the bench, although a stouter cover would have been an advantage in this respect.

The authors point out in their preface that steroid techniques have been omitted in this edition, since the volume of steroid work in paediatric practice is small. By inference one could assume that some of the more complicated techniques in this book are best carried out in specialist centres where there is sufficient work volume to maintain good quality control and familiarity with methods. Some methods then will only be applicable in a large paediatric centre, providing a comprehensive children's service, but there will also be much to interest those who cater only for a small children's ward.

In their selection of methods the authors have preferred accuracy to simplicity or speed and this could well be justified in a University teaching hospital. Many laboratories will find, however, that for some of the tests listed a simpler quicker method would suffice, and yet still give results of clinical value. This is an important point when large volumes of routine work are carried out by junior trainees or assistants, rather than by skilled staff technologists.

A very wide range of tests is included, with many additions since the third edition of six years ago. Of particular interest is the section on atomic absorption spectrophotometry, with methods listed for calcium, magnesium, iron, total iron binding capacity and zinc. A diffusion method for immunoglobulins is given, and while this method has proved its usefulness, it is perhaps unfortunate that no attention is given to immunoelectrophoresis.

Methods included which would interest some workers in haematology department are those for Hb electrophoresis, and various red cell enzyme screens.

The small number of diagrams are generally used in situations where wordy explanations would be cumbersome.

References are given directly following each test, and the ample documentation is an excellent feature of this book.

Although of special interest to laboratories with large paediatric sections, this book could still prove a useful addition to the library of any general laboratory.

J.M.

Measurement in Medicine. P. S. Oldham, M.A., F.S.S. 216 pages. English Universities Press, London. (1968). Price in U.K. 50s 0d.

In his introduction the author states: "This book is an attempt to provide explanations of what statistical techniques do, of the logical structure of a statistical argument . . ., of the connection between observations and the conclusions reached by statistical methods and, in general, explanations of why particular statistical techniques fulfil practical needs in medicine." I feel that he has achieved his aims and produced a comprehensive supplementary text especially suitable for those persons taking the New Zealand Certificate in Science Statistics course, or for medical personnel with some university statistical training.

The text is clearly set out and liberally illustrated with examples taken from a wide variety of sources. The final interpretation of results — a common source of mistakes — has been stressed throughout, and the chapters on the significance of frequencies and regression are particularly good. It is perhaps unfortunate that there is little mention of multivariate regression analysis, for this type of analysis is frequently applicable and lack of knowledge of its existence does lead to invalid conclusions drawn from the concatenation of valid bivariate regression analyses.

This book is well worth a place in a medical laboratory. C.E.H.

The Peripheral Blood Film. T. A. Harper, M.B., B.S. (Madras), M.C. Path. 134 pages, illustrated. Butterworths, London (1968). N.Z. price \$4.00.

This book, as the title implies, deals with the cells, normal and abnormal found in peripheral blood. In the tabulation of cell type maturation, however, cells found in the bone marrow are described and illustrated.

Information is grouped under four main sections:—normal peripheral blood cells; abnormal peripheral blood cells; classification of blood film appearances and differential diagnosis; the blood film in disease.

In addition there is a short chapter on blood film preparation, an appendix on staining techniques and of haemotoxic drugs and chemicals.

The first three sections are illustrated with 60 photomicrographs, the majority of which are of good quality. However, they do suffer in value from being monochrome, a point freely acknowledged in the author's preface.

Section 4 consists of descriptions of the blood film findings in disease arranged in alphabetical order for easy reference.

While this book contains nothing that cannot be found in other texts, it does have the advantage that the information on this important aspect of haematology is contained in a concise, readily assimilated and abundantly illustrated form.

As an adjunct to a more expensive colour atlas or as a technologist's personal reference, this book could be useful.

B.W.M.

Reagent Grade Water: How, When and Why? Martha Winstead, M. T. (A.S.C.P.) 135 pages. American Society of Medical Technologists, Houston. (1967).

In 1962, water from a new well was introduced into the author's town water supply. The problems and investigations associated with this development resulted in the production of the text-book. When reading this book one is often struck with the thought "has the author been singularly unfortunate in her geographical location, or are many other laboratories living in a fool's paradise?" On the other hand, as the author points out, *alcaligenes* etc. growing in a bottle of *pure* water is clear

evidence of contamination, since presumably micro-organisms would be incapable of survival in chemically pure water. Innumerable examples are given of how insufficiently purified water can interfere in biochemical tests. The interference of silicates in the phosphorus colorimetric reaction, and in enzymatic assays, are especially noteworthy. Examples are given of difficulties all attributable to impure water in prothrombin assays, haemoglobin measurement, and of course in the using of the Coulter type particle counter. The official (U.S.A.) specification for reagent grade water are given, also a modified form of these in the light of the author's experience. The use of distillation is compared with deionisation (by using resins). The latter is given in more thorough detail. The reason for this may be that the author prefers deionisation to distillation. The pros and cons of various forms of water purification are especially interesting *e.g.* the choice of filters used to remove organic and particulate matter; whether to follow deionisation with distillation (or vice versa); and the quality results obtained with these various permutations.

It is a pity that so little is said about the use and selection of all glass distillation apparatus. Few laboratories would be prepared to use deionised water for all analyses. The newer commercially available systems of all-glass distillation incorporating "fail safe" features would have been worthy of comment.

The section on testing both raw and purified water could be improved. Frequently, the methods used employ commercially prepared reagents the constituents of which are always clear. In any case, unless the manufacturer of these reagents also supplies a sample of the water used in their preparation, one may wonder how a reagent blank may ever be relied upon. The use of solid reagents in the simple screening tests associated with the quality control of water would seem to be more logical.

However, these criticisms do not detract from the fact that this is a well-written, fully documented account of a fundamental problem. As such the book should be essential reading to everyone involved in laboratory management. If this text-book makes all clinical laboratories appreciate that some form of regular quality control of "pure" water is necessary, then the author will have made a valuable contribution to medical laboratory technology.

J.L.B.

Review of Medical Microbiology. 8th Edition. E. Jawetz, Ph.D., M.D., J. L. Melnick, Ph.D. and E. A. Adelberg, Ph.D. 502 pages. Lange Medical Publications, Los Altos (1968). Obtainable from N. M. Peryer Ltd., P.O. Box 833, Christchurch, at N.Z. \$6.50.

This publication, now in its eighth edition, has been used widely by medical students and editions have been published in several languages.

The first thirteen chapters deal with basic science as applied to microbiology. Succeeding chapters cover systematic bacteriology, medical mycology, rickettsial diseases, the agents of the psittacosis, lymphogranuloma venereum and trachoma, and viruses. The book concludes with an appendix on medical parasitology, a list of reference books and journals and the index.

Chapters contain well planned sections and subsections, many of which are further divided into tabulated paragraphs. The letter press is a concentration of factual and up-to-date information which demands close study and does not provide rapid and easy reading.

Except for the virus section, this volume varies little from the previous edition. Changes have been made in the chapter on microbial genetics and a section on the genetics of drug resistance has been included; the section on the coliform bacteria has been revised and enlarged; the revised chapter on the agents of psittacosis, lymphogranuloma venereum and trachoma (Bedsoniae, Chlamydiae) has been removed from the virus section and placed earlier in the book.

Most of the new information in this book is found in the chapter covering the general properties of viruses and in chapters dealing with

individual viruses. The former now includes sections on viral protein, nucleic acid and lipid, the replication of viruses, viral genetics and viral interactions. Informative, additional diagrams have been included.

Technologists and advanced trainees will find the basic science part of this book most useful, and the virus section offers a ready source of information concerning properties of viruses and viral infections. The sections on systematic bacteriology, medical mycology and parasitology are less well suited to their requirements.

This book continues to be very good value.

N.P.M.

Techniques in Blood Grouping. Second Edition. I. Dunsford, Ph.D., M.C. Path., F.I. Biol. and C. C. Bowley, M.B., B.S., F.R.C.O.G., F.C.; Path. 534 pages in two volumes. Illustrated. Oliver and Boyd, Edinburgh. (1967). N.Z. price \$14.50.

The greater part of Volume One of this book is an introduction covering almost all aspects of a transfusion centre's function, and organisation problems. Throughout this section the whole question of sources of error in blood banking procedures is dealt with in a clear and thorough fashion.

Section Two contains carefully planned summaries of the more important blood groups, rendered down to a routine level and presented in carefully graded steps of increasing complexity. A concise glossary of about 950 entries makes up a useful third section. Section Four, which is the technical section of this book, shows a rather unimaginative approach to the techniques available for blood grouping. It is unfortunate that in this, a technical book, two rather bad technical practices should be recommended. One is the suggestion that a Pasteur pipette should be held vertically in the reverse position, which would be a likely source of pipetting error; and the second is a recommendation that a pool of up to six separate cell types should be used for screening for blood group antibodies in ante natal sera.

Dunsford and Bowley's book gives the impression of being a report on the activities of the Sheffield Regional Transfusion Centre, together with a set of local laboratory technical notes. While these may be admirable for use in their own service, it must be appreciated that they are not necessarily acceptable elsewhere. Although this book is not strongly bound, which physically limits its life in a routine laboratory, it could be of limited value in a small hospital blood bank and for trainees studying Blood Group Serology.

R.D.

Book Received

Exfoliative Cytology of the Stomach. D. D. Gibbs, B.A., D.M., M.R.C.P., D.C.H. 147 pages, illustrated. Butterworths, London. (1968) N.Z. price \$7.75.

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The first of a new series of automatic analytical gas chromatographs which will operate completely unattended for periods exceeding 24 hours is announced by *Pye Unicam Ltd.** of York Street, Cambridge, England.

It is known as the Pye Series 106 Model 6, and is based on a standard laboratory chromatograph incorporating a flame ionisation detector and isothermal control of column temperature — the Pye Series 104 Model 4 — with the addition of an ingenious electro-mechanical system which automatically injects up to 36 solid samples in succession at intervals of up to 90 minutes. This is shown diagrammatically in Fig. 2. The automatic injection system may also be purchased separately and fitted to any existing chromatograph in the series 104 range.

Applications

The instrument is suitable for the analysis of many solid and liquid samples of low volatility, proving particularly useful where samples are normally received as dilute solutions in a volatile solvent.

It was in fact originally designed for the routine estimation of steroids in blood plasma and urine, the technique here being sufficiently rapid, sensitive, accurate and inexpensive to be used as a standard screening test for the early detection of breast cancer and various endocrine disorders. Typical detection limits are 0.01 μg with the standard flame ionisation detector and 0.0001 μg with the electron-capture detector fitted to other Series 104 chromatographs. The use of solid samples minimises interference of solvent peaks with steroid peaks and in trials, no catalytic decomposition of steroids has been detected. A special glass sample-holder has been developed on which adsorption of steroid samples is negligible.

Technical Details

The automatic injection system consists of two units: the injector mechanism itself, and a control unit. In addition there is a heatable loading-rack for preparing the samples. The first operation is to load up to 36 glass sample-holders — 0.6 in (15 mm.) long with a capacity of 40 μl into a magazine. The magazine is then laid horizontally on the loading-rack and the samples, in solution, are injected by means of a syringe. An indexing device points out the holder being filled and indicates its number for logging purposes. When all the holders are full, the heater is switched on to evaporate the volatile solvent. The magazine is then transferred to the injector unit (See Fig. 2) and the controller switched on.

The magazine is held upright so that the sample-holders are stacked vertically. A signal from the control unit then energises a solenoid coil so that a metal plunger pushes the bottom sample-holder out of the magazine. A second solenoid withdraws the plunger. The sample-holder falls into a stainless-steel guide-tube fitted to the top of the column, where it is heated by a small electric element. The sample rapidly evaporates and is flushed into the column by the carrier gas. After about a minute a third solenoid is energised and another metal plunger ejects the used sample-holder into a glass receiver.

To prevent contamination, the carrier gas is introduced as close as possible to the top of the column, and small bleeds are provided on each limb of the injector unit. A port for manual injection of samples is also provided.

Right —

Fig. 1. The Pye Series 106 Model 6 gas chromatograph fitted with automatic injection system for solid samples. The injector unit can be seen on top of the main oven; the controller is on the right with the magazine-heater/loading-rack on top.



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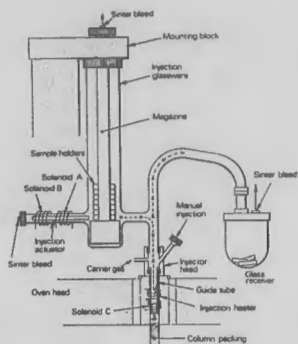


Fig 2

Left —

Fig. 2. Schematic diagram of the injector unit of the Pye Series 106 Model 6 chromatograph.

Control

The control unit consists essentially of a) a cam timer with a period of two minutes, which controls the injection sequence by sending pulses of current to the three solenoids, and b) a second timer which can be set to re-initiate this cycle at intervals of between 10 and 90 minutes. An indicator dial shows which sample is being processed, and a limit-switch on the dial switches off the timer after the last chromatogram. Before every injection, pulses from the cam timer automatically zero the recorder and make a distinctive mark on the recorder trace to indicate the number of the sample.

Provision is made for the injection sequence to be remotely initiated by a programme timer or a computer if desired. Other parameters such as amplifier attenuation-level and column temperature may then be programmed also.

New Models

Further chromatographs of Series 106 are under development and will be introduced shortly. All will be based on the Pye Series 104 instruments with the addition of automatic injection systems for different kinds of sample.

Further information from: Pye Unicam Ltd., York Street, Cambridge, England.

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Adherence to the following instructions is necessary in order to ensure uniformity of presentation, and all contributors are urged to study them before submitting their manuscripts.

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ILLUSTRATIONS

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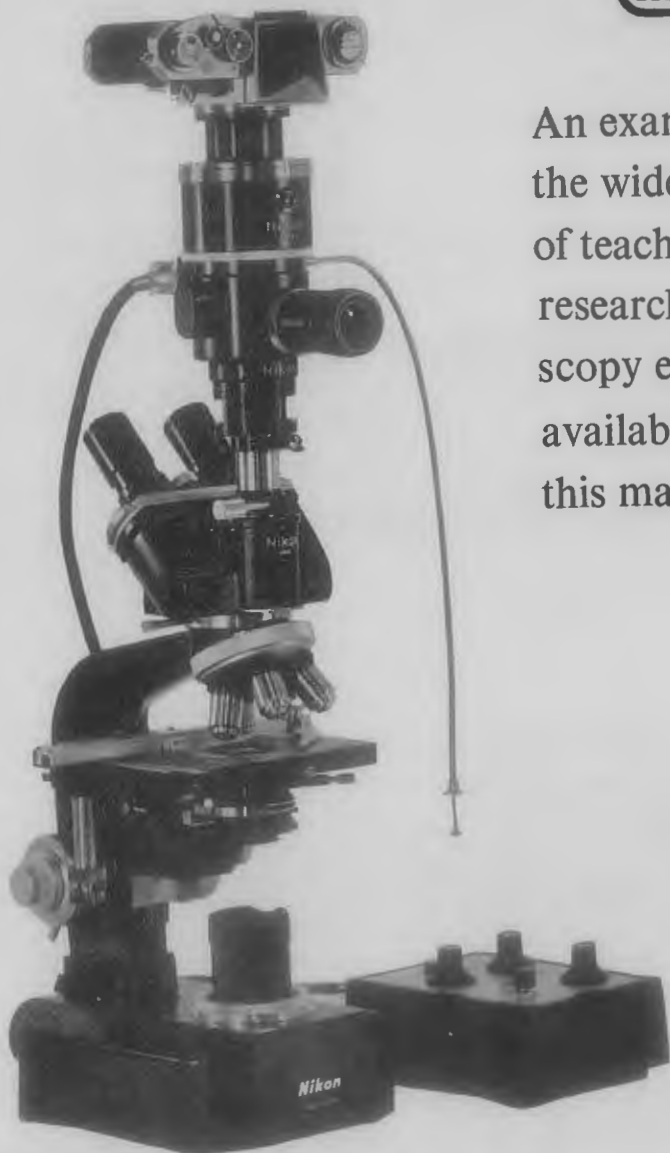
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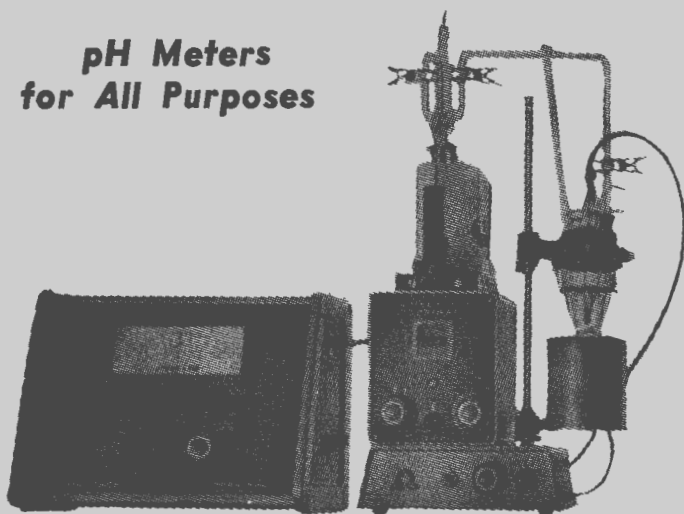
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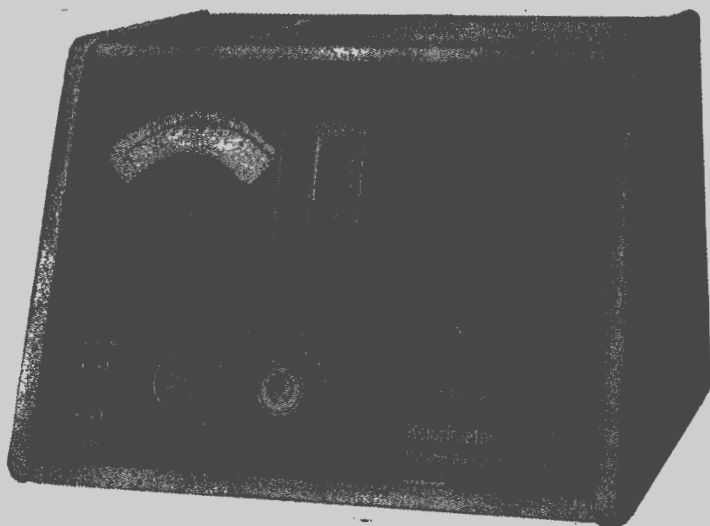


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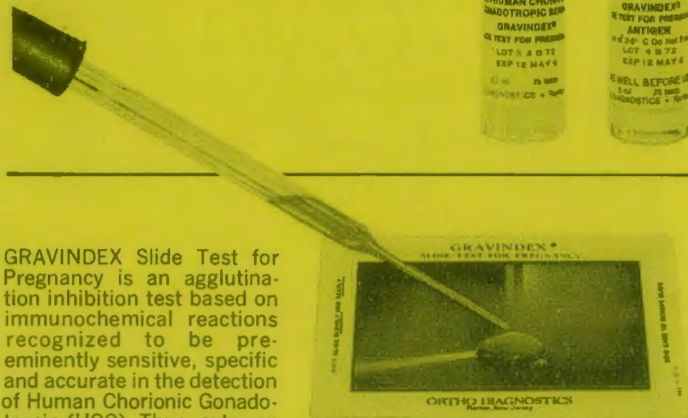
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