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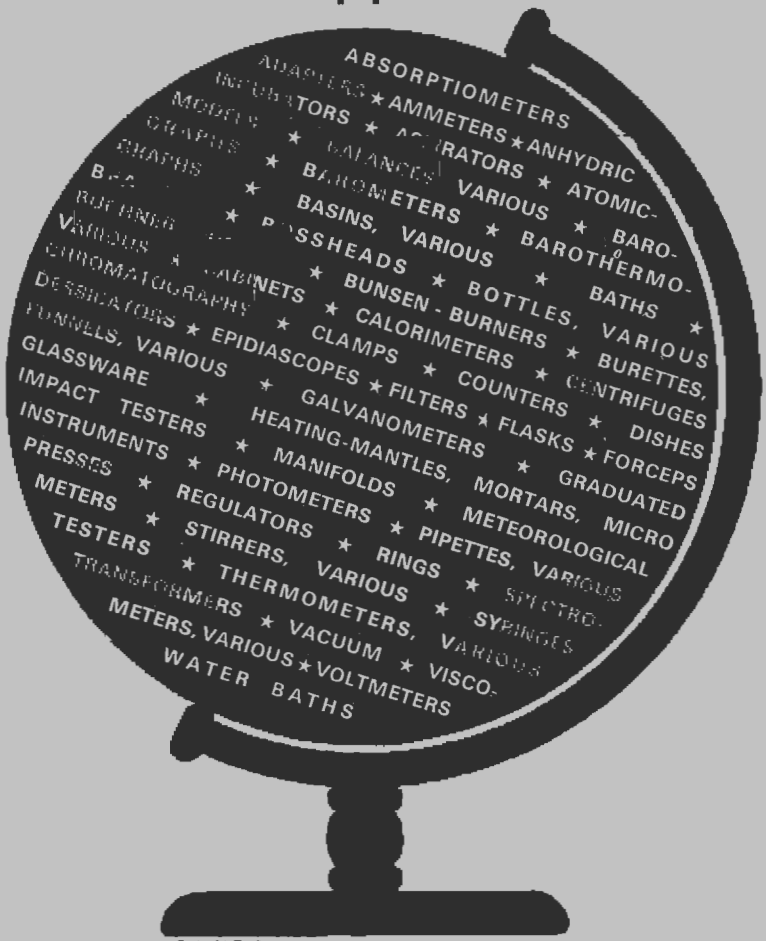

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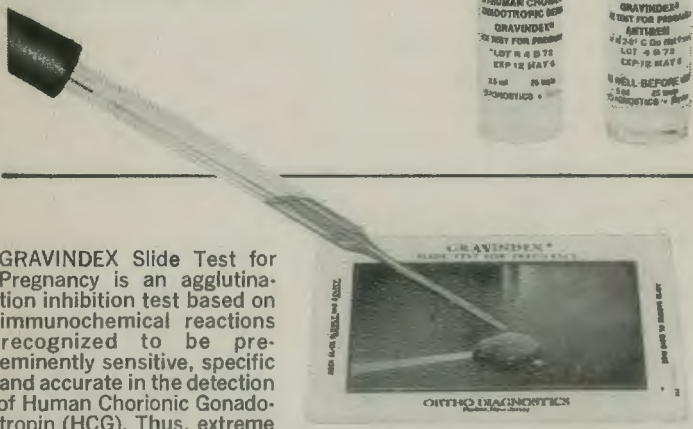

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Quality Control in Clinical Chemistry

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Received for publication August, 1966.

Introduction

The often-encountered reservation on the part of the clinician towards the results of the biochemistry laboratory is not entirely unwarranted, for the results of inter-laboratory surveys have revealed an alarming lack of precision in many laboratories. Institution of quality control on a national scale is the first step towards rectifying this situation.

Quality control is a system of monitoring the performance of every test over a period of time. The data obtained from these control procedures yields information about the quality (*i.e.* the accuracy and precision) of the results. This fundamental information distinguishes the satisfactory from the unsatisfactory methods, thus leading the way to any necessary improvements. Quality control of satisfactory methods ensures the constant validity of the specimen results by continually monitoring the accuracy and precision of the methods.

Accuracy

Accuracy concerns the efficiency of measuring the true amount of substance present, and is obtained in the laboratory by the use of tested methods, pure reagents and daily calibration of reliable instruments with accurate reference standards. The monthly performance of recovery experiments provides a check on the percentage of substance that is actually being measured by the method. These experiments are conducted by adding a weighed amount of pure chemical to a known volume of serum or urine. The expected resultant concentration is compared with that obtained by actual measurement. Table I shows the results of one such experiment—for fasting blood glucose.

The accuracy, or "pitch," of a method may be checked by the analysis of carefully prepared standard solutions and of commercially prepared sera containing weighed-in amounts of constituents. It is important that the technologists be unaware of the value of the prepared standard solutions and of the fact that a particular sample is a commercially prepared serum, the values of the constituent levels of which are readily accessible. The possibility of any unconscious or conscious bias is thus eliminated.

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Date	Original serum	Original serum with 88 mg./100 ml. glucose added		
		Observed value	Percentage recovered	Mean percentage recovered
13/6/66	94	172	95	98
14/6/66	115	202	99	
15/6/66	106	192	99	

Table I. Results of a recovery experiment on the method for measuring fasting blood glucose.

NOTE: (1) All results are given in mg./100 ml.
(2) Each batch was in control.

The commercial sera are standards-in-serum; *i.e.* they are standards of composition similar to that of the test samples. They are therefore of greater value than non-serum standards in assessing the accuracy of a method, since they provide a truer indication of just what the method is measuring in the test samples of serum.

The accuracy of any result also depends on the specificity of the method, which is evaluated as satisfactory or not, only after thorough investigation.

Intra-batch precision

Precision is concerned with the reproducibility of results. Intra-batch precision, or precision within a single batch, is essential if the results are to be accredited with any significance at all.

Intra-batch precision is controlled by the inclusion in each batch of a duplicate of one of the patients' specimens in that batch. The technologist is unaware of which specimen has been split by the quality control supervisor, thus any bias towards a particular result or extra-careful treatment of one or two specimens is avoided. From a minimum of 30 pairs of duplicates the mean difference between duplicates may be calculated. Benson and Freier[1] published the following formula:

$$\begin{pmatrix}\text{maximum permissible difference}\\ \text{between duplicates}\end{pmatrix} = 3.27 \begin{pmatrix}\text{mean difference}\\ \text{between duplicates}\end{pmatrix}$$

They gave significance to the value thus obtained for the maximum permissible difference by stating that if the actual difference between specimen duplicates is greater than this maximum permissible difference, the batch is out of control and requires investigation. But this permissible difference is so very generous and cannot detect any change in the "pitch" of the results, that it is felt that too much reliance should not be placed on it. The values of this difference are included in Table II and Figure 1 for interest.

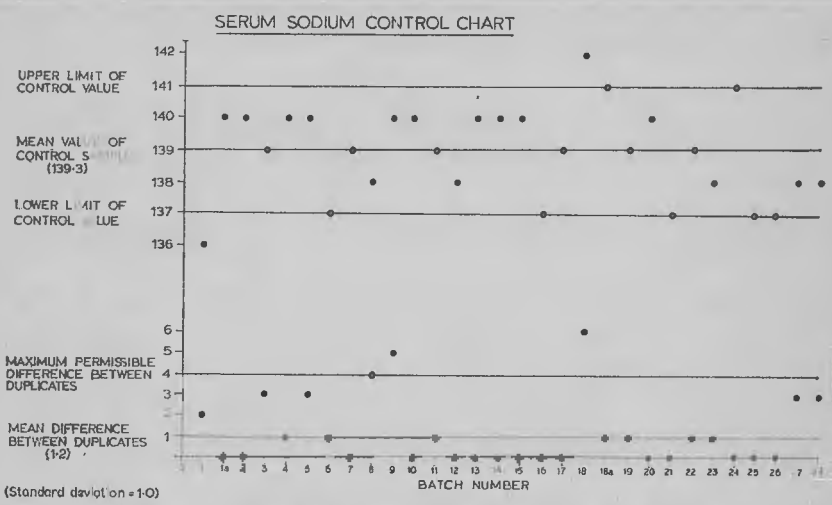

Figure 1. Portion of a typical serum sodium control chart.
NOTE: Repeated batches are numbered with the suffix, "a".

This data on intra-batch precision is recorded on a control chart, on the vertical axis of which are marked the mean difference between duplicates and, for interest, the maximum permissible difference between duplicates. The batch number or date of analysis is marked along the horizontal axis. Figure 1 is a portion of a typical routine serum sodium control chart for 30 consecutive batches.

Inter-batch precision

A satisfactory level of precision between batches must be maintained in order that results obtained on one day may be directly comparable to those obtained on any subsequent day.

Inter-batch precision is controlled by the inclusion in every batch of a sample from a control pool. The value obtained for the relevant constituent in this control pool is the key to the control of inter-batch precision. How this value can be used to control a method is explained in the following paragraph.

The Theory of Quality Control

Elementary statistical theory provides the basis of quality control[2]. If any quantity is measured many times the results obtained will not be identical, but will differ from each other by small random deviations. A plot of the value obtained (X) against the number of times it was obtained (n) will give a symmetrical curve, the normal, or Gaussian, distribution curve (Fig. 2). Note that the curve does not touch the X axis. This means that it is possible to obtain occasional values on either side very far from the peak of the curve, which represents the most

frequently occurring value. Any area under the distribution curve corresponding to an increase from X_1 to X_2 gives the probability that a single sample will yield on X value lying in the range X_1 to X_2. Clearly, the most probable value to occur is that at the maximum of the curve, and this corresponds to the arithmetic mean of all the samples, namely $\overline{X}$. In mathematical terms:—

$$\overline{X} = \frac{X_1 + X_2 + \ldots\ldots\ldots\ldots + X^n}{n} = \frac{1}{n} (\lesssim X)$$

The standard deviation, σ (sigma), of the batch of samples is an important quantity. Its significance is clear from the fact that it measures the spread of the curve, or the extent to which the individual results deviate from the mean. In Fig. 3, curves A and B have the same mean value, but different degrees of spread, or standard deviations. On the average, batch A shows greater deviations from $\overline{X}$ than does batch B.

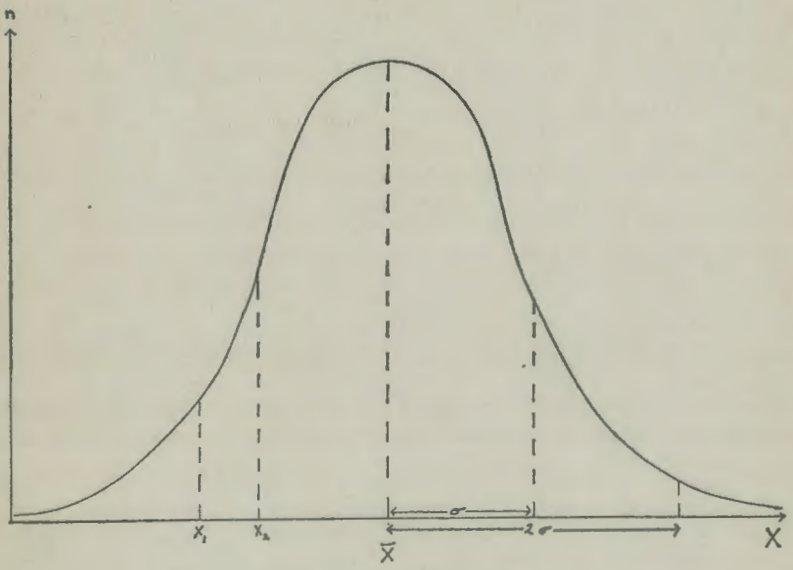

Figure 2. The Gaussian distribution curve.

Notation:

X = value of quantity being measured.
n = number of times each value is obtained.
$\overline{X}$ = arithmetic mean of all X values.
X_1, X_2 = randomly selected values of X.
σ = standard deviation.

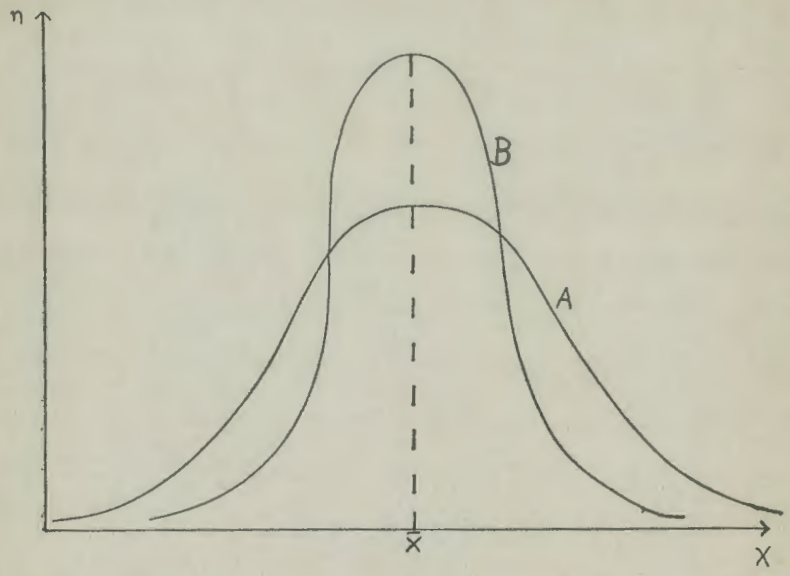

Figure 3. Two distribution curves, A and B, with the same mean, $\bar{X}$, but different standard deviations.
Notation: As for Figure 2.

The standard deviation is equal to the square root of the sum of the squares of the differences between the mean and the individual values, divided by one less than the number of samples. In mathematical terms:

$$\sigma = \sqrt{\frac{\leqslant (\bar{X} - X)^2}{n-1}}$$

It can be shown that 68.2% of the observations deviate by less than one standard deviation from the mean, and that 95.5% deviate by less than two standard deviations. Only 1 in 400 should deviate by more than 3 standard deviations, and any such results are highly suspect and are likely to be due to gross mistakes.

The Practical Application of the Theory

This statistical theory is related to quality control thus: the standard deviation is a statistical measure of the reproducibility of the results of a certain procedure in the laboratory. This reproducibility is monitored by the inclusion in every batch of a sample from a large pool of serum or urine, which is kept deep frozen so that its composition remains constant for six months to a year. A tube of this pooled control sample is removed from the deep freeze each morning by the quality control supervisor, thawed at room temperature, thoroughly mixed by vigorous shaking, and distributed between the batches that are to be analysed that day.

The limits inside which the daily values of the control serum or urine must lie for the batch results to be termed "in control" are set at two standard deviations above and below the mean, which has been calculated from the values obtained for the control sample on at least thirty different days. These control limits are really 95.5% confidence limits that fix the error that is allowed in determining the control value without affecting the validity of the values of the patients' specimens. If the control value falls outside these limits, the batch is repeated, and the cause of the discrepancy discovered and rectified. Since only 95.5% of all results are expected to fall within these limits, one result in twenty is expected to lie outside them.

This data on the control of inter-batch precision is recorded on the same control chart as that used for intra-batch data. On the vertical axis is marked the mean value of the relevant constituent of the control sample, together with the upper and lower control limits (Fig. 1).

For the system to be of value it is essential for the analyst to mark in the daily control result as soon as it is obtained, for not only does it provide visible confirmation of the batch results, which can then be sent out; but it also provides a rapid assessment of the testing procedure up to the most recent batch if for any reason a batch or single specimen result is in doubt. Equally essential is the regular examination of all quality control data by the departmental head himself, who supervises the long-term effectiveness of the control programme.

Precision is conveniently expressed as the coefficient of variation, or percentage error. The mathematical definition is:

$$\text{coefficient of variation} = \frac{\text{standard deviation}}{\text{mean}} \times 100\%$$

Table II presents some statistical data obtained in this laboratory for 10 routine serum methods.

To account for any small variations that may occur in the laboratory, either suddenly or gradually, with the result of altering the test procedure slightly, the mean, standard deviation, and normal range (see later) of controlled methods should be re-estimated at monthly or bi-monthly intervals.

Control pools

The control pool of urine can be collected from urine specimens remaining from the day's tests or from any normal person over a 24-hour period. Preservatives must be added if necessary, of course.

The control pool of serum may be composed of human, ox or bovine serum, or a mixture of the two. Table III presents the values of six arbitrarily selected constituents of the first human, ox and mixed pools collected. Out of the 20 constitutents analysed, this particular pool of human serum had normal values for 12

Serum constituent	Range of values of control sample	Mean value of control sample	Standard deviation	Coefficient of variation	Range of differences between duplicates	Mean difference between duplicates	Maximum permissible difference between duplicates
Albumin g./100ml.	2.8-3.5	3.1	0.3	9	0-1.0	0.1	0.3
Alkaline phosphatase KA units	13-20	14	2	12	0-3	1	3
Calcium mg./100ml.	8.0-9.2	8.7	0.3	4	0-1.4	0.5	1.6
CO_2 content mEq/l.	15-22	17	1.5	9	0-4	0.5	2.5
Creatinine mg./100ml.	1.5-1.9	1.7	0.1	5	0-0.6	0.1	0.4
Glucose mg./100ml.	66-82	76	4	5	0-8	2	6
Magnesium mg./100ml.	1.6-1.9	1.8	0.1	3	0-0.4	0.1	0.2
Potassium mEq/l.	4.7-5.2	5.0	0.1	3	0-1.3	0.1	0.3
Sodium mEq/l.	136-146	139	1	1	0-9	1	4
Total protein g./100ml.	5.7-7.0	6.4	0.3	4	0-2.4	0.3	0.9

Table II. Statistical data for 10 routine serum methods.

NOTE: (1) All methods except those for calcium and magnesium are automated.

(2) All results have been reduced to a number of significant figures that are of PRACTICAL use. Thus, for creatinine the mean difference between duplicates to 2 significant figures is 0.12, leading to a maximum permissible difference of 0.392, which for practical purposes is called 0.4.

(3) All values are based on at least 30 separate determinations.

Serum constituent	Pool of human serum	Pool of ox serum	Pool of mixed serum
Alkaline phosphatase KA units	12	14	8
CO_2 content mEq/l.	12	17	21
Glucose mg./100ml.	84	76	67
Sodium mEq/l.	138	141	141
Total protein g./100ml.	6.8	6.4	7.7
Urea mg./100ml.	159	16	68

Table III. Values of some constituents in 3 control pools: Human serum, ox serum, and an approximately equal mixture of the two.

NOTES: (1) The ox serum in the mixture is not from the same pool as the pure ox serum.

(2) The values given for the constituent levels in the human pool and the ox pool are mean values of at least 30 separate determinations. Those given for the constituent levels in the mixed pool are mean values of at least 20 separate determinations.

(3) The urea level of the mixed pool has been raised by the addition of pure urea. It is obvious that the levels of CO_2 content and glucose could also be raised, by the addition of sodium carbonate and glucose, respectively.

(4) Electrophoresis of the proteins in both the ox pool and the mixed pool yields the abnormal result that only 4 different proteins are separated.

constituents, the ox pool for 11, and the mixed pool for 10. Further techniques of obtaining control pools of suitable composition are described in references 9 and 10.

The protein-bound iodine (PBI) pool must be composed of left-over PBI sera, and glassware specially cleaned to be free of iodine traces must be used, since ordinary laboratory glassware can cause very high irreproducible results.

The chief drawback to using non-haemolysed, non-lipaemic human serum as a control pool is the slowness of accumulation of the large pool necessary—a minimum of, say, 3 litres for a large laboratory. Daily collections of the serum remaining from the biochemical tests rarely amount to more than 50 ml., thus requiring at least 3 months to elapse before control samples may

even begin to be run to determine means and standard deviations, which usually takes 6 weeks.

There is no such delay if ox serum is used: a bucket of blood is obtained from the nearest abattoir, and is allowed to stand in water at 37°C. (approx.) for 3 hours. Cutting of the clot into 8 equal pieces causes it to retract and the serum to flow out. Overnight incubation or refrigeration will produce greater stress in the clot, with consequently greater retraction and a larger volume of serum, but will alter the concentrations of some constituents considerably, especially enzymes. If overnight incubation is employed, it is recommended to incubate 100-200 ml. for 3 hours only, in order to obtain an enzyme control pool. Wherever possible weighed amounts of those constituents which have decreased should be added to the pool.

The serum thus obtained is filtered through cotton wool to remove small pieces of clot, centrifuged, mixed thoroughly and placed into clean glass test tubes with polythene caps, in aliquots of about 10 ml., an average daily amount. The large volume of serum is shaken regularly during dispensing to ensure adequate mixing. The tubes are stored in covered tins and deep frozen.

The importance of thorough mixing of any frozen specimen cannot be over-emphasised, since the layering-out of different constituents is very marked. Before a control pool is used up, the mean and standard deviation of the next pool are determined while the method is still in control.

Commercially prepared sera containing weighed amounts of constituents can be used as control pools, provided the expense involved is not excessive. They do, however, suffer from one serious disadvantage: the values of the constituent levels are known to the technologists, who may therefore show unconscious or conscious bias in obtaining results. Hence when these results are used to calculate mean values and standard deviations, the limits of control that they set for a method may be falsely narrow. If they are the method is accredited with an erroneously high reproducibility, which cannot be obtained with any other type of sample.

Disadvantages

The most obvious defect of this quality control system is that the technologist is aware that a certain specimen is the control sample, and could treat it with greater care than the patients' specimens. The knowledge of the limits within which the control sample value is expected to fall could lead to unconscious or conscious bias in producing results. The counter to this defect is found in an experiment conducted by Weinberg and Barnett[11] who showed an absence of bias in this respect in the laboratory investigated.

Even though the value of the control sample may lie between the acceptable limits, any one of the patients' specimens may be

out of control—by faulty dilution, contamination, etc. No system of quality control will be able to detect this.

A further defect of the system is that the control samples are treated differently from the patient specimens in that the former are not obtained by venepuncture and are not therefore subjected to the many small influences encountered in transport from the ward to the laboratory and in preparation for analysis.

The system does not eliminate the possibility of clerical errors, which can account for as much as 5% of laboratory errors.

Advantages

One advantage of the system of quality control described above lies in the fact that the instrument of control (the pool) has the same composition as the unknown specimen, and it also passes through all the stages of the test; neither of these advantages is possessed by standard solutions.

This method is independent of the proportion of normal specimens received for analysis, and also requires no waiting for a specified number of routine specimen results to accumulate before a computation may be performed to provide some control data.

If the batch is out of control *immediate* action can be taken by the technologist. No time need elapse while a supervisor makes his rounds to check the control values for each batch. Since the technologist is permitted to know the control value, he feels trusted and is gratified when the control values fall within the correct range.

This method of quality control is employed in this laboratory since it is felt that these advantages more than offset the defects mentioned above.

The Quality Control Programme in Brief

It must be stressed that the control samples are but a single facet of the whole quality control programme, which extends throughout the laboratory and involves the entire staff. This programme may be summarised as one comprising the following elements: adequately trained staff; the use of efficient equipment, methods, reagents and specimen collection; accurate non-serum reference standards and standards-in-serum, calculations and clerical work; and the special techniques of recovery experiments, split specimens and pooled control samples. Wherever possible results from two or more tests should be correlated to provide mutual checks. Much information can be obtained from participation in provincial and national clinical chemistry surveys.

Future Developments

Calculation of the normal ranges of all the methods in use in the laboratory is an essential part of quality control. For the results of any test to be meaningful, the normal range as estimated by that method with those instruments and operators must be

known. Normal ranges are ideally computed from Gaussian distribution curves for all normal persons, but since this is impossible in practice it is assumed that the curve for 60 normal persons resembles that for millions. If 60 normal specimens are not procurable, methods based on clinical specimens must be used. Hoffmann has published a graphical percentage probability method of computing normal ranges[3], but it is inapplicable when the proportion of abnormal specimens exceeds that of the normal specimens. This has often been the case in this laboratory where, for example, 75% of 484 consecutively determined fasting blood glucoses were abnormal, and similarly 70% of 260 serum creatinines were abnormal.

Quality control samples should ideally be run at three different constituent levels; below, within, and above the normal range, especially where the technique is difficult, as in protein-bound iodine estimations[8], or where the normal range is narrow. This has yet to be established here, though it has been done exhaustively by Plym[7], who showed that for some tests the coefficient of variation differs for different levels of constituent. This variation in precision is indicative of a need to run control samples at more than one level.

Alternative Systems

There are other methods of quality control available: Hoffmann and co-workers have published three methods using clinical specimens[4, 5, 6]. All these methods require the accumulation of a fixed, though sometimes small number of specimen results before the calculation of the control data may be performed.

Communication with the Clinicians

The institution of a quality control programme with its attendant increase in the accuracy and precision of the laboratory results is in vain unless the facts of its existence are made known to the large number of clinicians who use the laboratory. Communication of information about laboratory procedures is effectively wrought through circulars and displays of quality control data. Perusal of control charts yields useful information on the stability of the tests through time.

It must be stressed to the medical staff that the validity of any test result depends on the handling of the specimen from the time of sampling up to the time of issue of the report form. Thus the stringent quality control precautions that are carried out to minimise random influences in the laboratory on the specimen must be supplemented in the ward by careful preparation of the patient and accurate techniques of sampling, and, if necessary, storage.

Intelligent interpretation of laboratory results demands a knowledge of the normal ranges and percentage errors of each method. These should be quoted on all report forms.

Conclusion

The operation of an efficient quality control programme in the laboratory, together with the maintenance of efficient communication channels to the medical staff, will effect a disappearance of the distrust of biochemistry now prevalent among clinicians. In its place will appear an awareness of the strenuous efforts of self-criticism at present being made towards increased precision and accuracy in biochemical measurements.

Summary

A system of quality control using daily control samples of serum and urine is described, together with its advantages and disadvantages. The control of both accuracy and precision is discussed. The theoretical statistics which form the basis of the method and practical details of preparation, storage and distribution of control samples are included. Reference is made to some alternative systems of quality control and to the possible course of future developments.

Acknowledgement

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A Modified Aminophenazone Procedure for the Assay of Serum Alkaline Phosphatase Activity

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Introduction

The procedure of King and Armstrong[2] for the assay of serum alkaline phosphatase has been modified by King[2] and Kind and King[1], whose modifications are used in an automated continuous-flow procedure devised by Marsh *et al.*[4], and used in the Technicon *AutoAnalyzer* equipment. The substrate common to all of these modifications is a carbonate buffered solution of disodium phenyl orthophosphate, and enzyme activity is assayed by measurement of the amount of phenol liberated from the hydrolysed substrate. The major modification of the original procedure has been the introduction of a phenol colour reagent (1 phenyl-2, 3-dimethyl-4 aminopyrazolon-5), which is also known as '4 aminophenazone' or 'amino-antipyrine.' The use of this reagent obviates the need for deproteinisation as required in the use of the phenol reagent of Folin and Ciocalteu.

In the adaptation of Nothstein and Ellerbrook[5], reactants are added manually in the same order, and in approximately the same proportions and concentrations as those of the automated procedure. The omission of added alkaline buffering components in the colour reaction system of these procedures does not affect the specificity and stability of the colour reaction.

A manual procedure is here described in which the colour reagent is included in the buffered substrate, so that only one reagent addition is required for colour development. The method is compared with that of the original King and Armstrong procedure as described by King and Wootton[6], and studies on the degree and variability of the 'serum blank' are described.

Materials and Methods

The King and Armstrong procedure was carried out according to King and Wootton, using the reagents described by them. Reagents used in the modified aminophenazone method are as follows:—

1. Aminophenazone buffered substrate

Sodium carbonate	6.36 g.
Sodium hydrogen carbonate	3.36 g.
di-Sodium phenyl orthophosphate	2.18 g.
4-aminophenazone	0.5 g.

are dissolved in about 900 ml. of distilled water, brought to the boil, cooled, and made up to 1 litre. The pH is 10.0. A few drops of chloroform are added as preservative.

2. Potassium ferricyanide 0.5 g./100ml.
3. Stock phenol standard. Pure crystalline phenol, 1.00 g. is dissolved in, and made up to 1 litre with 0.1N hydrochloric acid.
4. Working phenol standard (10mg./100ml., equivalent to a serum activity of 10 K.A. units/100ml.). The stock standard is diluted 1 in 10 with distilled water.

The chemicals used are Merck GR, but any analytical grade reagents should be satisfactory. All solutions described above are stable for at least 2 months at 4°C. The same stock phenol standard was used for both assay procedures.

The assay technique used in the modified aminophenazone method is as follows:—

2.0ml. of aminophenazone buffered substrate is measured into each of a series of matched cuvettes, which are placed in a waterbath at 37°C.

0.1ml. of test serum is added, and the contents of each tube mixed thoroughly.

After 15 minutes incubation the tubes are removed from the waterbath, and 1.0ml. of potassium ferricyanide solution is added. The contents of each tube are then thoroughly mixed. An orange to red colour appears according to the amount of phenol present.

The optical density of the test solution is measured at a nominal wavelength of 505 mμ, within 5 minutes, using an incubated reagent blank (2.0ml. of aminophenazone buffered substrate) to which 1.0ml. of ferricyanide solution has been added. The phenol standard (0.1ml. of working standard plus 2.0ml. of aminophenazone buffered substrate incubated for 15 minutes) is treated in the same way as the tests.

All colorimetric measurements for the comparative tests were made with Bausch and Lomb *Spectronic 20* instruments. The sera used were a random selection, some were slightly haemolysed or lipaemic. Assays by the different assay procedures were performed on the same groups of sera on the same day.

Results

The results of 40 serum assays by each method are shown in Table I, which also shows the serum blank for the modified aminophenazone method in Optical Density units. Fig. 1 shows linearity of the phenol colour reaction, and Fig. 2 an absorption curve of a standard phenol solution as used in the modified aminophenazone method.

Correlation of results obtained by the two methods may be assessed by calculating the statistical factor *t*, which from this series is found to have a value of 1.2. The limiting value of *t* for a series of 40 paired observations is 2.0. Statistically therefore, there is no significant difference between the results obtained by the two

Serum	King Armstrong method units/100 ml.	Modified aminophenazone method units/100 ml.	Serum blank O.D. units
1	12.6	15.1	.062
2	9.0	8.7	.030
3	14.0	15.0	.033
4	3.2	4.2	.027
5 a	27.5	28.5	.032
6	8.9	10.2	.026
7	7.3	7.6	.025
8	9.1	9.7	.027
9	5.4	4.8	.030
10	6.6	6.5	.034
11	4.5	5.4	.030
12	7.2	8.0	.029
13	8.1	7.1	.030
14	6.3	6.1	.018
15	4.3	4.7	.042
16	11.8	10.7	.030
17	10.3	10.5	.029
18	11.1	9.9	.028
19	7.8	6.7	.024
20	9.8	8.4	.026
21	6.8	5.4	.029
22	6.1	4.4	.042
23 b	23.2	31.0	.020
24	8.5	8.1	.022
25	10.9	10.7	.015
26	4.7	4.4	.012
27	5.4	5.5	.030
28	23.0	30.0	.022
29	5.3	7.3	.013
30	12.9	12.0	.017
31	7.1	8.0	.034
32	5.8	7.7	.013
33	5.9	5.4	.035
34	7.1	7.5	.033
35	6.1	8.2	.028
36	9.3	9.6	.036
37	18.6	19.8	.017
38	14.3	12.8	.026
39	5.4	4.7	.027
40 c	17.9	18.1	.013

Table I. Showing results obtained by the modified aminophenazone method, and by the King-Armstrong method. The serum blank was obtained by adding 0.1 ml. of serum to 2.0 ml. of buffered substrate, and immediately adding 1.0 ml. of potassium ferricyanide solution, and read against a reagent blank. Sera a, b and c are commercial controls.

methods. The standard error (2 S.D.) for each method was determined by a method of duplications. This was found to have a value of 7.4% for the King-Armstrong procedure, and 4.6% for the modified aminophenazone method. The mean serum blank reading using the modified aminophenazone method was 0.027 O.D., with a standard deviation of 0.007 O.D. for the 40 sera tested.

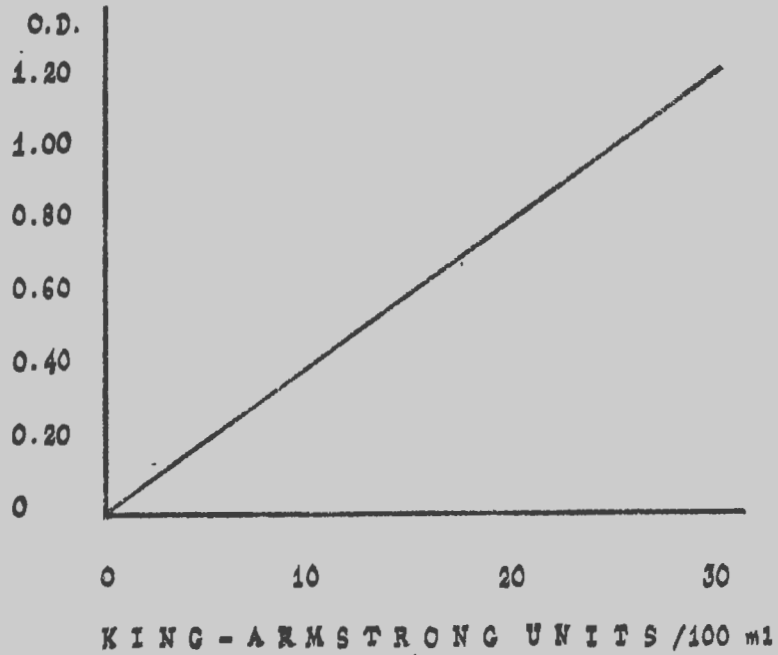

Figure 1. Graph showing relationship between Optical Density and alkaline phosphatase activity, aminophenazone method.

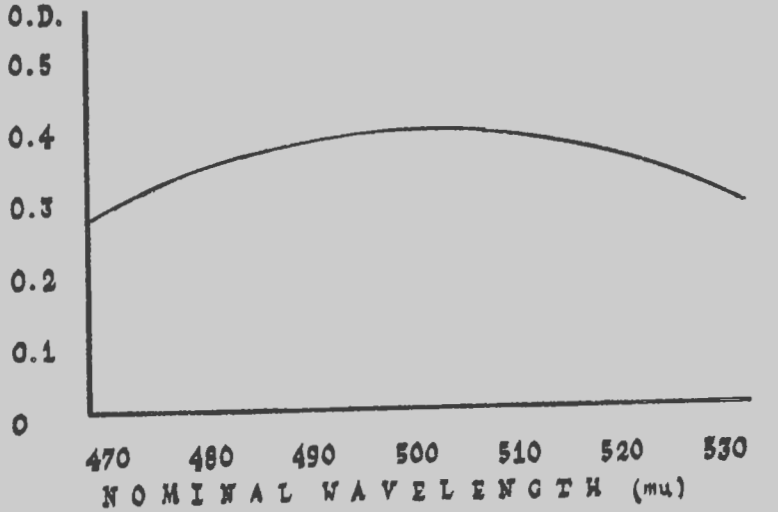

Figure 2. Absorption curve for phenol standard equivalent to 10 King-Armstrong units/100 ml, *vs* reagent blank, aminophenazone method.

Conclusions

The advantage of technically simple methodology in manual analytical procedures is obvious, provided that no concessions in precision or reliability have to be made. The modified aminophenazone procedure for assay of serum alkaline phosphatase described above has been in routine use in this laboratory for over 18 months and no technical problems have arisen. The omission of a serum blank in this procedure appears justified in view of the low and constant value obtained, although it must be recognised that an occasional lipaemic serum, or serum containing abnormal paraproteins may cause appreciable turbidity, and that this may be corrected by using a serum blank. The relative lack of precision of the King and Armstrong procedure can be attributed largely to the random variation of the serum blank in that procedure. This is presumably due to the non-specificity of the Folin and Ciocalteu reagent for phenol, and the difficulty of maintaining identical conditions of temperature, degree of mixing etc. for both test and blank solutions, particularly when large numbers of samples are being assayed simultaneously. The modified aminophenazone procedure has been found to be relatively free of the pipetting and transfer errors common in the more complex procedure of King and Armstrong. This modified aminophenazone procedure may also find application as a standby procedure in laboratories where serum alkaline phosphatase assay is normally performed by automated analysis with a phenyl phosphate substrate.

Summary

A modified aminophenazone procedure for assay of serum alkaline phosphatase is presented in which the phenol colour reagent is included in the buffered substrate. Only one reagent addition is required for colour development, and a serum blank correction is not normally required. The results obtained by this procedure are compared with those obtained with the procedure of King and Armstrong.

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Evaluation of Factor V Assay Methods

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Introduction

In 1949 Owren[2] described the result of an investigation into the effect of liver damage on the level of factor V in the blood.

A factor V deficient person (parahaemophilia) provided the substrate, and the patient's test plasma was added to this, the coagulation time being taken as being proportional to the level of factor V activity present. From these experiments he drew the following conclusions:—

1) The formation of factor V is dependent on normal liver function. The concentration of factor V decreases with increasing damage to the liver parenchyma.
2) The formation of factor V is independent of the supply of vitamin K.
3) In parenchymatous hepatitis, a concentration of factor V under 50% of the normal value is a bad prognostic sign; a steadily falling factor V concentration indicating the development of malignant hepatitis with fatal ending. Persistently sub-normal values point to the development of chronic hepatitis.
4) In obstructive jaundice factor V is normal or increased. In vitamin K deficiency from intestinal diseases factor V is normal.

It was obvious that if an accurate assay of factor V was available, the level of factor V could be used as a guide to liver function: especially when considered with the prothrombin level. However, in practice the number of parahaemophiliacs is very small and, hence, naturally occuring factor V deficient plasma very scarce.

Recently several methods of assaying factor V have been published, and these were investigated with the view of establishing a rapid and accurate factor V assay for liver function tests. A summary of the methods investigated is now presented.

Methods Investigated

Two methods were based on the labile property of factor V. Wolf[5] incubated oxalated plasma at 37°C for 24 hours, noting that longer incubation inactivated some of the prothrombin. He also found that dilutions in saline affected the results. The saline acts as an anticoagulant, whereas water resembles a physiological solution as diluent. Thus all the graphs were made up using water instead of saline. However, the 100% coagulation time was too long to achieve the desired accuracy.

Quick[3] prepared the factor V-free plasma by taking oxalated human plasma and storing it at 4°C. until the prothrombin time was increased to greater than 60 seconds. It was found that it took much longer for the prothrombin time to increase to 60 seconds in our laboratory than the time specified by Quick. However, this seemed a relatively easy method and was adapted for use in the coagulation unit of the Central Laboratory. The reagents were freeze-dried for convenient storage and rapid preparation. These were reconstituted with distilled water and then diluted to optimum concentration with buffered saline.

The original method of Quick was to mix:—

0.1ml. deficient reagent.
0.1ml. thromboplastin.
0.1ml. test plasma dilution.

Incubate for 1 minute, then add 0.1ml. M/40 $CaCl_2$ and take the time for coagulation to occur.

Note: 100% test plasma is a 1 in 10 dilution of plasma in Owren's diluting fluid II. Using the test plasma at 100% and 10% dilutions, the optimum concentration of thromboplastin was determined. It was found that a 1 in 3 dilution of thromboplastin in glyoxaline buffer gave the fastest coagulation time.

The substrate plasma gave optimum reaction using it undiluted.

With the established times, the $CaCl_2$ was tested at the 100% and 10% test plasma level, at concentrations of M/50, M/40, M/30. M/40 gave the best reaction time.

Incubation times of 30 seconds, 60 seconds, 2, 3, 4, 5, 8 and 10 minutes before recalcification were tried, an incubation between 3 and 6 minutes giving the most satisfactory results. Using these optimum conditions a graph was prepared, plotting test plasma concentration against time in seconds.

It was at this stage that problems occurred. The 100% concentration varied from 27 seconds to 35 seconds. Apart from this length of time being too long for the 100% level, the variations in time between dilutions were too great for accurate work.

A standard technique was developed and every precaution was taken, but the times could not be duplicated. No explanation was forthcoming as to why this should be so, and the method was abandoned.

Speers, Borges and Wadley[1] obtained factor V-free reagent by removing factor V with a mixture of inosithin and celite. Due to the difficulty encountered in obtaining the inosithin from overseas, this method was not investigated further.

In 1960 Borchgrevink[1] published a method for the estimation of factor V, and this method was investigated. The method proved ideal for the purpose and with only slight modification was adapted for use in the Central Laboratory.

The following are the reagents and methods that were used:—

Principle

Use has been made of the fact that Russell Viper venom, (R.V.V.) and cephalin, when mixed together with normal plasma at an alkaline pH, will remove the factor V activity from the plasma without removing any other "intrinsic activity." The R.V.V. and cephalin also act as a thromboplastin/proconvertin complex. Thus, on the addition of a test plasma to the reagent, clotting times on recalcification will be proportional to the factor V activity of the test plasma.

Preparation of Reagents

Calcium chloride: M/30.

Cephalin: 20g. of acetone-dried brain is covered with ether and left overnight in a stoppered flask. The supernatant is filtered and evaporated with an air stream. The material is suspended in 40ml. of acetone at room temperature. Wash twice more and then with boiling acetone. 3g. are suspended in 3ml. of saline and the volume brought up to 100ml. slowly. A yellowish tint makes the material unsatisfactory. Pale yellow to white is satisfactory. Store at −20°C.

Factor V substrate: Take 4ml. of 3% cephalin and add to 160ml. Owren's buffer. Use this suspension to wash out 16 ampoules of Russell Viper venom (*Stypven*) 0.1mg./ampoule. To this solution add 160ml. fresh platelet-free citrated plasma. Adjust pH to 8.5 ± 0.1 with NaOH. Distribute in 10ml. volumes in unsiliconed glass tubes (5″ x $\frac{5}{8}$″), and place in the 37°C. bath with frequent mixing. Leave until "blank" clotting time has reached 70 seconds.

Test as follows:—

0.2ml. reagent.

0.1ml. Owren's diluting fluid II.

Warm 2 minutes.

0.1ml. M/30 $CaCl_2$.

Three to four hours incubation is needed before the 70 seconds is reached. Repool plasma and adjust pH back to 7.3 with 1N HCl (the blank time will probably shorten slightly).

Store at 20°C. in 10ml. aliquots in glass 5″ x $\frac{5}{8}$″ test tubes.

For use:—

Thaw out 10ml. of the reagent and distribute in 3″ x $\frac{3}{8}$″ test tubes in exactly 0.2ml. volumes. Refreeze. When required for testing, thaw tubes immediately before use.

Owren's buffer

pH 7.35
sodium barbitone 5.873g.
sodium chloride 7.335g.
O.1N HCl 215ml.
distilled water to 1,000ml.

Owren's diluting fluid I
3.13% trisodium citrate 100ml.
physiological saline to 700ml.

Owren's diluting fluid II
Owren's buffer 200ml.
3.13% trisodium citrate 48ml.
distilled water 152ml.
physiological saline 600ml.

Method

0.2ml. reagent (from the deep freeze). Place in the 37°C bath for exactly 5 minutes, then add 0.1ml. test plasma dilution. (1 in 20 dilution in Owren's diluting fluid II). Mix and stand for exactly two minutes at 37°C. Recalcify with 0.1ml. M/30 $CaCl_2$. Take clotting times in duplicate. Another dilution of the test plasma is made and two more clotting times are determined, (that is, a total of four clotting times on the one test plasma). The times are averaged.

Discussion

The following points should be noted.

1) Specimens are collected using a citrate anticoagulant. 1ml. of 3.13% trisodium citrate and 9ml. of blood are added together and mixed by inversion in a siliconed tube.
2) Plasma should be rendered platelet-free before testing as platelets have factor V activity adsorbed onto their surface.
3) The thawed reagent is reasonably stable at 4°C for 1-2 hours, but to keep test standardised use fresh from deep-freeze as described in method.
4) Plasma dilutions appear to remain stable for at least half an hour but, where possible, use immediately after dilution is prepared. Store plasma at 4°C, if the test cannot be done immediately after centrifuging.

It was found that the factor V activity of the test plasma could be maintained for long periods of time without any deterioration if it was treated as follows:—

After centrifuging at high speed to remove the platelets, (done in a refrigerated centrifuge), the plasma is placed in a glass tube which has previously been siliconed. The tube is then placed in a beaker which contains a mixture of alcohol and dry ice (solid CO_2). The alcohol is kept in the deep-freeze prior to mixing. The mixture has a temperature of -70°C and this snap freezes the plasma. It is then stored in the deep-freeze. After thawing, the factor V estimation is carried out as before with no detectable loss of activity over a 24 hour storage period.

Preparation of a standard curve

Fresh pooled plasma was diluted using Owren's diluting fluids I and II to give concentrations ranging from 100% to

	DILUTING FLUID I	DILUTING FLUID II
0%		0.1 ml.
1%	1 ml. 10% dilution + 9 ml. diluting fluid I	1/20 (1.9 ml. + 0.1 ml).
2½%	2.5 ml. 10% dilution + 7.5 ml. diluting fluid I	1/20
5%	5 ml. 10% dilution + 5 ml. diluting fluid I	1/20
10%	1ml. plasma + 9ml. diluting fluid I	1/20
20%	1ml plasma + 4 ml. diluting fluid I	1/20
30%	1 ml. plasma + 2.33 ml. diluting fluid I	1/20
50%	1ml plasma + 1 ml. diluting fluid I	1/20
100%	—	1/20

Table I. Dilutions of fresh pooled plasma used to plot standard graph.

1%. Refer to Table I. A standard time/concentration curve was plotted on log.log 1 cycle x 1 cycle paper. There was a straight line relationship between the concentrations of 10% to 100% and the times for coagulation to occur. The end point was definite and easily seen. A difference of 0.5 seconds between the highest and lowest time for coagulation to occur in the four tubes was attained with a little practice.

Summary

Different methods of obtaining factor V-free plasma are described. The method found most suitable for use in the coagulation unit of the Auckland Hospital Central Laboratory is described in detail.

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Fluorescent Treponemal Antibody Testing

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Introduction

Since the fluorescent antibody technique was first used for the detection of treponemal antibodies[5], many reports have appeared to evaluate the procedure for the diagnosis of syphilis, with particular reference to its use in place of the complicated *Treponema pallidum* Immobilisation test (TPI). However, to eliminate non-specific results sera have to be diluted at least 1 in 200[6], and there is agreement that this test, designated FTA-200, is (except in early syphilis) less sensitive than the TPI test and will not detect many cases of late and latent syphilis. Kiraly *et al.*[11], using a 1 in 50 dilution of serum in a screening test, found the method less sensitive and less specific than the TPI test. Leibovitz *et al.*[13] used a 1 in 100 serum dilution and claimed good agreement with TPI results, but they did not demonstrate the specificity of their procedure in normal controls.

A number of technical difficulties are also apparent with the fluorescent antibody method. Reproducibility of results is related to the degree of reactivity of sera[2, 7], and consequently borderline results cannot be regarded with great confidence. Miller *et al.*[14] do not regard the FTA-200 test as a dependable routine procedure for the diagnosis of syphilis. They reported difficulty in obtaining adherence of treponemes to slides. This was resolved by suspending the organisms in saline rather than Nelson's medium. Other workers have reported difficulties with adherence of treponemes.[4, 10, 13] Leibovitz *et al.*[13] treated treponemes with sodium hypochlorite and suspended them in saline with dimethyl sulphoxide and rabbit serum. It was claimed that this antigen readily adhered to slides and was stable when stored frozen. These techniques cannot be used, however, in laboratories where fresh suspensions of *Treponema pallidum* are not available, and commercial reagents must be used. It is not known what suspending medium is used in the commercially available lyophilised antigens, and initial experiments in this laboratory using several different commercial antigens did not produce encouraging results. The treponemes were invariably washed off the slide, and the test did not seem very sensitive. Recently Kiraly[12] has reported that treponemes freeze-dried in 5% dextrose are stable and are as sensitive as those prepared in saline. Otherwise there is general agreement that the use of lyophilised treponemes reduces sensitivity, probably by about 50%.

As described below, attention to details of technique has helped solve the problem of adherence of antigen to the slide. Use of the absorption technique (FTA-abs), introduced by Hunter *et al.*[9], where the serum is absorbed with a solution of disrupted Reiter treponemes to block non-specific antibodies and then tested in low dilution, has solved the problem of sensitivity without sacrificing specificity.

Method

Reagents:

1. Phosphate-buffered saline
   0.01M pH 7.2
2. Carbonate-bicarbonate buffer
   0.2M pH 9.0
3. Fluorescent antihuman globulin:
   Commercially available *fluorescein isothiocyanate antihuman globulin conjugates were used. They should be stored undiluted in the deep freeze and optimal dilutions prepared in phosphate buffered saline on the day of use.
4. Treponemal antigen:
   Commercially available** lyophilised Nichol's treponemes, reconstituted with distilled water immediately before use.
5. FTA Sorbent***
   Commercially available preparation of disrupted Reiter treponemes.

Procedure

Clean slides are soaked in alcohol and wiped dry with a clean cloth. Two circles approximately 1 cm. in diameter are inscribed with a diamond pencil on each slide. A small amount of reconstituted antigen (about 0.003 ml.) is placed within each encircled area and spread to give a thin film. Slides are then air-dried and fixed at approximately 56°C. for 3 minutes, followed by immersion in acetone for 5 minutes. Films are then again air-dried, with the aid of gentle heat if the weather is cool or humid. Sera to be tested are inactivated at 56°C. for 30 minutes. Stored sera, previously inactivated, do not need re-inactivating. Sera are then diluted 1 in 4 with FTA Sorbent, and a drop of the dilution placed on the slide next to the encircled area. With a pasteur or capillary pipette the serum is carefully spread to cover completely the encircled area and then, tilting the slide to one side, excess serum is pipetted off so that only a thin film remains. Slides are placed in a wet chamber to prevent evaporation, and incubated at 37°C. for 30 minutes.

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* The Sylvana Company, Millburn, New Jersey. Difco Laboratories, Detroit, 1, Mich., U.S.A. Baltimore Biological Laboratories, Baltimore 18, Maryland.

** Institute Sieroterapico e Vaccinogeno Toscano "Sclavo", Siena, Italy. Baltimore Biological Laboratories. Difco Laboratories.

*** Difco Laboratories.

The slides are then rinsed in phosphate buffered saline to remove excess serum, and soaked for a further 10 minutes in two changes of phosphate buffered saline. They are then drained for a minute, and excess fluid blotted away from around the encircled area. Optimally diluted fluorescent conjugate is applied to the slides with the same technique as used in applying the serum, and the slides are re-incubated at 37°C. for 30 minutes.

They are then washed and drained as before, being careful not to allow the encircled area to dry completely, and mounted under coverslips with buffered glycerol, pH 9.0 (1 part carbonate-bicarbonate buffer to 9 parts glycerol).

Microscopic equipment used was a Leitz microscope with monocular head, X 40 dry objective, X 10 eye-piece, dark field condenser 1.2A, and an H.B.O. 200 watt mercury lamp. Filters used with the ultra-violet light were heat-absorbing filter K.G.1., red-absorbing filter B.G.38, primary fluorescence filter 2 mm. B.G. 12, and a yellow-orange ocular filter to exclude all wavelengths under 500 mμ. Glycerol was used between the condenser and slide.

The condenser is adjusted for maximum illumination with each slide and the treponemes are located with the regular dark ground illumination. They are then examined under ultra-violet illumination and classified as invisible, just visible, weakly fluorescent or fluorescent. With the absorption technique, weak degrees of fluorescence are considered positive. The tests should be carefully compared with controls consisting of a weak positive; a non-specific positive, showing staining unabsorbed but negative after absorption; and an antigen control treated with fluorescent conjugate only.

Discussion

Technical Considerations:

A. *Antigen*

The " Sclavo " product, supplied in vials of 0.2 ml., was quite suitable. It should preferably be used on the day that it is reconstituted, as storage in the refrigerator results in clumping of the organisms with loss of adhesion and a reduction in staining intensity. There seemed to be a definite variation between vials; some were used successfully after storage at 2°-4°C. for up to one week. Other commercial antigens consistently failed to adhere to the slides after storage in the refrigerator. They were quite satisfactory if used immediately after reconstituting, but unfortunately are supplied in 1 ml. quantities, enough for over 150 duplicate tests. It is possible to prepare and fix slides with freshly reconstituted antigen and store them in the deep freeze. Antigen kept in this way is probably not completely stable, as some of the treponemes appear to suffer a reduction in staining intensity. Generally, however, slides stored in this way have been found to

give reasonable results after up to about six weeks, and it is possible that at temperatures below −20°C. they are stable for longer periods. Apart from the use of freshly reconstituted antigen, the most important point in the technique was to use a minimal amount of antigen. If 0.01 ml. was used there was masking of organisms and a loss of adherence to the slide. A large amount of serum applied to the antigen spot also tended to dislodge the treponemes.

B. *Conjugate*

Of the various fluorescein isothiocyanate antihuman globulin conjugates used, all were satisfactory if the titres of the individual bottles were checked with known positive sera. Some of the titres were not as high as that claimed by the manufacturers, possibly due to difference in technique or deterioration on storage. None produced non-specific staining of treponemes, even in low dilution, but background cellular debris was stained to a varying degree. This was troublesome only with one conjugate if it was used at twice the concentration of its optimum titre. The ampoules of lyophilised dilute conjugate supplied with the "Sclavo" antigen were not very satisfactory when used in this laboratory. It is more sensible for the individual laboratory to obtain concentrated conjugate and determine the optimum titre under its own experimental conditions. The undiluted conjugate should be stored in small aliquots in the deep freeze and the optimum titre redetermined from time to time. Diluted conjugate cannot be stored frozen for more than a few days without marked loss of activity. It has been found convenient to dilute conjugate about 1 in 10 with phosphate buffered saline, to lyophilise it in small aliquots and store in the refrigerator. The optimum titre is then redetermined. Although accompanied by some loss of activity, this procedure is very convenient, and there is no wastage of conjugate.

C. *Effect of pH*

The observations of Pital[17] on the effect of pH upon the staining intensity of fluorescein isothiocyanate conjugates have been confirmed, and we have mounted slides at pH 9 to obtain maximum intensity of fluorescence. The increase is not great, but is worthwhile taking advantage of. Depending upon the stability of the antigen antibody complex, it is also logical to mount slides at pH 8-9, where small variations in pH will not produce any change in fluorescence intensity. It should be noted that the 0.01 M. phosphate buffers commonly used with glycerol as a mounting medium have insufficient buffering capacity to maintain some samples of gycerol at an alkaline pH. As shown by Chadwick and Fothergill[3], there is a serious loss of fluorescence if the pH drops below 7.

D. *Other Technical Details*

These have been reviewed by Neilsen and Idsoe[15]. Methanol may be used as a fixative. There is some contradiction as to the

desirability of inactivating sera, rotating slides during incubation and carrying out the test at 37°C. We have used inactivated sera as they are first tested in complement fixation reactions as a routine. Rotation of slides is omitted because of the loss of antigen during such a procedure, and 37°C. is used for incubation to obtain maximum sensitivity. We have used a 1 in 4 rather than a 1 in 5 dilution of serum, because of the reduced sensitivity of the lyophilised antigen.

Evaluation

Absorption experiments with Reiter's treponemes have been carried out by several workers, and it has been shown that after absorption sera from cases of treponematoses produce specific staining of Nichol's treponemes[8, 16, 18].

The FTA-abs test has now been evaluated by several other workers[1, 2, 10] and it seems that it is not only very specific but is more sensitive than the TPI test. In their original study Hunter *et al.*[9], in 76 cases of primary syphilis, obtained a sensitivity of 80.7%, compared with 36.8% for both TPI and FTA-200 tests. Of 46 cases defined as late treated, untreated or inadequately treated syphilis, 100% gave positive results. The comparative figures for TPI and FTA-200 tests were 91.2% and 19.5% respectively. Of 148 controls, including biological false positive reactors, no positive FTA-abs tests were obtained. In a series of 216 cases of syphilis Bradford *et al.*[2] obtained a sensitivity of 88% for FTA-abs, 64% for TPI and 50% for FTA-200 tests. Wuepper *et al.*[19], in testing 67 cases of late and latent syphilis with the FTA-abs test, obtained 100% positive results. Of 101 cases tested with the TPI and FTA-200 tests there were 90 and 66 positives respectively. Of 50 cases with chronic false positive reactions in non-treponemal tests for syphilis, none were positive with the FTA-abs test. A large proportion of these patients had antinuclear antibodies, rheumatoid factor or elevated serum globulins.

Further large-scale testing in a variety of diseases will have to be performed before it can be assumed that the test is 100% specific. However, because of its sensitivity and greater simplicity when compared with the TPI test, it is likely to become the standard reference procedure in the serological diagnosis of syphilis. The TPI test may still be useful in some circumstances, in view of the fact that it seems to detect a different antibody. Although lyophilised antigen is not as suitable as that which is freshly prepared from rabbits, attention to details of technique permit its satisfactory use. The availability of a stable antigen will greatly increase the number of laboratories capable of performing the test.

Summary

The difficulties of fluorescent treponemal antibody testing are discussed with particular reference to the use of lyophilised antigen. An FTA-abs procedure is described which has been used success-

fully with commercially supplied reagents. The value of FTA-200, FTA-abs and TPI testing is discussed with reference to the literature. The FTA-abs procedure is as specific as the TPI test, and is considerably more sensitive. It will detect approximately 80% of cases of primary syphilis and close to 100% of cases in all other stages of the disease.

Acknowledgments

I wish to thank Andor Fischman for his valuable advice during this work, and in preparing this paper.

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Technical Communications

Contributions ore invited for this new section of the JOURNAL, which is intended as a regular feature. It is hoped that it will appeal to contributors who have information to offer, which is not considered worthy of being presented as a formal paper, but which will, nevertheless, be of interest to our readers.

Communications should be addressed to The Editor, and the usual instructions regarding double spacing and the presentation of references should be followed.

Screening for Galactosaemia

Sir,

Screening tests for inborn metabolic errors are currently of interest and the Guthrie test for Phenylketonuria[4] is used to screen most of the 60,000[6] odd babies born in this country.

The frequency with which this disease occurs is calculated to be about 1 in 20,000[7] and this means that an immense effort is required to find the small number of cases (3) which should occur. One would of course expect that this painstaking screening would turn up more than this number.

Galactosaemic infants are liable to die very quickly and those who survive may suffer from cataracts, liver damage and mental defects. A frequency of 1 in 70,000[7] has been quoted but it has been deduced on the basis of heterozygous gene frequency that the expected incidence would be 1 in 16,000[3]. Beutler and Balluda (1964)[2] (1966)[3] discuss the implications of this and suggest that a number of galactosaemic infants may be lost by intrauterine death, or cause of death may be undiagnosed. The mortality of infants under one year due to various causes is around 1,200 *per annum* in this country[6].

This problem has received recognition in that several screening tests have been suggested.

I have tried the technique described by Beutler and Balluda (1964)[2] using Sigma reagents namely Uridine Diphosphoglucose, ∝ galactose—1—phosphate and N.A.D.P. (T.P.N.). For the gassing procedure for which CO is specified I used the domestic gas supply. This worked satisfactorily.

The essential defect is a lack of the enzyme galactose—1—phosphate uridyl transferase.

GAL-1-P + UDGP + TPN ——> G-1-P (Glucose-1-Phosphate)

G-1-P + phosphoglucomutase (in haemolysate) ——> G-6-P

G-6-P + phosphohexase isomerase (in haemolysate) ——> β G-6-P

β G-6-P + glucose-6-phosphate dehydrogenase (in haemolysate) ——> 6 phosphoglucomate which in turn oxidises to ribulose-5-phosphate

The two latter steps result in the reduction of T.P.N. which is visualised by the reduction of methylene blue to a leuco-form. The actual technique only involves pipetting 0.1ml. of heparinised capillary or venous blood into 0.7ml. of reaction mixture, gassing and incubating under controlled lighting conditions (100 watt bulb at 10 cm.) and observing the time of decolourisation.

I have been unable to trace a genuine uridyl transferase deficiency to use as a control and have had to resort to a known glucose—6—phosphate dehydrogenase deficiency (a non spherocytic congenital haemolytic anaemia). It can be seen from the reaction that this enzyme is required to give reduced T.P.N.

A later modification of this technique identifies the reduced T.P.N. by spotting the reaction on filter paper and examining the spots under ultraviolet for the characteristic fluorescence. It is suggested that this test could be applied to the dried blood spots used in the Guthrie test and is therefore potentially a most useful test.

Of the other screening tests Guthrie (1964)[5] reported that a trial was in progress using a modification of the microbiological method. Thus a "galactosaemic" mutant of *Escherichia coli* is inhibited by a suitable medium containing galactose which the organism cannot metabolise. The blood spot requires fixing with glacial acetic acid vapour.

The Association of Clinical Pathologists publish a broadsheet[1] describing a method of observing the O_2 uptake or respiration of blood added to a galactose—1—phosphate substrate in narrow glass tubes with bulbed ends. The bore is only 1mm. and considerable manipulation is involved. A number of controls are also required. It would appear that some simple screening test is required since galactosuria is not an invariable finding, and that it should be applied to all newborn infants who are underweight, vomiting or jaundiced for no apparent reason or in fact in any suspicious circumstances.

R. D. ALLAN,
Dunedin Hospital, April 12, 1967.

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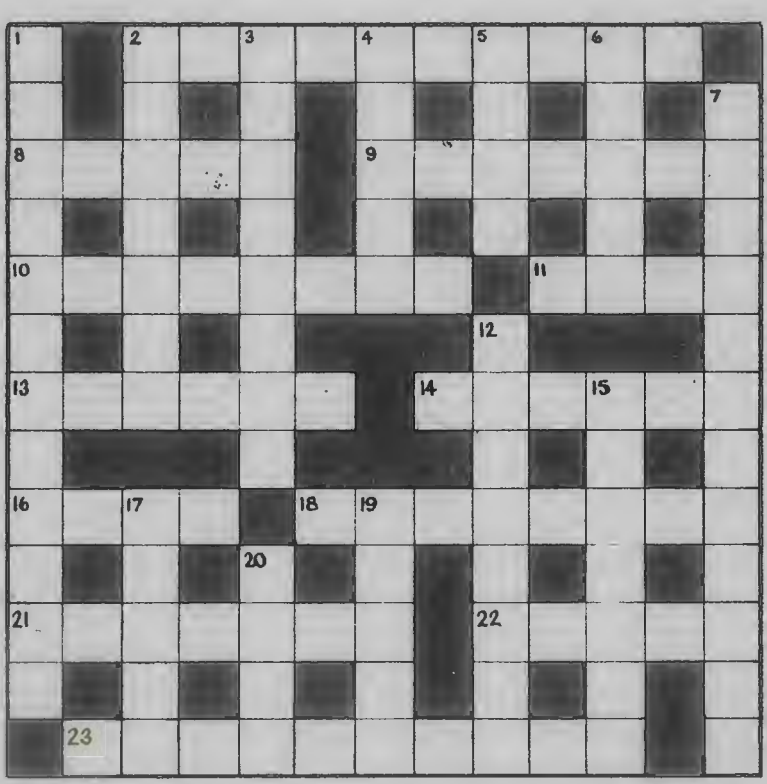
Laboratory Crossword (No. 7)

Compiled by D.S.F. and H.A.H.



Clues Across

2. An early exponent of 1 down.
8. The beginning of a rolling stone.
9. An outspoken yeast?
10. The mania confuses a blood pigment.
11. Fermented law maker!
13. Deserves.
14. Pertaining to digestion.
16. Chemical substance is last and confused.
18. Attached to.
21. A test for ketones? O, rather!
22. A branch of a nerve.
23. An endless electron and a familiar Philip get together in a cell.

Clues Down

1. Be my historic science.
2. An early bacteriologist from the beginning of the Rue Stapillion.
3. The clot grows smaller.
4. Add nothing to three hundred and one and mix for bacteria.
5. A ventilating organ.
6. Kidney secretion.
7. I cross my coat around a picture of oversized cells.
12. Concerning the hunt carried out in some laboratories.
15. Relating to 20 down.
17. A fluid measure that may be relit.
19. Chemical group.
20. The Territorial Army's high explosives produce this.

Solution on page 131.

Selected Abstracts

Contributors to this issue: R. D. Allan, J. Case, J. D. Drummond, B. Glynn-Jones, J. Hannan, J. Marr, D. Tingle.

BLOOD BANKING

Three Examples of a New Red Cell Antibody, Anti-Co^a. Heisto, H.; van der Hart, Mia; Madsen, Grethe; Moes, Mieke; Pickles, M. M.; Race, R. R.; Sanger, Ruth and Swanson, Jane (1967), *Vox Sang (Basel)*, **12**, 18.

The sera of three patients were found to contain the same antibody, which reacts with almost all random blood samples and has been shown to be distinct from "public" antigens previously reported.

The new antigen was found to be absent in only four bloods out of 1,727 random blood donors.

Delayed Haemolytic Transfusion Reactions Simulating Auto-Immune Haemolytic Anaemia. Crookston, Marie C. and Crookston, J. H. (1967), *Vox Sang. (Basel)*, **12**, 32.

Seven cases are described in which blood group incompatibility was unsuspected until several days after transfusion. In one case the rapid appearance of multiple antibodies produced signs and symptoms simulating acquired haemolytic anaemia. In two of the seven cases antibodies were detectable in the pretransfusion serum only when cells representing a double dose of the antigen were used.

Infectious Mononucleosis Complicated by Hemolytic Anaemia due to Anti-i. Troxel, D. B.; Inella, Filomena and Cohen, R. J. (1966), *Amer. J. clin. Path.*, **46**, 625.

Anti-i is quite commonly present in infectious mononucleosis, but in this case it caused severe haemolytic anaemia. Procedures are outlined for the differentiation of anti-i from other apparent non-specific cold agglutinins, and evidence is brought to show that the anti-i is different from heterophile antibody. The association of anti-i with diseases involving reticulo-endothelial proliferation is discussed.

Incidence of Irregular Antibodies in a General Hospital. Iammarino, R. M. (1966), *Amer. J. clin. Path.*, **46**, 573.

The incidence of atypical antibodies amongst the patients in a large general hospital was 1.9%, and the incidence amongst blood donors was 0.46%. A trend of increasing antibody incidence is evident.

The Indications for Transfusing Freshly Drawn Blood. Oberman, H. A. (1967), *J. Amer. med. Ass.*, **199**, 129.

The use of freshly-drawn blood without sufficient justification is wasteful, as it depletes the available donor pool and upsets the blood bank inventory.

This article reviews the variety of clinical situations in which freshly-drawn blood is requested and concludes that this is only really justified on rare occasions. In most situations an appropriate blood component, or relatively fresh rather than freshly-drawn blood is adequate.

Selection of "Safe" Group O Blood. Grove-Rasmussen, M. (1966), *Transfusion (Philad.)*, **6**, 331.

According to the results of this study on 100 random group O blood donors, a saline screening test at 1 in 50 for agglutination is not a satisfactory method of selecting safe universal donors.

A test at 1 in 20 for immune anti-A and anti-B and a test for haemolysis are recommended.

In the series of 100 tested, 23 gave positive results by the 1 in 50 screening test; 40 gave positive results by the 1 in 20 screening test for immune antibodies; and 46 gave positive haemolysin tests.

Rh Antibody Type in Haemolytic Disease of the Newborn. Murray, Sheilagh (1967), *Vox Sang. (Basel)*, **12**, 81.

The acceptance of incomplete antibody as representing a state of hyperimmunity is not necessarily valid according to this study, which

resulted from the observation that in some women with successive infants affected with haemolytic disease of the newborn saline antibodies reappear after a pregnancy in which only incomplete antibodies were detectable.

No explanation is offered as to how saline antibodies which do not cross the placenta can be related to severity of disease in the infant, but an examination of a very large series of cases certainly suggests that it is so related.

[The concept that incomplete (or albumin-reacting antibodies can block complete (or saline-reacting) antibodies is well-accepted and adequately proven, and there is ample evidence to suggest that, as the subject becomes even more hyper-immune, a third order of antibody develops which in turn blocks incomplete antibodies. It seems therefore not unreasonable to suppose that an explanation for the phenomenon dealt with by this author is that third order antibodies (which also cross the placenta) interfere with the capacity of incomplete antibodies to block complete antibodies, thus permitting saline agglutination to reappear. Since there is now in the patient's serum a very high level of antibody capable of crossing the placenta, a severely affected infant is a foregone conclusion, notwithstanding an apparent increase in saline-reacting antibody activity.—Ed.]

A Nebulous Antibody Responsible for Crossmatching Difficulties (Chido). Harris, Jean P.; Tegoli, J.; Swanson, Jane; Fisher, Nathalie; Gavin, June and Noades, Jean. (1967), *Vox Sang. (Basel)*, **12**, 140.

A group of cases is mentioned in which difficulty is encountered in finding compatible blood. The relationship between the separate cases is established by the fact that each serum gives a negative reaction against the cells of each of the others, but the difficulty in distinguishing between negative and weakly positive results has made it impossible to effect a clarification of the corresponding antibody to a point at which it would be justified to postulate phenotype and gene symbols.

The antibody works by the indirect antiglobulin technique and the authors have reported the class, to which the name Chido has been given, in the hope that a more powerful example will be recognised, thereby enabling more precise definition to be made.

The First Example of Auto-Anti-Xg^a. Yokoyama, M., Eith, D.T. and Bowman, M. (1967), *Vox Sang. (Basel)*, **12**, 138.

In a case of acquired haemolytic anaemia, the autoantibody was identified, after tests with the patient's serum and with eluates from her cells, as being specific for the sex-linked antigen Xg^a. The antibody failed to work with some antiglobulin reagents unless fresh complement was present during the incubation of cells and serum. The patient's serum also contained anti-S.

Irregular Antibodies in Pregnant Women. Smith, B. T., Haber, Jane M. and Queenan, J. T. (1967), *Obstet. Gynec.*, **29**, 118.

In a study of 12,297 cases over a five year period 113 women were found to have atypical antibodies in their sera, not including anti-D. In 26 of the immunised women it was necessary to crossmatch blood at some stage and haemolytic disease of the newborn requiring exchange transfusion occurred in 7 infants.

Sul, a New Blood Group Antigen in the MN System. Konugres, Angelyn A. and Winter, Nancy M. (1967), *Vox Sang. (Basel)*, **12**, 221.

A new low-incidence antigen, named Sul (Sullivan) has been identified and recognised as belonging to the MN system. Anti-Sul is a common antibody, and 4 examples were found in 119 normal sera, reactive in saline at room temperature. The incidence of the antigen is low; no further examples were found in 4,935 bloods.

Screening for Irregular Blood Group Antibodies—Selectogen^R Procedure Compared with Other Methods. Dybkjaer, E. (1966), *Scand. J. Haemat.*, **3**, 332.

It has become common practice in recent years in the U.S.A. to employ commercially prepared test-cell suspensions. One of these test systems, Selectogen (Ortho Pharmaceutical Corp., Raritan, N.J., U.S.A.), has now become available in Denmark. In the present study, a comparison is made between the Selectogen method and other methods of investigation, including a two-stage papain technique.

291 selected sera, showing a positive antibody screening test or some other indication of the presence of an irregular antibody, were investigated. Of these sera, 133 contained irregular antibodies which could be analysed in detail. The two-stage papain technique was specifically positive in 125 sera but only 48 of these sera were positive by the Selectogen albumin technique after 15 minutes at 37°C.

A disadvantage of the two-stage papain method is the many nonspecifically positive reactions. This disadvantage is balanced, however, by the increased number of irregular antibodies revealed by the use of this technique. J.H.

CHEMICAL PATHOLOGY

Rapid Identification of Barbiturates in Blood by Thin Layer Chromatography on Microscope Slides. Steen, G. O. (1966) *Scand, J. clin. Lab. Invest.*, 18, 557.

A chloroform extract is evaporated, redissolved and applied to half a slide, the other half carries mixed standards. The solvent is petroleum ether, methanol, acetone and water. Run for 15 minutes. Diphenylcarbazone followed by mercuric sulphate-permanganate are the two sprays. Identification by colour and position in relation to standards. R.D.A.

Vanilmandelic Acid Screening Test for Phaeochromocytoma and Neuroblastoma, Brown, W. G., Owens, I. B., Benson, A. W. and Henry, S. K. (1966), *Amer J. clin. Path.*, 46, 599.

This can be performed in 20 minutes on cellulose acetate, using the pH 8.6 barbitol buffer as for proteins and spraying with diazotised p. nitroaniline. Beckman Microzone Electrophoresis 250 V 10 applications of 0.25 µl gives spot if over .4 mg./ 24 hours. R.D.A.

Simplified Method for the Estimation of Inorganic Phosphorus in Body Fluids, Goldenberg, J. and Fernandoz, A. (1966), *Clin. Chem.* 12, 871.

A simplified procedure in which the protein precipitant, trichloracetic acid, contains ferrous iron stabilised with thiourea. The supernatant is decanted and a small volume of molybdate reagent added. A stable colour develops. Figures are produced to demonstrate the small error resulting from decantation. R.D.A.

A Comparison of Transaminase Determination by Various 'Kit' Outfits, Craig, Griffiths, B. and Higgins, G. (1966), *Proc. Ass. clin. Biochem.* 4, 109.

Boehringer, Hyland, Sigma, Staynes, Transac kits. A reasonable correlation for G.P.T. was obtained. In 16% of the sera tested for G.O.T., serious discrepancies were found. Calibration curves prepared at intervals altered in the Transac. and Hyland kits. No one kit could be singled out as being at fault. Variation in one sample from 20 to 50 units and 18 to 43 units are quoted with the different kits. It is concluded that it is essential to establish a normal range for the kit used, to make sure the reagents are stable and to use known controls daily. R.D.A.

Rapid Concentration of Protein and Electrolytes from Frozen Urine by Centrifugation. McErlean, B. A., (1966), *J. clin. Path.*, 19, 632.

Fluid is frozen to —6°C. overnight in the plugged barrel of a 20ml. plastic syringe. A 5ml. glass bottle is placed at the bottom of a 100ml. centrifuge bucket and the syringe barrel with the plug removed is placed

on top of the bottle surrounded by wadding to insulate. Spin for 1 minute at 3,500 r.p.m. 94% of protein and sodium is claimed to be recovered. The fluid fraction which contains the protein and electrolytes escapes from the lattice of ice crystals which remain frozen. I have found this to work well with an International U.V. Centrifuge at 2,900 r.p.m. R.D.A.

Determination of Glutamic Oxalacetic Transaminase Activity by Coupling of Oxalacetate with Diazonium Salts. Sax, S. H., and Moore, I. I., (1967), *Clin. Chem.*, **13**, 175.

This is an example of a manual method using materials found useful for an automated method in the first instance. Fast Ponceau L or Fast Red P.D.C. is found to give a negligible blank colour with α oxoglutarate at pH 4.2. However acetoacetate reacts with this dye and a blank is required in cases where this is expected. R.D.A.

Determination of Sodium in Urine by Specific Ion Electrode. Annino, J. L., (1967). *Clin. Chem.*, **13**, 227.

Good correlation obtained with flame photometry and mean "recoveries" 97%. The urine is diluted with Tris buffer and the sodium acitivity measured with a sodium electrode and calomel reference electrode using the millivolt reading from a pH meter. A wide range of concentrations 0-200 mEq./l. can be read with one dilution and the calibration curve is reasonably stable. R.D.A.

A New Photometric Method for Serum Urea Nitrogen Determination. Searcy, R. L., (1967). *Amer. J. med. Technol.*, **33**, 15.

An emerald green colour results when dilute ammonia solutions react with sodium salicylate, nitroprusside and alkaline dichloroisocyanurate. This is the basis of this method which permits specific and reproducible results on micro volumes (20μl) of serum. The solutions are less toxic and corrosive than those used in the Berthelot reaction but, like it, is a urease technique. R.D.A.

Use of Sephadex 9-10 for the Chromatographic Investigation and Estimation of 5 Hydroxyindoles in Human Urine. Contractor, S. F., (1967), *J. clin. Path.*, **20**, 107.

Details are provided for the adsorption and elution of 5 H.I.A.A. and fluorometric measurement. A recovery of over 92% is claimed. (Report to 77th A.C.P. general meeting.) R.D.A.

Liquor Bilirubin Levels in Normal Pregnancy. Morris, E. D., Murray I. and Ruthven, C. R. J., (1967). *J. clin. Path*, **20**, 108.

Six out of 29 Rh positive pregnant women gave results indicative of affected infants in Rh negative subjects. A complicating factor in this test, namely the wide range of liquor volume, could be minimised by expressing the bilirubin: protein ratio. This ratio did provide a more correct forecast in a group of iso-immunised women. R.D.A.

Minipette System, a Convenient Ultramicro Performance of Routine Clinical Chemistry by means of a Semi-Automatic Pipet (Minipette) and a Drain-System Colorimeter of New Design. Sasaki, M. (1966), *Bull. Yamaguchi med. Sch.*, **13**, 19.

The author's group has developed an original system which enables simultaneous and semiautomatic chemical analysis of 14 to 15 components with only 0.5ml. of serum being used for the complete group of analyses.

Two pieces of apparatus were created: (1) A drain-system photometer for the rapid measurement of solutions of 1ml. in succession and (2) The "minipette" for semiautomatic pipetting of reagents in quantities as small as 0.1 to 0.2ml.

The system has been in use for about one year; the reproducibility was found to be better than that of conventional micro analysis. J.H.

High Resolution Acrylamide Gel Electrophoresis: Some Practical Aspects of its Problems. Ritchie, R. F. (1967), *J. Maine med. Ass.*, 58, 15.

In the investigation of serum proteins what had been a simple five fractions on paper and free electrophoresis has grown to a complex 50 bands in newer techniques.

Although all electrophoretic methods require careful execution, the new high resolution systems require meticulous attention to detail and are fraught with problems which may severely compromise the results.

Difficulties may be encountered with electrophoretic techniques even in the hands of experienced personnel. Some of the more common causes for suboptimal resolution are discussed together with practical recommendations for their avoidance. J.H.

Critical Analysis of Blood Sugar Measurements in Diabetes Detection and Diagnosis. Whichelow, M. J., Wigglesworth, A., Cox, B. D., Butterfield, W. J. H. and Abrams, M. E. (1967), *Diabetes*, 16, 219.

Capillary blood taken from the warm ear lobe gives an accurate reflection of the arterial blood sugar level. Compared to the arterial level the venous blood sugar level is unpredictable and differs from the arterial to a varying extent, *e.g.*, from +26mg. per 100ml. to −4mg. per 100ml. at two hours in the glucose tolerance test, depending on many factors.

Studies of the macromethod in the AutoAnalyser indicate that although the reproducibility is good, the volume of blood pumped by the proportioning pump is inaccurate (too small). Analysis of the micromethod shows that the reproducibility is also good, though not quite so good as the macromethod. However, the sample volume taken for analysis by the proportioning pump is accurate.

Reasons are given for recommending that capillary, not venous, blood be taken, and that the ferricyanide-reducing micromethod on the AutoAnalyser be used for blood glucose estimation.

It is well-known that the glucose concentration of plasma is higher than that of blood, and in this connection proper mixing of the blood from which the aliquot is drawn up by the pump is an important consideration. J.H.

An Examination of Commercial Kits for the Determination of Glutamic Oxalacetic Transaminase (GOT) and Glutamic Pyruvic Transaminase (GPT) in Serum. Hollands, M. and Logan, J. E. (1966), *Can. med. Ass. J.*, 95, 303.

Nineteen transaminase kits sold in Canada by nine manufacturers were evaluated for accuracy, precision, stability of reagents and for adequacy of instructions. Testing of two kits could not be completed because of the instability of the reagents. Four kits yielded values which were not in agreement with those obtained by the reference method. The precision of the five SGOT kits and the four SGPT kits so tested was found to be acceptable for routine clinical laboratory use.

Some kits did not contain instructions that were complete enough to ensure that the user would be aware of factors which could introduce serious errors into the performance of the test. J.H.

Atomic Absorption: A Milestone in Spectroscopy. Burkhart, C. R. (1966), *Va med. Mon.*, 93, 524.

Atomic absorption spectroscopy is an extension of flame photometry (emission spectroscopy). Both depend upon the capacity of an atom to change from an unexcited (ground state) to an excited state following the absorption of a certain amount of energy. Conversely, the change from the excited state back to the ground state emits energy. Such energy is detectable as absorption and emission spectra specific for each element.

The sensitivity of emission spectroscopy is limited because of the very small percentage of available atoms which can be excited in an ordinary flame. There is a much larger number of atoms which at ground state can absorb energy.

The critical factor in atomic absorption spectroscopy is a light source of the proper wavelength. For the light source, electronic tubes are used in which the cathode is composed of the element to be determined. When a voltage is applied to the tube the element sputters off, is excited and returns to the ground state, emitting light in the spectral bands of the element. If this light is directed through a fog of atoms of this element, these wavelengths are absorbed in proportion to the concentration, in the fog, of the element. (A flame is used to produce the fog.)

Among the elements which may be determined are: calcium, copper, iron, lead, mercury and zinc. J.H.

The Clinical Value of Creatinephosphokinase (CPK) Determination in Myocardial Infarction. Grudzinska, W. (1966), *Polskie Archwm Med. wewn.*, **37**, 513.

CPK activity was determined in 45 patients with a clinically overt recent myocardial infarction, 35 patients with acute coronary insufficiency without infarction and 16 patients with miscellaneous diseases: pulmonary infarction, pneumonia, heart failure and pericarditis.

The results indicate a considerable clinical value in the CPK determination in the early diagnosis of myocardial infarction and in the differential diagnosis between infarction and acute coronary insufficiency.

It is claimed that the test is of particular value in diagnostically dubious cases, *e.g.*, when lesions of other parenchymatous organs are present simultaneously. J.H.

Enzymic Determination of Uric Acid by a Colorimetric Method. Lorentz, K. and Berndt, W. (1967), *Analyt. Biochem.*, **18**, 58.

Uric acid is oxidised to allantoin and hydrogen peroxide in tris buffer at pH 8.5. The hydrogen peroxide formed oxidises o-dianisidine by means of peroxidase to a brownish quinonediimine, which is proportional to the uric acid originally present. Addition of concentrated sulphuric acid transforms the faintly coloured and slightly soluble compound to a stable and intense purple dye with maximal absorbance at 530mμ. Colour development is linear up to uric acid values of 8mg. per 100ml.; higher concentrations require dilution before deproteinisation or use of less material or less supernatant. J.H.

Urinary Lactic Dehydrogenase Activity in Patients with Diseases of the Urinary Tract. Glenert, J., Hemmingsen, L., Juul, P. and Sogaard-Andersen, J. (1966), *Nord. Med.*, **76**, 1274.

By means of colorimetic analysis (backward principle) the urinary lactic dehydrogenase activity was studied in 64 normal subjects and 40 patients, including 35 with urinary tract disease.

No case of elevated activity was found among the normal subjects. Out of 28 patients with benign diseases of the urinary tract 20 had raised values, in all cases possibly attributable to the presence of RBCs, WBCs, bacteria or protein in the urine. Three out of five patients with malignant diseases of the urinary tract had elevated activity, whereas the other two showed a normal amount.

It was concluded that these determinations were of no value in the diagnosis of urinary tract diseases. J.H.

HAEMATOLOGY

A One-Step Method for Counting Leukocytes and Platelets. Miescher, P. A. and Gerarde, H. W. (1966), *Amer. J. clin. Path.*, **46**, 576.

Using the Unopette system and a single dilution of blood in a fluid

consisting of a mixture of ammonium oxalate and potassium EDTA, leucocytes and platelets are enumerated in the same counting chamber. Platelets can be counted without the use of phase contrast microscopy.

Further Evidence for a Simpler View of the Coagulation of Blood. Mann, F. D. (1966), *Amer. J. clin. Path.*, 46, 612.

Observations on mixing experiments with Factor X deficient plasma and certain other facts lead to the conclusion that some coagulation defects are due to inhibitor substance firmly bound to coagulation factors, rather than to lack of specific coagulation factors themselves. Accordingly it is unnecessary to postulate specific coagulation factors to explain each observed abnormality of coagulation.

1963 Prothrombin Time Test Survey. College of American Pathologists, Standards Committee, Sub-committee on Coagulation. Miale, J. B. and Lafond, D. J. (1967), *Amer. J. clin. Path.*, 47, 40.

This report is an analysis of a survey in which 1,177 laboratories participated. Every laboratory was asked to estimate the prothrombin time on three samples of plasma, each of which had been carefully estimated in a reference laboratory by carrying out twenty determinations on each, using ten different commercially available thromboplastin reagents.

The statistical analysis is presented in two sections, the first giving the raw data, and the other presenting conclusions and recommendations based on the data.

An Evaluation of a Capillary Micro Partial Thromboplastin Test in Hemophiliac Disorders. Mochir-Fatemi, F. and Leikin, S. (1967), *Amer. J. clin. Path.*, 47, 91.

This simple technique is claimed to be well-suited for the detection of coagulation disorders in infants and children.

Tests were carried out, in parallel with the venous test, on 35 normal children; and similar studies, in addition to Factor VIII and IX assays, were made on a group of children with untreated coagulation defects.

No discrepancies were noted, and by applying the test to haemophiliacs after plasma therapy, it was shown that the test is sufficiently sensitive to mild haemophiliac states.

A Flow-Through Cuvette for the Coulter Counter. Bull, B. (1967), *Amer. J. clin. Path.*, 47, 107.

This cuvette, which is obtainable commercially, eliminates the need for large numbers of beakers in the operation of the Coulter counter. The cuvette is easily kept clean, and is free from the defects present in an earlier development for the same purpose.

The Forgotten Red Cell Count. Sirridge, Marjorie S. (1966), *Curr. med. Dig.*, 33, 1983.

In all anaemic patients (haemoglobin below 10.0 g./100ml.), particularly those in whom the diagnosis is not obvious, the RBC count gives information which cannot easily be gained in any other way. Its primary value is that it allows one to determine the approximate size and haemoglobin content of the RBCs when the RBC count is compared to the values for haemoglobin and haematocrit.

If every physician had time to study the peripheral blood films of his anaemic patients or if he had someone whom he could trust to do this for him, he could probably, in most instances, make a moderately accurate estimate of the size and colour of the RBCs without ordering a RBC count. But usually this is not a practical procedure, and the wise physician will order a RBC count or mean corpuscular values to gain help in determining what type of pathologic process is responsible for his patient's anaemia.

New electronic counting devices have made the counting of RBCs not only easier but also more accurate, so there should be less reluctance on the part of the physician to rely on this test. It is of little value in following patients, but should never be forgotten when the diagnosis of anaemia is the problem at hand. J.H.

The Peripheral Blood Smear. Cohen, F. B. (1966), *J. Newark Beth Israel Hosp.*, 17, 228.

As laboratory medicine becomes more complex, closer to basic science and more theoretical, the practitioner often feels completely dependent on machines and experts of whose activities he has no real understanding, and who may present him with information that often seems mystifying rather than enlightening. It seems advisable to recall, therefore, the importance of the ancient process of inspection and observation as applied to the diagnosis and treatment of the many disorders which affect the morphology of the peripheral blood. It has been said that an intelligent study of the stained film together with an estimation of the haemoglobin content will yield 90 per cent. of the diagnostic information obtainable by examination of the blood.

There is a discussion of blood film findings in relation to protein abnormalities, RBCs, WBCs and platelets.

The author states, in summary, that an attempt has been made to emphasise the value of a simple technique, namely the inspection of a stained peripheral blood film. With this technique and with practice, any physician can, often in a few moments, obtain data of great variety and significance concerning his patient's general condition and perhaps his specific disease. Just as the "Purloined Letter" was in the most obvious place and yet went unnoticed, so the answer to many of the patient's problems can be found in a simple blood film, right below our noses through the lenses of our microscopes. J.H.

Studies on Infectious Mononucleosis V. The Arneth Count (Preliminary Observations). Cantow, E. F. and Kostinas, J. E. (1967), *Amer. J. med. Sci.*, 253, 221.

The significance of a shift to the right in the Arneth count has not been widely appreciated although, as pointed out by Herbert, hypersegmentation of the neutrophils may precede all other signs of folic acid or vitamin B_{12} deficiency. The absence or presence of hypersegmentation can be determined by utilising Herbert's criteria. His normal percentages for the various cell types are as follows: two-lobed nuclei, 20-40%; three-lobed nuclei, 40-50%; four-lobed nuclei, 15-25%; five-lobed nuclei, 3% and cells with more than five lobes, none.

In the authors' study of infectious mononucleosis a shift to the right was seen. 130 serial peripheral blood films from 41 consecutive patients with infectious mononucleosis were studied. 45.4% of the Arneth counts were abnormal, utilising the increase over the normal range of four-, five- and six-lobed neutrophils. The greatest number of abnormal counts occurred late in the course of the illness, from the fourth to the sixth week.

A relative folic acid deficiency is postulated as the aetiological reason for the rightward shift and possible mechanisms are discussed. J.H.

Vitamin E Deficiency — A Previously Unrecognised Cause of Hemolytic Anemia in the Premature Infant. Osaki, F. A. and Barness L. A. (1967), *J. Pediat.*, 70, 211.

The late anaemia of some premature infants has been found to be due to haemolysis at six to ten weeks. This anaemia is correctable by the administration of vitamin E.

Irregularly contracted erythrocytes in peripheral blood films are described as being associated with the condition. J.M.

The Selective Hemolytic Action of Drugs — Clinical and Mechanistic Considerations. Zinkham, W. H. (1967), *J. Pediat.*, 70, 200.

The selective haemolytic action of drugs may be related to one of three different pathogenetic mechanisms. These are a deficiency of an enzyme or metabolic pathway within the cell (enzymopathy); the presence of an abnormal haemoglobin (haemoglobinopathy); or the formation of antibodies against the drug or a drug-red cell complex.

This report reviews the clinical presentation and laboratory definition of these disorders. J.M.

HISTOLOGY

Uses and Limitations of Frozen Section in Diagnosis of Lesions of the Breast. Desai, S. B. (1966), *Brit. J. Surg.*, **53**, 12.

This article presents a technique for the preparation of frozen sections for urgent diagnosis and a review of 1006 breast lesions. Unfixed tissues are frozen with carbon dioxide gas. Sections 7-10 mu. are dipped in dilute Terry's neutral methylene blue rinsed and mounted in water. The nuclei stain blue to purple, collagen a reddish colour and elastic and muscle shades of blue. Cryostat sections were prepared in selected cases. In 97% of the cases diagnosis was correct and conclusive, the remaining 3% requiring paraffin sections for a definite diagnosis. D.T.

Preparation of Fibre Cross Sections. Grieve, M. C. and Paterson, M. D. (1967), *Lab. Pract.*, **16**, 2.

A technique to prepare cross sections of fibres, *e.g.*, polyurethane elastomas, viscose rayon, vegetable fibres and human and animal hairs is given. An embedding mould is prepared using pieces of a 3 x 1 slide, the fibres are stretched across the central well and embedded in 'H.T. Cement' (a methacrylate polymer), then hardened for 5 hours at 70°C. Sections are cut on a sledge microtome, floated off the microtome knife on to a slide coated with 2% albumen and dried. The 'H.T. Cement' is removed from around the sections with acetone, they are then mounted in 'Uvinert.' A staining technique is given, using chlorazol sky blue, which assists in the identification of rayon fibres. D.T.

Fuchsinophilia in Early Myocardial Infarction. Puley, R. W., Forbes, C. D. and Hall, M. J. (1964), *Arch. Path.*, **77**, 3.

A simple staining technique is described using acid fuchsin which is claimed to show the earliest changes of myocardial infarction. The method is applicable to the routine laboratory and although the results need careful evaluation. it is worthy of consideration. B. G.-J.

A Simple Silver Method for the Demonstration of Reticulin Fibres. James, K. R. (1967), *J. med. Lab. Technol.*, **24**, 1.

The method described is certainly simpler than the techniques in common use. Consistent results are claimed, with the risk of granular deposits and loosening of sections from the slides decreased. B. G.-J.

IMMUNOLOGY

Practical Utilisation of the AutoAnalyzer for Serodiagnosis of Syphilis (A Study of 1,009 Serums) Using Cardiolipid Antigen. Gaillon, R., Ripault, J., Studievic, C. and Dausset, J. (1966), *Path. Biol., Paris*, **14**, 952.

A "semi-automatic" method is described, using the AutoAnalyzer for routine serodiagnosis of syphilis by the complement fixation method with cardiolipid antigen.

There was no contamination of a negative serum by a positive serum which had been through the apparatus previously.

The sensitivity of this method was superior in 4% of the cases to the classical manual method. The sera found to be positive by the AutoAnalyzer came only from syphilitic patients. J.H.

MICROBIOLOGY

Survival of Bacteria on Swabs. Ellner, P. B. and Ellner, C. J. (1966), *J. Bact.*, **91**, 905.

It is well known that bacteria fail to survive for long periods on cotton wool swabs. Unsaturated fatty acids inhibit many organisms. A number of these substances have been demonstrated in cotton wool.

The study was undertaken to compare a number of materials known to prolong bacterial survival with untreated materials, and cotton wool untreated and treated with polyvinyl alcohol. Various organisms were used.

Results showed poor survival of bacteria on cotton wool swabs. There is an apparent superiority of transport medium with ordinary swabs.

J.D.D.

Malignant Cells in Sputum: A Simple Method of Liquefying Sputum. Taplin, D. J., *J. med. Lab. Technol.*, **23**, 252.

A non-enzymatic method for liquefying sputum is described. It disinfects the specimen and stops enzymatic autolysis. The cells are concentrated by centrifuging, the morphology and staining reaction are well preserved, and no artefacts are produced. The method is simple, not time-consuming and requires no special equipment. J.D.D.

Reliability of a Commercial Triphenyltetrazolium Chloride Reduction Test for Detecting Significant Bacteriuria. Parker, R. H., Nord, N. M., Croft, G. F. and Hoeprich, P.D. (1966), *Amer. J. med. Sci.*, **251**, 260.

Examination of 200 clean, voided specimens of urine for significant bacteriuria was accomplished by three methods: (1) quantitative and qualitative culture, (2), commercial triphenyltetrazolium chloride (TTC) reduction test (Uroscreen) and (3) microscopic examination of a Gram-stained drop of uncentrifuged urine. Specimens were refrigerated and in most instances tests were completed within 4hrs of collection. No specimen was examined, despite refrigeration, if more than 12hrs had elapsed after collection.

More than 100,000 bacteria per ml. were present by culture in 68 specimens. The TTC test was positive 57 times. However, 21% of the positive TTC tests were associated with insignificant bacteriuria by culture. A negative TTC test was found in 31% of specimens with significant bacteriuria.

In contrast with the results of the TTC test, microscopic examination of a Gram-stained drop of uncentrifuged urine was found to be a reliable index of bacteriuria. A 3 mm. bacteriological loopful of urine was placed, without manual spreading, on a slide and allowed to dry. Following heat fixation and Gram-staining, the preparation was examined by oil-immersion. If bacteria were not seen within 2 to 3 minutes of scanning, the examination was considered to be negative. The classification of any bacteria as Gram-negative or-positive, bacilli or cocci, may be valuable information for clinical management.

No explanation is offered for the unusually high percentage of specimens showing significant bacteriuria. J.H.

Rapid and Easy Method of Determining the Drug-Resistance of Tubercle Bacilli. Tyrakowska, J. (1966), *Pol. med. J.*, 5, 533.

To determine drug sensitivity, drug-containing media [Lowenstein-Jensen] (primary culture) were seeded with tubercle bacilli and stained on the fourteenth day of incubation. For staining, the surface of the medium was covered with hot (80°) carbol fuchsin reagent, the excess of the dye was decanted and the medium washed three times with water. The medium was then decolourised with Ebner reagent. Its surface, which had been stained red with the carbol fuchsin reagent, regained its original green colour after treatment with acid alcohol. For inspection, the medium was removed from the tube by means of a suitably shaped wire, placed on a Petri dish and inspected under a strong side-illumination.

This method was compared with the one previously employed; the two methods yielded identical results in over 90% of the tests.

Preliminary studies showed that the presence of colonies of tubercle bacilli in the stained medium could be established between the fifth and tenth day of incubation only under a magnifying glass at 90X magnification. Macroscopically, the colonies became evident by the fourteenth day of incubation. J.H.

A Study of the Uroscreen Test in 1,000 Gynecological and Obstetrical Patients. Widholm, O. and Rautalinko, T. (1966), *Gynaecologia*, **162**, 88.

Since only 50% of the patients with bacteriuria have symptoms of infection, it is understandable that if a simple and rapid chemical reaction could be found, it would be of great value in attempts to combat kidney ailments resulting from chronic infection.

The purpose of this investigation was to study the suitability of the Uroscreen test as a preliminary test for detecting urinary infections, compared with the examination of urinary sediment and bacterial cultures.

In ward patients the occurrence of urinary infection, based on Gram staining and bacterial culture, was 10%. The corresponding figure with the Uroscreen test was 6.6%. In 500 out-patients the Uroscreen test revealed that 10% of the patients had significant bacteriuria.

There were no false-positive Uroscreen results. No correlation was found between the Uroscreen test and pyuria, the limit of which was considered to be 5 WBCs per field at 300- to 400-fold magnification.

The authors feel that the Uroscreen test may be of value in mass examinations, *e.g.* in maternity welfare centres where the aim is to detect asymptomatic urinary infections at any early stage. J.H.

Escherichia coli Diarrhoea in Children. Aggarwal, S. C. and Ramakumar, L. (1966), *Indian J. Pediat,* 33, 233.

During the past few years, considerable stress has been laid on the role of *E. coli* in producing diarrhoea in young children and 22 *E. coli* serotypes have been isolated during epidemics of diarrhoea in children all over the world.

In the present study, bacteriological examination of stools from 90 cases of diarrhoea in children up to the age of five was carried out; *E. coli* was present in 23 cases. Twelve serotypes were found but only one of them (055) belonged to a recognised enteropathic variety. Among the rest, 09:K(A):H— occurred frequently. Complete absence of *Shigella* and *Salmonella* organisms was observed in the present series. J.H.

Bacterial and Mycotic Flora in Cases of Chronic Suppurative Otitis Media. Arya, S. C. and Mohapatra, L. N. (1966), *J. Indian med. Ass.,* 47, 369.

In this study of 164 cases, the predominant pathogenic organisms isolated were *Ps. aeruginosa, Staph. aureus, Proteus* organisms, *E. coli* and *Kl. aerogenes,* which accounted for 86% of these cases. Only in 45 cases (27%) was a fungus found in addition to the bacteria. The predominant species were *Aspergillus, Rhizopus, Alternaria* and *Cephalosporium,* all of which are ubiquitous. In almost all cases there was a pathogenic bacterial agent which was thought to be primarily responsible for this condition. The system used for isolation is described. J.H.

The So-Called "Paracolon" Bacteria. Fields, B. N., Uwaydah, M. M., Kunz, L. J. and Schwartz, M. N. (1967), *Amer. J. Med.,* 42, 89.

The proposal by Ewing and Edwards of a system of classification for the *Enterobacteriaceae* eliminated the indiscriminate grouping of the poorly-related bacteria which have been assembled under the catch-all designation, "paracolon." Much of the confusion regarding family interrelationships was resolved by the disposition of many of these previously so-called "paracolons" into the discrete taxonomic groups, *Serratia, Citrobacter, Providence, Hafnia, Edwardsiella* and *Arizona,* and by the recognition that other "paracolon" bacteria are merely biochemical variants of classic species such as *Escherichia coli* and *Klebsiella pneumoniae.*

With the means at hand for precise differentiation among the various *Enterobacteriaceae,* the authors have evaluated the clinical significance of the respective groups of "paracolons" in human infections. Bacteriological methods employed are described.

The modern classification of the *Enterobacteriaceae* has introduced a number of new, precise designations with which the clinician is not completely familiar. J.H.

A Clinical Evaluation of Blood Cultures. Munro, J. F., Duncan, L. J. P. and Durie, T. B. M. (1967), *Brit. J. clin. Pract.,* 21, 71.

It has been suggested that septicaemia may present as a medical emergency more frequently than is commonly believed. This paper presents

the results and assesses the value of blood cultures taken from in-patients in a general medical unit from October 1962 to September 1965.

Blood-borne infection was detected bacteriologically in 8.4 per, 1,000 admissions, a total of 91 positive cultures being obtained from 28 patients. The actual occurrence rate was, however, probably much higher because some patients with septicaemia may not have had blood taken for culture and in others negative cultures may have been obtained.

Any patient whose condition is more serious than can be attributed to the primary cause of the illness should have blood taken for culture even if the temperature is normal.

There is a description of the bacteriological methods employed. J.H.

A Community Attack on Bacteriuria. Kunin, C. M. (1966), *Hosp. Pract.*, **1**, 67.

Early detection and careful urological characterisation of urinary tract infections in children permits the hope that chronic infections of the kidney and their sequelae may be partially or completely prevented.

The programme described in this article has shown that the prevalence of bacteriuria in a population of over 8,000 schoolgirls is 1.2% among white children, 0.9% in Negroes. No case of bacteriuria was found in the first 1,600 boys screened, hence the programme was thereafter confined to girls.

Examination, under high dry magnification (500x), of unstained sediment from centrifuged portions of fresh urine (using reduced light for best contrast) showed that an abundance of bacteria in the sediment almost always coincided with a bacterial count of well over 100,000 per ml. This method was found to be more reliable than any of the commercial chemical tests. J.H.

UNCLASSIFIED

The Evolution of a "Teaching Program" on Examination of the Urine. Weller, J. M., Greene, J. A., Jr. and Geis, G. L. (1966), *Univ. Mich. Med. Cent. J.*, **32**, 135.

Study of urinalysis presents the teacher with the problem of obtaining suitable specimens to enable the student to have actual experience in examining urine samples representing a variety of abnormal states. The microscopic examination has been a particularly difficult skill to acquire; current textbook expositions do not appear to be conducive to ready learning of this subject matter. Observation suggests that clinical laboratory diagnosis courses are also deficient in imparting such knowledge.

It was the authors' opinion that a teaching programme in the form of a text in combination with selected colour photomicrographs of actual urine sediments would prove most effective in teaching. A trial of such a programme showed that it offers much advantage in presenting this type of material and is a very effective tool. J.H.

A Rapid Direct-Reading Latex Agglutination Pregnancy Test. Fink, H. and Frie, A. (1966), *Obstet. Gynec., N. Y.*, **28**, 660.

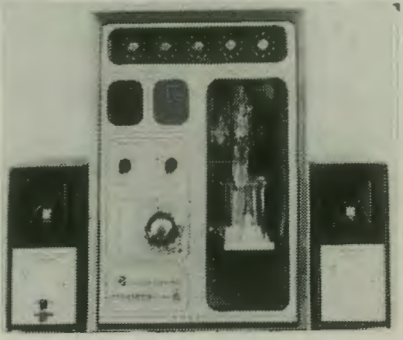
This direct latex agglutination test (developed and supplied by Pfizer Diagnostics) uses polystyrene latex particles coated with anti-human chorionic gonadotrophin. One drop of urine is place within a ring on a slide and one drop of antiserum-coated reagent is added. After mixing and tilting, a positive test is indicated by agglutination.

Two hundred urine specimens were tested. In normal pregnancy, diagnostic accuracy of the direct latex agglutination test was 95%; of a latex agglutination inhibition test (Gravindex), 92%; and of a haemagglutination inhibition test (UCG-Test), 94%. In all three tests, as a rule, false negative results were due to either early or advanced pregnancy.

In the absence of pregnancy, the accuracy of the direct latex agglutination test was 100%; of the latex agglutination inhibition test, 95%; and of the haemagglutination inhibition test, 99%. J.H.

What's New

A NEW AUTOMATIC CELL COUNTER



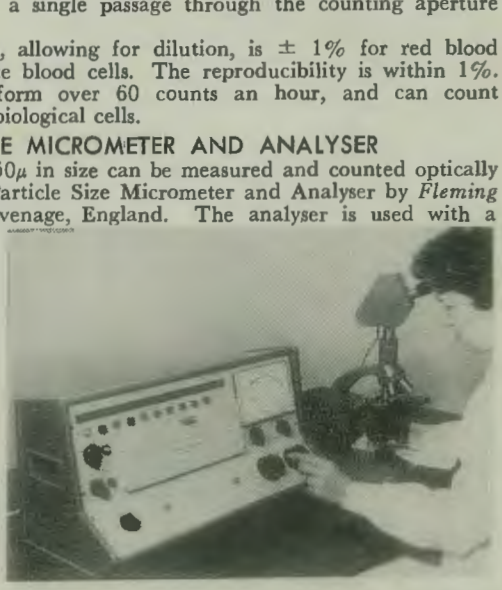
A new automatic cell counter is announced by *Coulter Electronics Ltd.*, Dunstable, England. The instrument, known as the model F, is used in haematology, cytology and bacteriology, and is directly developed from the company's Model A counter. It is a single unit. No microscope is required, and all glassware is self-contained within the cabinet. Particle size is assessed on a volume basis; therefore the accuracy of the count is held constant regardless of particle shape.

The sample is suspended in an electrolyte solution and the particles are counted and sized in a single passage through the counting aperture of the instrument.

The overall accuracy, allowing for dilution, is ± 1% for red blood cells and ± 3% for white blood cells. The reproducibility is within 1%. The instrument will perform over 60 counts an hour, and can count and assess the size of any biological cells.

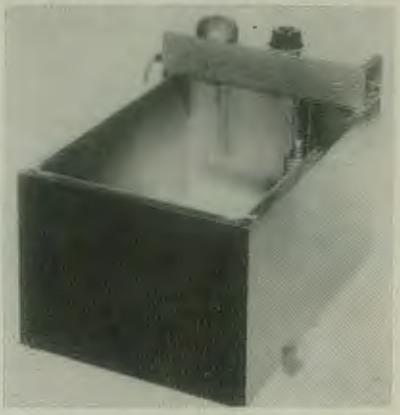
PARTICLE-SIZE MICROMETER AND ANALYSER

Particles from 1 to 250μ in size can be measured and counted optically with the new Type 526 Particle Size Micrometer and Analyser by *Fleming Instruments Ltd.*, of Stevenage, England. The analyser is used with a microscope, and between 200 and 600 particles can be classified in ten minutes. Operator fatigue is low, and close agreement in the results by different operators can be relied on.



The instrument consists of a control unit and an image-splitting unit which can be fitted to any microscope using a standard diameter eyepiece. The image splitter is a vibrating mirror which causes a light beam to scan the particle from two positions. The distance between the two images as seen in the microscope is known as the shear, and this distance depends on the amplitude of the mirror movement, which is in turn directly related to the amount of current fed into the vibrator. The current is calibrated as microns on the indicator meter in the control unit. When the two images just touch, the meter shows the diameter of one particle. Up to ten size groups can be pre-set. The image shear between the upper and lower limit of each group is automatically presented and the particles seen to be between the limits are counted and set into 4-digit registers with a foot switch. The accuracy is ±2% or ±0.5μ, whichever is the greater.

THERMOSTATIC LABORATORY WATER BATHS IN STAINLESS STEEL



New models of thermostatic waterbaths have been introduced by *Grant Instruments (Cambridge) Ltd.* The baths are made of stainless steel with black formica-covered outer cases, with all joints welded and treated to prevent weld-corrosion. Fitted over one end of the bath is a removable control unit comprising a heater, a thermostat and a stirrer. The bath has a false bottom to assist the forced circulation which maintains a uniform temperature throughout the bath.

A device for maintaining the water level can be obtained and should be used to counteract excessive evaporation when the water temperature is 60°C. and above. There are four sizes of baths, and both flat or sloping lids can be supplied. An automatic 1500 watt boost heater for rapid warming up can be fitted, and an inlet beside the stirrer can be connected to the water supply.

A NEW PHOTOELECTRIC COLORIMETER

Gallenkamp Ltd. have produced a new photoelectric colorimeter, which is known as the Gallenkamp Mk 3. It has a transistorised stabiliser, which prevents mains voltage or frequency fluctuations from interfering with measurements made by the instrument. It also reduces the instrument's drift, after a brief initial warming-up period, to negligible proportions.

An optical sub-assembly incorporates a 3-position polypropylene cell holder which can accommodate rectangular cuvettes, test tubes or an accessory flow-through cell; a pre-set focus lamp working at reduced voltage; and an encapsulated photocell. Both lamp and photocell are accessible for easy replacement, and the indicating meter has an anti-parallax mirror scale calibrated in optical density units between zero and one.

A NEW APPROACH TO ELECTROPHORESIS

In order to save technologists time in preparing samples and media for electrophoretic analysis, a new electronic apparatus has been developed, known as the Spectrophor I, produced by *Bausch and Lomb* in the U.S.A. A major feature of this machine is its high degree of automation which, coupled with the elimination of the need to "dry and stain" the separated products, results in elimination of most sources of variability in the results.

Eight protein separations may be achieved within one hour, with quantification adding a further two minutes per sample. The usually difficult LDH isoenzymes require only two hours with this instrument. Sea Kem brand Agarose is used as the supporting medium for the electrophoresis and there is a direct readout of proteins using the absorbance of the peptide bond at 250 mμ.

In the case of serum protein excellent resolution is obtained; in addition, the pre-albumins and split beta globulins not usually seen in conventional electrophoresis are in evidence. It is interesting to note the

ease with which spinal fluid proteins can be fractionated. Prior concentration is not required, and 10-20 microlitres of sample results in acceptable curves. A method for haemoglobin electrophoresis has been developed.

This is a promising development, since easily performed isoenzyme assays should find ready acceptance in the diagnostic clinical chemistry laboratory. It affords a great potential as a sensitive indicator of tissue damage.

Other possible applications might be envisaged such as the analysis of other isoenzymes, nucleotides or even urinary proteins which will help signal such damage. J.H.

*Harris, S.G. (1967), *Wld. med. Electron.*, 5, 43.

A NEW TRANSPORT SYSTEM FOR HAEMOLYTIC STREPTOCOCCI

Manufactured by *Falcon Plastics,* the new *Culpak-Kit* provides a method of transport and recovery of haemolytic streptococci from swabs.

The sterile unit consists of a small filter paper strip enclosed in an envelope. The swab from the suspected site is immediately rolled and scrubbed onto the surface of the sterile strip, which is then replaced in the envelope and sent to the laboratory. On arrival the strip is placed, exposed side downwards on a blood agar plate, which is then incubated for four hours. The strip is then removed, the plate streaked and the plate re-incubated for 18 hours.

This technique eliminates the preparation of transport media and is especially useful when samples have to be mailed to the laboratory.

Free samples on request to Gateway International, 401 South Vermont Avenue, Los Angeles, Calif. 90005, U.S.A.

Teaching Aids for Medical Technology

PARASITOLOGY:

A problem which presents itself in teaching parasitology is the provision of a continuous supply of material for students to examine. One parasite for which an adequate supply exists is the nematode *Ascaris lumbricoides.*

Pigs are commonly infected with *Ascaris lumbricoides* var. *suum* which is morphologically indistinguishable from the human variety. A trip to the local abattoir when pigs are being slaughtered should provide an ample supply of worms. Once the worms have been collected, it is a simple task to manufacture a faeces sample containing ova.

Select the female worms, dissect out the uteri and gently homogenise them in a small quantity of saline. Add the washings to a faeces sample and mix. The ova are readily recovered on concentration and show typical morphology.

FILM:

Introduction to Ion Exchange.

National Film Library catalogue number C668. This colour film, of 20 minutes running time, was made by the Permutit Company and provides an excellent basis to the understanding of the action of ion exchange resins. The film demonstrates the nature of exchange resins, both anion and cation exchangers, the production of distilled water, purity checks and other uses of ion exchange resins.

Tutors interested in obtaining the film *Robert Koch—Benefactor of Mankind* from the National Film Library, should note the new catalogue number B3008/1.

R.T.K.

The Health Department Examinations — November, 1966

INTERMEDIATE EXAMINATION

CHEMICAL PATHOLOGY

Time allowed. 3 hours. *All questions to be answered.*

1. Describe in detail any manual method of estimating blood non-protein nitrogen *or* blood urea *or* blood urea nitrogen. Give reasons for each step of the procedure. Indicate chemical changes occurring and show the calculations involved in obtaining the result. *(25 marks)*
2. Discuss the fractional test meal under the following headings:—
   a) Reasons for being performed.
   b) Instructions to the patient and the collection of specimens.
   c) Analysis of the specimens in the laboratory and the method of reporting the results. *(25 marks)*
3. i) Sketch and describe briefly any type of water still.
   ii) Describe your method for detecting occult blood in faeces, giving reasons for the steps involved.
   iii) Outline briefly some of the important functions of (a) the liver and (b) the kidney.
   iv) Give examples of biochemical estimations where a misleading result would be obtained in each case if:—
      a) the blood specimen had been several days in the mail before reaching the laboratory.
      b) the blood specimen was badly haemolysed.
      c) the specimen was collected with the wrong anticoagulant.
      d) the specimen was infected.

   (5 marks for each part, total 20)
4. i) Define the following : molar solution; normal solution; buffer solution.
   ii) Why is optical density usually used in colorimetry, rather than percentage transmission?
   iii) For very accurate determination of serum chloride it is necessary to collect blood under oil. Why is this?
   iv) How do you test for the presence of urobilinogen in urine?
   v) Outline your method for detecting acetone in urine.
   vi) List the different kinds of pipettes used in biochemistry and state how they are used.
   vii) Why is a single pan balance considered to be superior to a double pan balance?
   viii) List some inflammable, corrosive or toxic chemicals and liquids commonly found in chemical pathology laboratories.
   ix) Indicate differences between direct and indirect acting bilirubin.
   x) What two main considerations are important when selecting the wavelength of light to be used for reading a test solution in a photoelectric colorimeter or spectrophometer.

   (3 marks for each part, total 30)

HAEMATOLOGY

Time allowed. 3 hours. *All questions to be answered.*

1. Describe in detail how you would stain a prepared blood film with prepared Leishman's stain. Give reasons for the various steps taken. Describe the naked-eye appearances of the prepared specimen. Describe the appearances and distinguishing features of a late normoblast.
2. Describe in detail the procedure for the one-stage prothrombin time test, What controls should be used? What is the purpose of the test?
3. Describe the full procedure for routine ABO grouping of a specimen of clotted blood from a potential blood donor. Discuss expected and

anomalous results. What would be the possible ABO groups of the parents and children of a group AB man?

4. Describe in detail the procedure of Coombs test on the cord blood of a new born baby. What information is gained from this investigation?
5. You are provided with a single cell photoelectric colorimeter reading only in optical density. Describe how you would calibrate this instrument for the routine estimation of haemoglobin by the cyanmethaemoglobin method. What are the advantages of the cyanmethaemoglobin method?
6. Write short notes on a) Chronic myeloid leukaemia; b) iron deficiency anaemia; c) Arneth count; d) reticulocytosis; e) panagglutination. (Technical procedures may be referred to by name without description of the method.)

MICROBIOLOGY

Time allowed. 3 hours. *All questions to be answered.*

1. Describe in detail how you would isolate *Haemophilus influenzae* from a specimen of C.S.F. and confirm its identity. *(15 marks)*
2. Discuss briefly the preparation of the following media and give reasons for the inclusion of the chief ingredients in each. a) Robertson's cooked meat medium; b) selenite F broth; c) Lowenstein-Jensen medium; d) thioglycollate medium; e) MacConkey bile salt agar. *(20 marks)*
3. Write short notes on: a) The life cycle of *E. granulosus;* b) testing for "free" coagulase; c) relative centrifugal force (R.C.F.); d) pour plate method for counting bacteria; e) cellulose membrane filters. *(20 marks)*
4. Describe the methods used in your laboratory to demonstrate the presence of organisms in the blood of a person suspected of having septicaemia. *(15 marks)*
5. How would you confirm the identity of a yeast thought to be *Candida albicans?* *(10 marks)*
6. Discuss the ways in which moist heat may be used as a means of sterilisation. *(20 marks)*

SUCCESSFUL CANDIDATES

Auckland Centre

Adamson, Miss J. R.	Rotorua
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Bosley, I. S.	Auckland
Bree, A. D.	Auckland
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Cottle, M. L.	Auckland
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Haines, D. J.	Hamilton
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Dodd, G. W.	Dunedin
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Gray, Miss M. J.	Gore
Holland, Mrs D. T.	Christchurch
Hurring, J. R.	Invercargill
McIvor, R. K.	Dunedin
McKenzie, Miss J. R.	Invercargill
McQuorrie, Miss A. M.	Dunedin
Marshall, Miss K. A.	Dunedin

Morris, Miss S. L.	Christchurch
Pomeroy, D. B.	Invercargill
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Tizzard, J. L.	Christchurch
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McMillan, Miss B. A.	Wellington
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Tizard, Miss S. M.	Wellington
Vause, Miss C.	Wellington
Phillips, Miss L. A.	Wellington

Qualifying Triple Examination

CHEMICAL PATHOLOGY

Time allowed: 3 hours. *All questions to be answered.*

1. Outline the forms in which cholesterol is present in the plasma. Give details of one method for the quantitative estimation of cholesterol, and indicate some conditions associated with abnormal levels.
2. a) Describe briefly two serum flocculation tests and indicate the mechanism of their action. Discuss the relationship of these to liver function.
   b) Give details of a method for the estimation of serum uric acid. Under what circumstances are abnormal levels found?
3. a) The strength of a solution of oxalic acid in water was determined by using it to titrate a sodium hydroxide solution known to have a strength of exactly 5N. 10 ml. of the sodium hydroxide was neutralised by 35.2 ml. of the oxalic acid.

   Calculate the strength of the oxalic acid in g. per litre. Atomic weights: H = 1; C = 12; O = 16. Oxalic acid has the formula $(COOH)_2.2H_2O$.

   b) During a determination of serum phosphate, 0.2 ml. of plasma was deproteinised by addition of 0.8 ml. of water and 1.0 ml. of 10% trichloracetic acid. 1.0 ml. of the protein-free supernatant fluid was used for the estimation and was compared with a standard solution of phosphate containing 0.5 mg. of phosphorus (as phosphate) per 100 ml. The optical densities of reagent blank, standard and test were 0.022, 0.340 and 0.583.

   Calculate the concentration of acid-soluble phosphate present in the plasma in mg. per 100 ml.
4. Describe in detail a method for the estimation of the concentration of serum bilirubin. Discuss the possible errors of the method, the preparation of a calibration curve and the clinical application of the determination of direct acting bilirubin.
5. Write notes on:— a) the precautions required in the handling of poisonous reagents such as cyanide in the laboratory; b) the construction and mode of action of one form of thermostat; c) microgram, mu, equivalent weight, valency; d) the regulation of the pH of the blood; e) ketone bodies in serum.

HAEMATOLOGY AND BLOOD BANK SEROLOGY

Time allowed: 3 hours. *All questions to be answered.*

1. What features in a blood film might suggest the presence of a haemolytic anaemia? Describe your scheme for the investigation of such an anaemia, indicating the way in which the tests can help to determine the type of haemolytic anaemia.
2. a) Give an outline of the current theory of blood coagulation. b) An elderly patient is to be investigated for prolonged postoperative bleeding. Describe briefly the laboratory tests necessary for a full investigation of such a case.

3. Discuss the manner of inheritance of the ABO and Rhesus blood groups.
4. Write notes on: a) leucocyte alkaline phosphatase; b) leucocyte peroxidase activity; c) quality control in haemoglobinometry; d) Westergren sedimentation rate estimation.
5. NOTE: Details of techniques are *not* required in the following:—
   a) What steps would you take to identify an immune antibody in the blood of a pregnant woman?
   b) What immune antibodies may give rise to haemolytic disease of the newborn? By what methods are they demonstrated?
   c) Enumerate the reasons for false results in the anti-human globulin test. How may they be eliminated?
   d) What tests would you carry out in the investigation of a blood transfusion reaction?

MICROBIOLOGY

Time allowed: 3 hours.

Qestions 1 and 2 *must* be undertaken, and *two* of the remaining three questions.

1. Write brief notes on:—
   i) *C. diphtheriae* virulence test; ii) reduced oxygen tension; iii) freeze drying; iv) fimbriae; v) agglutinin absorption; vi) routine test dose as used in bacteriophage typing; vii) Griffiths typing; viii) bimetallic thermostats; ix) versene; x) Rideal Walker test.
2. Give one method for:—
   i) testing for acetyl-methyl-carbinol; ii) the detection of cytochrome oxydase; iii) the demonstration of proteolysis by a bacterial culture; iv) the demonstration of lecithinase activity; v) spore staining; vi) sterilising carbohydrate media; vii) estimating the number of viable bacteria in a water sample; viii) concentrating faecal specimens for examination for ova; ix) testing for tuberculin sensitivity; x) the titration of anti-streptolysin O in blood.
3. An outbreak of food poisoning has occurred due, it is suspected, either to home-made brawn or to creamed meringue. What specimens should be examined to confirm or disprove this suspicion? Outline the methods you would use in examining the specimens.
4. You are given a Robertson's meat broth culture containing the follow-organisms:—
   Cl. tetani, Cl. welchii, Staphylococcus pyogenes aureus, Streptococcus pyogenes, Esch. coli and *Proteus vulgaris.*
   Describe the procedure, with a short statement of the principles involved, that you would adopt to obtain each of these as a pure culture, and how you would identify them.
5. Describe the methods and media you would employ to isolate:—
   i) dermatophytes from infected skin; ii) streptococci from the throat; iii) *N. gonorrhoeae* from a cervical swab; iv) the causative organism of pneumonic plague.

SUCCESSFUL CANDIDATES

Alexander, E. B.
Waikato Hospital
Anderson M. H.
Auckland Hospital
Bryan, P. Green Lane Hospital
Bryce, Miss A. M.
Mater Hospital, Auckland
Conder, Miss M. L.
Rotorua Hospital
Hooley, Miss P. K.
Mater Hospital, Auckland
MacDonald, R. Auckland Hospital
McQuinn, Miss M. E.
Auckland Hospital
Martin, Miss B. E.
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Orchard, I. R.
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Peters, Mrs D. J.
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Pittman, Mrs P. E.
Tauranga Hospital
Sturm, Mrs M. S.
Auckland Hospital
Way, Mrs J. M.
Palmerston North Hospital

Single Subject Final Examinations

CHEMICAL PATHOLOGY—ORDINARY LEVEL

(WRITTEN)

Time allowed: 3 hours. *All questions to be answered.*

1. Discuss the glucose tolerance test under the following headings:— a) method of performing the test; b) errors and difficulties in the performance of the test; c) the handling of specimens; d) relative merits of different methods for measuring blood glucose; e) errors and difficulties in the method of glucose estimation with which you are familiar.
2. How would you carry out a complete quantitative serum protein analysis by electrophoresis and total protein determination?
3. Describe four enzyme tests which bear on liver function and the nature of the variations that occur in disease. Outline briefly the method of estimation for one of these.
4. a) During standardisation of a caustic soda solution, 10 ml. of the solution under test required 56.2 ml. of standard hydrochloric acid to neutralise it. If the hydrochloric acid has a strength of 1.12N, what is the concentration of the caustic soda solution in grams per litre? Atomic weights: Na = 23, O = 16, H = 1.
   b) Describe a method for the quantitative estimation of the protein content of cerebrospinal fluid. How would you prepare a calibration curve? Discuss the possible errors in the method.
5. Write notes on the following:—
   a) types of anticoagulant used for blood samples withdrawn for chemical analysis; b) construction of the direct vision spectroscope and the way in which it resolves white light into its constituent wavelengths; c) chromatography—principles and an application you know of; d) the handling of infective specimens in the biochemical laboratory; e) photoelectric cells, their construction and operation.

(PRACTICAL)

Time allowed; 3 hours. *All questions to be attempted.*

1. Calibration of a 0.1 ml. pipette. (Allow 30 minutes.)
   Pipette 0.1 ml. of the sulphuric acid solution provided (approx. 10N) into approximately 2.0 ml. of water and titrate with the dilute alkali solution of accurately known strength (0.05N) using one drop of bromphenol blue as indicator.
   Repeat this procedure as many times as possible during a period of twenty minutes, carefully recording each titration figure.
   Calculate the mean value for the titration figure, and given the exact strength of the sulphuric acid, work out the volume contained by the 0.1 ml. pipette.
   b) Calibration of a 5.0 ml. pipette. (Allow 30 minutes.)
   Weigh the small flask provided. Using the labelled 5 ml. pipette, deliver 2.5 ml. of the special solution A into the flask and reweigh. Determine the weight of the solution added.
   Repeat this estimation using the same portion of the 5 ml. pipette (use the upper portion from 0 - 2.5 ml.) as many times as possible within the space of 20 minutes.
   Calculate the mean value for the weight of the solution delivered, and given the density of this, calculate the mean volume delivered.
2. a) Determine the total protein concentration of the plasma sample A by the biuret method.
   Use protein solution B as your standard, the protein content of which is given.
   Set up six tubes of the unknown and two of the standard. A supplementary method sheet is provided, giving the quantities of reagent which should be used.

Work out the mean of the standard and test optical densities and calculate the value of the unknown.

b) Does the urine sample C provided contain ketone bodies or glucose?

c) Examine urine sample D visually and indicate briefly the type of abnormality which might be expected in this sample. What tests would you apply to this urine?

3. a) Write notes on the pieces of laboratory equipment provided.

e) Measure the optical density of the coloured solution E, using one cuvette filled with water as instrument blank.

Carry out at least 9 separate determinations, draining the cuvettes carefully between readings.

f) Work out the average value for the optical density of the coloured solution E and write down the differences between the individual readings and the mean.

SUCCESSFUL CANDIDATES

Bumstead, W. J.
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Burrows, Miss E. M.
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Carthew, P. L.
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Charleton, G. J.
Drs Fitchett & Fairbrother, Hamilton
Collier, Miss D. J.
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Dold, G. E.
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Low, Miss Y. S. W.
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McConnell, D. S.
Christchurch Hospital
MacDonald, Miss C. C.
Christchurch Hospital
MacDuff, D. A.
Dunedin Hospital
McIntosh, J. I.
Waikato Hospital
McLaren, G. R.
Dunedin Hospital
Martin, Miss F. S.
Royston Laboratory, Hastings
Paine, N. C.
Dr Alexander, Lower Hutt
Paton, Miss M. J.
Dunedin Hospital
Sims, R. I. C.
Palmerston North Hospital
Smith, Miss D. I. A.
Wellington Hospital
Tanner, Miss K. M.
Norfolk Laboratory, Tauranga
Titheridge, A. C.
Christchurch Hospital
Turner, Miss A. S.
Rotorua Hospital
Wylie, Mrs E. McC.
Dunedin Hospital
Young, Miss P. A.
Wellington Hospital

HAEMATOLOGY AND BLOOD BANK SEROLOGY—ORDINARY LEVEL

(WRITTEN)

Time allowed: 3 hours. *All questions to be answered.*

1. You are given an adequate sample of clear serum from a rabbit which has had an immunising course where the "antigen" has been sterile human serum from a group O donor.

a) Outline your procedure in determining whether this is a suitable Coombs' serum. (10)

b) How would you prepare it for use? (5)
c) How would you store it and what precautions would you take to ensure the avidity of the serum was maintained? (5)
(20 marks)

2. The results of tests with five different Rhesus antisera on four sets of cells (1, 2, 3 and 4) are given below:—

Anti—	D	C	E	c	e
1)	+	+	—	+	+
2)	+	—	+	+	+
3)	—	—	—	+	+
4)	+	+	—	—	+

a) Give the possible genotypes appropriate to the above reactions.
b) Which of the possible genotypes is most likely?
c) How would you proceed to establish the true genotype?
(16 marks)

3. Write *brief* notes (5-10 lines) on any *eight* of the following:—
a) bovine albumin; b) *Lactobacillus caseii;* c) 47 chromosomes; d) deep frozen red cell panel; e) potassium in stored blood; f) Vel antigen; g) ovalocytosis; h) neutrophil alkaline phosphatase; i) intrinsic factor; j) sideroblast; k) factor VIII. *(24 marks)*

4. A patient presents with an anaemia and history strongly suggestive of paroxysmal nocturnal haemoglobinuria. Discuss briefly the tests you might carry out:—
a) to confirm the presence of haemolytic anaemia.
b) to confirm the diagnosis of paroxysmal nocturnal haemoglobinuria.
(20 marks)

5. Discuss fully the possible sources of error in the estimation of coagulation time.
Detail how you would set about establishing a normal range for the coagulation time as carried out in your own laboratory.
Why is the coagulation time within the normal range in some cases of very mild haemophilia?

(PRACTICAL)

Time allowed: 3 hours. *All questions to be attempted.*

1. A sample of serum supplied (X) contains an antibody. What is the antibody? Is the serum suitable for use as a typing serum? Note your steps and conclusions and briefly note your criteria.
2. Determine the percentages of alkali-resistant haemoglobin in the two stroma-free haemolysates (Y and Z) provided. Indicate very briefly but clearly your technique and calculations. From your results indicate the possible source or sources of the two haemolysates.
3. You are supplied with blood slides number 1 to 6 inclusive. Examine them microscopically, report fully and comment on your findings.

SUCCESSFUL CANDIDATES

Aldworth, Miss J. M.
Medical Laboratory, Otahuhu
Bent, Miss H. J.
New Plymouth Hospital
Brennan, Miss D. M.
Wellington Hospital
Bryant, D. J.
Green Lane Hospital
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Fagg, Miss F. E.
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Wellington Hospital
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Dr Alexander, Lower Hutt
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Drs Lynch, O'Brien & Desmond, Wellington
Graham, E. J.
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Hamilton, T.
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Grey Hospital, Greymouth
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Watson, Mrs C. J.
Auckland Hospital

MICROBIOLOGY—ORDINARY LEVEL

(WRITTEN)

Time allowed: 3 hours.

Questions 1 and 2 MUST be answered, and *two* of the remaining three questions.

1. Describe in detail how you would set up a microscope for the dark-ground examination of an exudate for the presence of spirochaetes. Draw a diagram to illustrate the principle of dark-ground illumination. How would you collect and prepare a specimen for dark-ground examination, and what special precautions would you take when handling the material? *(30 marks)*
2. Detail exactly how you would proceed to do a bacteriological investigation of a transfusion reaction which was thought to be due to contaminated blood. *(30 marks)*
3. If you were provided with a vaginal swab, describe how you would differentiate between an infection caused by *Candida albicans* and one caused by *Trichomonas vaginalis*. Use diagrams. *(20 marks)*
4. Describe one method for the sensitivity testing of *Mycobacterium tuberculosis* to streptomycin. What practical steps can you take to ensure reproducibility of results and stability of the drug concentration in the test medium? *(20 marks)*
5. State how you would prepare two types of culture media for the isolation of fungi from contaminated material. Using diagrams where necessary, describe the microscopical and cultural characteristics of *M. canis* and *T. mentagrophites*. *(20 marks)*

(PRACTICAL)

Time allowed: 3 hours first day, 2 hours second day. All questions to be attempted.

Indicate in detail the steps you have taken and give a clear and concise summary of your results and conclusions.

Media for subculture are available. Subcultures will be incubated overnight and returned for reading tomorrow.

1. Identify the organisms in the mixed cooked meat culture provided (Culture X).
2. Identify the organisms growing on the blood agar plate. (Culture Y).
3. Report on the five films provided. Three films (3a, 3b and 3c) are

stained by the Gram method, while 3d and 3e are fungal slide cultures.

4. Set up a range of penicillin concentrations from 0.005μg. per ml. to 5.0μg. per ml.
   Determine the minimal inhibitory concentration (M.I.C.) of the *Staphylococcus aureus* culture provided. (Culture Z).

SUCCESSFUL CANDIDATES

Anesi, Miss L.
Wellington Hospital
August, Miss P.
Grey Hospital, Greymouth
Colbourne, Mrs E. M.
Mater Hospital, Auckland
Crutch, E. R.
Wellington Hospital
Cuthbert, Miss J. E.
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Strutton, Miss C. J.
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Tracey, Miss R. J.
Napier Hospital
Yeates, N. J.
Middlemore Hospital
Yeoman, D. M.
Auckland Hospital

CHEMICAL PATHOLOGY—ADVANCED LEVEL

(WRITTEN)

Time allowed: 3 hours. *All questions to be answered*

1. Discuss the mechanism of the absorption of calcium and its excretion, and discuss briefly the types of condition in which abnormalities of calcium metabolism occur.
   What laboratory investigations would you use to demonstrate an abnormality of calcium metabolism, giving details of the laboratory procedures involved.
2. Why is the analysis of renal calculi important?
   Outline a scheme of analysis of renal calculi, indicating different types found, and discuss briefly the factors leading to the formation of renal calculi.
3. Discuss spectrophotometers, the relative merits of different types, and the proper use of each. Mention errors, difficulties and the precautions required in practice.
4. a) Calculate the relative centrifugal force(in multiples of G) on an object in a centrifuge of radius 15 cm., rotating at 3,000 revolutions per minute.
   (g980 cm./sec./sec.; $\Pi = 3.14$).
   b) Define the terms: specific extinction and molar extinction coefficient. Given that the specific extinction of coproporphyrin at 400 mμ is 7.47×10^3, find the concentration in micrograms per

litre of a solution of coproporphyrin which has an optical density of 0.34.

c) A solution of sodium hydroxide has to be adjusted in strength accurately to 20 g. per 100 ml. of solution. A preliminary titration using 10 ml. of the solution required 52.8 ml. of N hydrochloric acid, which has a factor of 1.02, for neutralisation.
Work out the concentration of the sodium hydroxide solution, and the amount of water which has to be added to a litre of the solution in order to bring the concentration to the correct figure.

5. Discuss critically the measurement of the carbon dioxide content of plasma, giving details of the various components measured and referring to the errors of the determination.

(PRACTICAL)

Time allowed: 3 hours. *All questions to be attempted.*

1. Given a stock sodium standard solution, calibrate the E.E.L. flame photometer for sodium determinations, and measure the sodium content of the serum samples M & N.
2. Prepare an N/20 solution of potassium hydrogen phthalate by weighing out the pure salt, dissolving it in water and making the volume up to 1 litre. (Equivalent weight 204).
Determine the factor of the N/10 hydrochloric acid solution provided (Solution 0), using for titration the standard acid solution prepared above and an N/20 sodium hyroxide solution made up from the stock solution of carbonate-free sodium hydroxide provided. Using the now standardised N/20 sodium hydroxide determine the titratable acidity of the urine sample P provided, by titrating to pH 7.4 (a pH meter is provided for this titration.)
3. a) Identify the abnormal haemoglobin derivative in the blood sample R.
b) Examine the urine sample S; suggest explanations for the abnormal colour.

SUCCESSFUL CANDIDATES

The sole candidate for this examination was unsuccessful.

HAEMATOLOGY AND BLOOD BANK SEROLOGY—ADVANCED LEVEL

(WRITTEN)

Time allowed: 3 hours. *All questions to be answered.*

1. In the serological investigation of a patient suspected of having auto-immune haemolytic anaemia:—
a) Outline a scheme of investigation.
b) Discuss the collection of samples of blood and serum for these investigations.
c) Give the principle of the gamma globulin neutralisation test in relation to the above. *(20 marks)*
2. The results of tests with six different Rhesus antisera on four sets of cells (1, 2, 3, and 4) are given below:—

Anti—	D	C	C^w	E	c	e
1)	+	+	+	—	—	+
2)	+	+	—	—	+	+
3)	+	+	+	+	—	+
4)	+	+	—	+	+	+

a) Give all the possible genotypes appropriate to the above reactions.
b) State which genotype is the most likely for each set of reactions.
c) How would you proceed to establish the true genotype?
d) Using the reactions of cell sample No. 4 as one example, illustrate your answer to (c). *(20 marks)*
3. Write *brief* notes (5-10 lines) on any *five* of the following:— Anti-Le^a; Haemoglobin A_2; Haptoglobin; Potassium in stored blood;

Heparin as an anticoagulant in a blood bank; Coombs' test on red cells in the presence of albumin; Cryoprecipitate. *(20 marks)*

4. List the radioactive isotopes used in the diagnosis of haematological disorders and list the test or tests in which each isotope is used. Indicate briefly the principle of each test mentioned. *(15 marks)*
5. Write a short essay on laboratory methods of haemoglobin estimation, with particular emphasis on the limitations of and the principles involved in both recent and older methods. Discuss the standardisation and quality control of one modern method of haemoglobin estimation. *(25 marks)*

(PRACTICAL)

Time allowed: 3 hours. *All questions to be answered.*

1. The sample of serum supplied (W) contains antibody. Classify any antibody and comment on the suitability of the serum as a standard typing serum. Note your steps and conclusions, and briefly note your criteria.
2. Freshly obtained citrated plasma and incubated whole serum is provided from a young man with a haemorrhagic state of long duration. Determine from the specimens provided the nature of the haemorrhagic state. Record clearly the full results of the tests you carry out.
3. You are supplied with blood slides numbered 7-12 inclusive. Examine microscopically, report fully and comment on your findings.

SUCCESSFUL CANDIDATES

Anderson, Miss P. Rae, B. A.
Drs Lynch, O'Brien & Desmond, Christchurch Hospital
Wellington

MICROBIOLOGY—ADVANCED LEVEL

(WRITTEN)

Time allowed: 3 hours. *All questions to be answered.*

1. Discuss the theory and practice of the phage typing of *Staphylococcus aureus.* How would you ensure that your phage set retained its standard typing pattern? *(25 marks)*
2. Explain the theory and practical application of antibody-antigen reactions, complement fixation and hypersensitivity as applied to diagnostic procedures. *(25 marks)*
3. Give an account of the cell components of β-haemolytic streptococci and indicate their importance in grouping and typing these organisms. *(20 marks)*
4. Outline in detail the procedure for the serological diagnosis of leptospirosis. Indicate what you would consider to be a positive result. *(20 marks)*
5. You are supplied with a turbid cerebro-spinal fluid from a patient suspected of having *Cryptococcus neoformans* meningitis. Give technical details for the procedure you would use to examine this specimen and isolate and identify the organism. *(10 marks)*

(PRACTICAL)

Time allowed: 3 hours first day; 2 hours second day.

Answer ALL questions. Indicate in detail the steps you have taken. Give a clear and concise summary of your results and conclusions.

Media for subculture are available. Subcultures will be incubated overnight and returned for reading tomorrow.

1. You are provided with a mixed culture in a cooked meat medium (Culture A). Identify the organisms present.
2. Identify the organisms growing on the MacConkey's plate supplied (Culture B).
3. The blood agar plate culture (Culture C) has been incubated in an atmosphere of air and 8% CO_2). Identify the organisms present.

4. You are provided with five different cultures of micro-organisms (Cultures D, E, F, G, H,). From the type of culture medium used, the colonial form of the organisms and by using the usual routine laboratory staining techniques, make a tentative identification of each organism.

SECOND DAY

5. Identify the micro-organisms in the four "spots" provided.

SUCCESSFUL CANDIDATES

Hilbourne, Mrs M. L.
Auckland Hospital

Thomas, J. R.
National Health Institute, Wellington

The Folin and Wu Protein-Free Filtrate

Who Was Folin?

A BRIEF BIOGRAPHICAL SKETCH

Otto Folin was born in Asheda, Southern Sweden, on April 4, 1867. He emigrated to the U.S.A. when he was 15 years of age and there supported himself whilst he worked through High School, eventually graduating in Science from the University of Minnesota. He went on to complete a dissertation on urethanes at Chicago University, and was granted his Ph.D. in 1895.

His decision was to concentrate on the subject of physiological chemistry and in order to gain wider experience he travelled and worked in several European centres before returning to the U.S.A.

Several papers were published by Folin, but openings in the relatively new field of physiological chemistry were difficult to come by.

In 1900 Folin was invited to set up a research unit at the McLean Hospital for the Insane in Massachusetts, to study chemical problems relating to mental disease. Having accepted the position, he decided that the best approach was to study in detail the compounds resulting from both normal and abnormal metabolism in the human body.

OTTO FOLIN (1867-1934).
Portrait by Smil Pollak-Ottendorf.
Photograph by courtesy of Harvard University, Bethesda, Md.

Although most of the urinary constituents were known qualitatively, the quantitative analyses of specimens was an extremely laborious task. Folin set about simplifying these procedures, and in so doing he introduced into biochemistry the colorimeter, an instrument which demonstrated the practical value of colour comparison as a basis for analysis.

Folin's work at the McLean Hospital resulted in 1905 in his classic paper, "'Laws Governing the Composition of Normal Urine." Further, his interpretation of the data he had collected led to the proposal that protein was absorbed from the gastro-intestinal tract in the form of

amino acid units, a concept which differed widely from the views held at that time.

In 1907 the first Chair of Biological Chemistry at Harvard University was offered to Folin. The position was accepted, and here Folin continued his work on the investigation into products of protein metabolism.

Amongst many techniques Folin developed, his work on demonstrating in blood a rise in the level of non-protein nitrogenous substances in kidney failure, proved to be of immeasurable and immediate clinical value.

Folin was actively engaged on the study of blood and urine chemistry until the time of his death on October 25, 1934.

R.T.K.

REFERENCES:

Schaffer, P. A. (1935), *Science*, **81**, 35.
Wardlaw, H. S. (1935), *Med. J. Aust.*, **i**, 69.

Book Review

Colour Atlas of Histopathology R. C. Curran, M.D., M.R.C.P. (Lond.) F.C. Path., F.R.S. (Edin.) (with 765 photomicrographs) Bailliere, Tindall and Cassell—Harvey Miller, London, 1966. Price in U.K. 75s.

This beautifully produced volume is designed to illustrate histopathology as an adjunct to standard descriptive texts. The author is the Professor of Pathology in the University of Birmingham, and brings his wide experience to bear on the choice of illustration and the helpful and detailed captions.

The book has been divided into chapters which illustrate broad topics related to the principles of pathology. Each page bears 9 coloured photomicrographs which are accompanied by explanatory notes.

Colour reproduction is good and comparable to actual microscopic appearances. Although intended for diagnostic pathologists, the book will interest histopathology technologists and stimulate them to broaden their appreciation of their medical colleagues' work.

One is impressed by the very high quality of the preparations from which the photographs have been taken. This serves to emphasise the very close relationship that exists between the morbid anatomist and his technical associates, and reminds the diagnostic pathologist of how dependent he is on these workers.

J.F.G.

Precautions in Handling Carcinogenic Aromatic Amines

A small booklet has been published by the Chester Beatty Research Institute at the Royal Cancer Hospital, London, which presents a recommended code of practice for laboratory staff when handling chemicals which may cause tumours of the urinary tract.

The substances most frequently used in laboratories which have been recognised as carcinogenic are the following:—α-naphthylamine, β-naphthylamine, benzidine, 4-aminodiphenyl (xenylamine), 4-nitrodiphenyl, *o*-tolidine and *o*-dianisidine or their salts; and the nitrosamines, nitrosophenols and nitronaphthalenes. In addition some preparations may contain small quantities of these substances as impurities.

Precautions listed include the labelling of these substances as "Dangerous"; storage in closed containers; education of all persons as to the risks and their avoidance; measures to be taken following accidental contact; measures to be taken following the use of dangerous chemicals; and the provision of impervious bench surfaces to prevent absorption into wood surfaces.

The booklet is available from the Chester Beatty Research Institute, and a copy of it should be in every laboratory.

R.T.K.

The Library

List of Current Acquisitions

Librarian: D. S. Ford, Blood Bank Laboratory, Dunedin Hospital.

Amer. J. med. Technol. Volume 33, No. 1. January-February 1967.

Contents: Professionalism and the Conquest of Inner Space; A New Photometric Method for Serum Urea Nitrogen Determination; Medical Technology as Applied in Circulatory Shock; Reflectance Densitometry of Cellulose Acetate Protein Electrophoresis; Tissue Preparation for Electron Microscopy; Demonstration of Six Antigenic Constituents of Histoplasmin by Immunoelectrophoresis; Use of Normalized T Scores in Grading Students.

Ann Med. exp. Biol. Fenn. Volume 44, No. 4. 1966.

Aust. J. biol. Sci. Volume 20, Nos. 1, 2. February, April 1967.

Canad. J. med. Technol. Volume 28, No. 6. December 1966.

Contents: Determination of LDH Activity; Dosage Rapide de l'Albumine Directement sur le Serum; Studies on the Staining of Amyloid with Congo Red; Absence of the Anti-B Isoagglutinin.

Volume 29, No. 1. February 1967.

Contents: The Ability of Tissues to Initiate Antibody Formation; The Enumeration of Bacteria; Deux Methodes d'Homogeneisation; Sequestrinized Fluids for Cytology; A Rare Antibody.

Filter. Volume 38, No. 4 December 1966.

Contents: Studies in the Determination of Isocritic Dehydrogenase; Clinical Laboratory Studies for Infectious Mononucleosis; Observation on Coccidiomycosis Immunodiffusion Test with Clinical Correlation; Rapid Tube Precipitin Test; Professional Organisations in Collective Bargaining.

Volume 39, No. 5. March 1967.

Contents: Blood Bank-o-Gram File; The Spectrum of Quality Control; Abstracts.

J. Amer. med. Technol. Volume 28, No. 6 November-December 1966.

Contents: Technical Characteristics of Commercial Instruments for Analysis of Prothrombin (Time) and Other Blood Coagulation Factors; Microchemistry or Macrochemistry—What's the Difference; Technical Characteristics of Commercial Hemoglobinometers and Other Test Instruments; Mycotoxicoses—The New Frontier; Millipore Filter and LE Phenomenon.

J. med. Lab. Technol. Volume 23, No. 4. October 1966.

Contents: Cytological Examination of Urine in a District Hospital; Canned Food and Food Poisoning; Simultaneous Hard and Soft Tissue Sectioning use Polyester Resin as an Embedding Medium; Composite Media to Differentiate Salmonella and Shigella from other Enterobacteriacae; Malignant Cells in Sputum: A Simple Method of Liquefying Sputum; Platelet Adhesiveness—A Preliminary Communication; An Improved Method of Obtaining Large Volumes of Blood from the Domestic Fowl.

Volume 24, No. 1. January 1967.

Contents: The Cytology and Cytochemistry of Acute Leukaemias—A Critical Review; Methods of Storing Viruses at Low Temperatures with Particular Reference to the Myxovirus Group; Seminal Analysis; Nitric Acid Leucofuchsin Technique for Myelinated Nerves using Cryostat Sections; A Simple Silver Method for the Demonstration of Reticulin Fibres; An Instrument for Preparing Needle Biospy Specimens from Human Adipose Tissue; Two Restraining Devices for the Sampling of Urine and the Intravenous Administration of Fluids in Mice; Cheap Labour-saving Method of Removing Stain from Methanol-Acetic Acid-Water (50-20-50) used in Differentiating Amidoschwartz 10B used in Protein Staining.

Lab. Management. Volume 5, No. 1-3 January, March 1967.

Lab. World. Volume 18, Nos. 1, 2, 3. January, February, March 1967.

Med. Technol. Aust. Volume 9, No. 1. January 1967.

Contents: Low Temperature Storage of Animal Semen; The Chromo-

somes of Man; Short Description and Life Cycle of the Intestinal Parasite *Trichuris trichiura:* Medical Technology in West Africa.

Volume 9, No. 2. April 1967.

Contents: The Cytology of Body Fluids; The Volemetron—A Semi-Automated Instrument for Blood Volume Determinations; An Automated Technique for Uric Acid Determination without the Use of Sodium Cyanide; A Rapid Gelatinase Detection Test Using X-Ray Film; Ventriculocordectomy on Dogs.

Med. Technol. Dig. Volume 3, No. 1 January 1967.

Contents: Cholesterol—Discussion and Test Procedure; Isotope Production; Units of Radiation; History of Medicine—Vaccination; Rules for Manipulation of Glass Volumetric Apparatus; Resume of Parasitology—*Trichomonas vaginalis;* Cholera—*Vibrio comma;* Chemical Test for Pregnancy.

Volume 3, No. 2 February 1967.

Contents: Baby's Sex Predicted by Prenatal Fluid Test; Epithelial Sex Chromatin Staining; Blood Glucose Determination by the Glytel Method; Radiation Protection; History of Medicine—Period of Theories and Systems; Is Ambition Affected by Uric Acid; Virology; Rules for Manipulation of Glass Volumetric Apparatus; Synovial Fluid Analysis in Disease; Preparation of Joint Tissue for Urate Examination.

Volume 3, No. 3 March 1967.

Contents: A Longer and Happier Life; Radiation Lecture Series—Measuring Radioactivity; Non-hemolytic Hyperbiluribinemia; Virology—Viral Characteristics; History of Medicine—The New World; *Hemophilus influenzae;* Cultivation and Identification of Hemophilus Organisms; Typing *Hemophilus influenzae.*

Volume 3, No. 4. April 1967.

Contents: Sickle Cell Anemia; Virology—Classification of Viruses; Basic Radiation Physics—Radiation Detection; Malaria II; New Fetal Blood Group Test; The Lactose Tolerance Test; History of Medicine—Indian and Colonial Medicine Men; Determination of Capacity of Glass Volumetric Apparatus.

Microbiologia (Buc.) Volume 11, No. 5. September/October 1966.

Contents: The Role of Organisms from the Proteus Group in Human Pathology; Benign Lymphoreticulosis with Erythemato-nodular Eruption after Inoculation*; Action of Antibiotics on Gas Gangrene Organisms; The Study of Intestinal Parasite Infections in Workers in a Stone Quarry*; Isolation of *Salmonella vom* from a Focus of Toxi-alimentary Infection*; Considerations on the Efficacy of the *Bordetella pertussis* Component of the Trivaccine Prepared by the Dr I. Cantacuzino Institute; Frequency of *B. Cereus* in Fresh and in Pasteurised Milk*; *Rattus norvegicus* as a Pathogenetic Carrier*; The New Nomenclature of Human Immune Globulins; Value of the Test of Ether-Soluble Bilirubin in the Positive and Differential Diagnosis of Infective Hepatitis*; A Simple Laboratory Method for the Early Diagnosis of Epidemic Hepatitis.

Volume 11, No. 6. November/December 1966.

Contents: The Genetic Code, Amino Acid Substitutions and Mutations' Photometric Measurement of Bacterial Growth*; The Role of Lysotypism in Determination of the Enterococcal Aetiology of Some Alimentary Infections*; Considerations on the Diagnostic Value of Anti-streptolysin O*; Contributions to the Study of the Efficiency of Anti-typhoid Vaccination; Simple Quantitative Method for Expressing the Sensitivity of Organisms to Antibiotics; The Sensitivity to Antibiotics of Shigella Strains Isolated 1960-64*; Rabies with an Incubation of 19 years and 6 months; The Action of Ultra Sound on Koch's Bacillus; Mass Carrier Episode with Salmonella; A New Group in the *Enterobacteriaceae* Family; Titration of Anti-Diphtheria Antibodies by Passive Haemagglutination.

* English summary.

N.Z. Hospital. Volume 19, Nos. 3, 4. January, March 1967.

Rum. med. Rev. Volume 20, Nos. 2, 3. April, September 1966.

Tonic Volume 4, No. 5. 1966/67.

Council Notes

A Council meeting was held on April 8, 1967, in the Boardroom of the N.Z. Registered Nurses' Association, Central House, Brandon Street, Wellington. Present were: Mr M. McL. Donnell (in the chair), Miss D. Hitchcock and Messrs C. W. Cameron, J Case, E. K. Fletcher, H. E. Hutchings, R. T. Kennedy, G. F. Lowry, J. D. R. Morgan and D. J. Philip.

Industrial Committee Report

The committee chairman, Mr H. E. Hutchings, reported to the Council that consideration had been given to the remits passed at the 1966 Annual General Meeting.

Remit 1 concerned the payment for penal rates for work on statutory holidays, and investigations had shown that very few groups in fact receive double time in addition to their normal hourly rate for such overtime. Those who do include hospital cooks and employees of engineering and carpentering firms. Public Service employees are paid double time only, as we are, although they receive in addition an extra day of annual leave for each holiday worked.

The Committee's recommendation was that this remit should be considered as operating contrary to our standard of semi-professionalism in view of the fact that only by trade union support would the specific points contained herein have hope of implementation.

Remit 2 called for an examination of the grading system with a view to acquiring improvements. The remit incorporates several points and some of these are already under consideration by the Council. Following the establishment of the new machinery for negotiation, a means may exist by which the Council can press for improvements.

Remit 5 required pressure for a more realistic fee to be payable for lectures given outside normal working hours. Investigation has revealed that the payment by Education Boards to part-time evening lecturers is 23/9 per hour, plus holiday pay amounting to 4d per hour. If the class is held after 5 p.m., the lecturer is also paid an extra hour's pay for every four hours of lecturing. These fees are paid by technical colleges to all lecturers and demonstrators who possess no academic qualifications (e.g. floral art demonstrators). Qualifications attract higher lecturing fees.

It was recommended that an immediate approach should be made by the Council to the Department of Health in an attempt to correct the anomaly.

The Council adopted the recommendations of the Committee.

Hospital Service Tribunal

It was reported that nothing further had been heard regarding the establishment of the Tribunal. In order that the Council might have proper liaison with the other interested groups, it was decided to ask if the Institute could be allowed additional representation on the Committee of Combined Hospital Employee Organisations. It was proposed that Mr Hutchings should join Mr Bloore on the Committee if this was acceptable.

Professional Committee Report

The draft of the President's submissions to the Medical Laboratory Technologists Board was considered and various points debated. It was eventually decided that the draft should be rewritten before final submission, and that points emerging during the discussion should be incorporated. It was also decided to incorporate a strong recommendation for the establishment of a qualification for laboratory assistants and to volunteer the Institute's willingness to administer the necessary examinations. A sub-committee was appointed to look into questions relating to such administration, under Mr Kennedy as Convenor.

Rules Sub-committee Report

Mr Fletcher reported on his drafts of proposed changes to the Rules and, following discussion, was instructed to prepare new drafts along

lines embodying the opinions of the Council, to circulate them to the members of Council and to present the final approved drafts for the Secretary to circulate as notices of motion for consideration at the A.G.M.

T. H. Pullar Memorial Address.

It was decided to institute a memorial address in honour of the late Dr T. H. Pullar, for delivery at the Annual Conference each year. It was envisaged that a distinguished pathologist would generally be asked to give the Address, and that its substance would be printed in the *Journal.*

Remits from 1966 A.G.M. regarding Examinations

The Secretary was instructed to write to the Medical Laboratory Technologists Board, conveying the wishes of the A.G.M. in two respects. These two resolutions of the A.G.M. are somewhat in conflict, but they request that, firstly, there should be a separate examination for Haematology and for Blood Group Serology at the "A" level, and that, secondly, the qualification system should be altered to bring it into line with the I.M.L.T. system in Great Britain.

New Members

Associates

Baily, E, L. Dept. of Agriculture,	Auckland	Gowan, R. N.	Hastings
		Marsland, Miss J. M.	Wellington
Bolitho, D. G.	Ashburton	Wills, Miss L. B.	Auckland

Members

Barron, Miss B. W.	Balclutha	Morey, Miss M. L.	Rotorua
Bartle, N.	Auckland	Morrah, Miss L. D.	Dunedin
Beardsley, Miss A. C.	Christchurch	Newton, J. D.	Christchurch
Brady, D. J. P.	Christchurch	Olsen, K. D.	New Plymouth
Bromley, Miss P.	Wanganui	Parke, Miss G. E.	Auckland
Cooper, Miss H. A.	New Plymouth	Parry, Miss M. E.	Auckland
Corkin, Miss P. A.	Christchurch	Pearson, R. D.	Palmerston North
Croot, Miss L. D.	Stratford	Pees, D. C.	Auckland
Davies, Miss J. B.	Wanganui	Pomeroy, D. B.	Invercargill
Erickson, G. A.	Christchurch	Powell, Miss C. A.	Balclutha
Flower, Miss S. E.	Christchurch	Riley, Miss A. M.	New Plymouth
Gilmore, Miss H. M.	Christchurch	Rumball, H. A.	Stratford
Guilliard, B. N.	Dannevirke	Sevren, Miss M.	Dargaville
Harris, Mrs H. A.	Dunedin	Thomason, Miss L. J.	Rotorua
Helliwell, Miss R. A.	Auckland	Tisch, G. W.	Christchurch
Herrick, Miss S. E.	Auckland	Tizzard, J. L..	Christchurch
Ingram, Miss W. P.	Tauranga	Tubbs, Miss G.	Dunedin
Kells, E. V.	Dannevirke	Tunzelman, Miss I. S.	Auckland
McCullough, Miss M.	Dunedin	Webster, Miss P. A.	Auckland
McDonald, Miss C. M.	Dunedin	Wilkinson, I. D.	Christchurch
McDonald, Miss F. R.	Dunedin	Williams, P. E.	Upper Hutt
Milicich, G. S.	Auckland	Yeoman, D. M.	Auckland

Reinstated

Associate

Loader, A. E.	Auckland

Member

Beech, M. J.	Auckland

Elected Associate

Anderson, M. H.	Dunedin	Pittman, Mrs P.	Tauranga
Duncan, Miss N. D.	Auckland	Shaw, Miss M. A.	Auckland
Holland, Miss S.	Auckland	Wadams, Miss V.	Auckland

Resignations

Christie, Miss J.	Hamilton	Kerr, Miss J.	Hamilton
Cox, Miss P. J.	Auckland	Smith, J. G.	Hamilton
Davis, Mrs G. M. *nee* Stark	Wellington		

Recruitment

Concern was expressed at the fact that many practising technologists are not members of the Institute, and the Secretary was instructed to appeal to all charge technologists to ensure that the benefits of membership should be brought to the attention of new entrants to their laboratories and to all other eligible persons.

Branch Reports

AUCKLAND

SECRETARY: I. C. King, Laboratory, Cornwall Hospital, Auckland.

One-day seminar. Preliminary organisation is under way for the annual one-day seminar of the Auckland branch. At this stage the date is set for Saturday, October 7, and the venue is the Gonzaga Hall, Mater Misericordiae Hospital, Auckland. The branch executive is at present working on the theme for the morning programme.

Branch meetings. The first meeting of the year, held on Tuesday, 14 March and attended by 59 persons, was addressed by Dr D. Nelson of the Department of Scientific and Industrial Research, who addressed members on *Forensic Medicine.* On Monday, 3 April, the branch met to discuss Institute and branch affairs, education and training, and items of interest in general.

Arrangements are being made in an effort to have future meetings addressed on *Renal Transplantation* and *Rheumatiod Arthritis,* whilst several members of the branch, who have recently returned from overseas trips, have indicated their willingness to address members on the state of medical laboratory technology in the countries visited. It would appear from preliminary conversations with these travellers that New Zealand is far from being "behind-the-times," at least in our own particular fields of endeavour.

Branch newsletter. This continues to appear at fairly regular intervals and has proved to be a good way to disseminate information and general gossip. On occasions we have managed 12 foolscap pages and would take this opportunity for thanking those who make the effort to have contributions available.

I.C.K.

CHRISTCHURCH

SECRETARY.—Mr D. S. McConnell, Pathology Department, Christchurch.

Officers elected at the Annual General Meeting were:—

Chairman.—Mr J. Walker.

Secretary/Treasurer.—Mr D. McConnell.

Committee.—Mr P. Skidmore, Mr K. McLoughlin, Mr T. Tanner,

E.P.S.N.

DUNEDIN

SECRETARY: D. S. Ford, Blood Bank Laboratory, Public Hospital, Dunedin.

This year's activities were started in fine fashion by a most successful barbeque held in March. The series of films and discussions for trainees was continued. On May 6, numerous members of the Branch attended the South Island Seminar held at Timaru Hospital. This meeting was very informative and members attending found both their mental and gastronomical appetites well satisfied.

Dr P. Middleton of the Medical School, Dunedin, gave a most interesting lecture on *The Work of a Virus Diagnostic Laboratory* to the Branch meeting held on May 9. This talk included details of the classification and identification of the numerous types of virus and some of the difficulties encountered.

The June meeting was held at the newly-opened laboratory at Gore Hospital, and consisted of photomicrograph slide quiz, with financial reward for the winning trainees. The meeting was followed by the social gathering at the home of the Charge Technologist.

There is a full and varied programme arranged for the remainder of the year:—

JULY.—Discussion of Conference remits.
JULY.—Branch party.
AUGUST.—Conference Report.
AUGUST.—Visit to Electron Microscope Unit.
SEPTEMBER.—Annual General Meeting.
SEPTEMBER.—Clinico-pathological lecture by Dr J. F. Gwynne.
OCTOBER.—Student demonstration evening.

D.S.F.

Regional Seminar Reports

Mid-North Island Seminar

PALMERSTON NORTH — 1 APRIL, 1967.

The first seminar for 1967 was held at Palmerston North Hospital on April 1, 1967, and was attended by over 60 technologists from an area bounded by the regions of the Hawkes Bay, Wairarapa and Taranaki Hospital Boards.

The date was chosen to enable the Senior Representative of Radiometer, Copenhagen, who was in New Zealand, to lecture and give practical instruction on the Astrup method of acid-base evaluation.

The programme was opened by Mr K. R. Archer, Medical Superintendent and consisted of forums on Haematology, Chemical Pathology and Microbiology, with time allowed for the discussion on various topics raised by members present.

HAEMATOLOGY FORUM (Chairman: Mr H. E. Hutchings).
Thrombocytopenic Haemolytic Anaemia Mr E. K. Fletcher.
Chromosome Culture Mr H. E. Hutchings.
BIOCHEMISTRY FORUM (Chairman: Mr L. Margolin).
The Astrup Method of Acid-Base Evaluation Mr H. B. Thomasen.
Clinical Applications etc. Dr M. G. Kirk.
Blood Alcohol Levels—Personal Experiences Mr D. L. MacDonald.
MICROBIOLOGY FORUM (Chairman: Mr G. D. C. Meads).
Leprosy Dr K. N. P. Mickleson.
Salmonellae—A Double Infection Mr G. D. C. Meads.

Discussion topics included relieving in small laboratories, Casoni tests, blood urea estimation, prothrombin times, colony counts, the collection of 24-hour urine samples, kitsets and hospital quotas for annual conferences.

C.H.C.

South Island Seminar

TIMARU — MAY 6, 1967.

The 4th Annual South Island Seminar was held in the Nurses' Tutorial Block, Timaru Public Hospital, on Saturday, May 6, 1967.

There was an attendance of at least 75 technologists consisting of members from public and private laboratories from Invercargill, Dunedin, Timaru, Christchurch and Nelson.

Opening address was given by Dr L. A. Faigan, Pathologist, followed by a very full programme which was ably chaired by Mr B. W. Main (Dunedin).

The following papers were presented:—
Platelet Adhesion—B. Rae.
A Case of Klebsiella Septicaemia—Mrs M. Hudson.
Genetically Determined Diseases—J. Braidwood.
Screening Tests for Inborn Errors of Metabolism—J. Braidwood.
A Screening Test for Galactosaemia—R. Allen.
One or Two Serological Curiosities—J. Case.
Biochemistry Worksheet—B. Smith.
E. Histolytica *in an Anal Ulcer*—M. Cattermole.
Preliminary Report on a Manual Method for Serum Alkaline Phosphatase—C. Cameron.
Urine Bacteriology and Bacterial Counts—L. Taylor.
Serum Lipase Estimations—F. Corey.
Factor VIII Assay Problems—B. Rae.
pCO_2 Analysis using the 'Aimer' Apparatus—M. Harris.
A Case History of Listeria Meningitis—G. Rose.
Clotting Time vs *PTTK*—B. Main.
Creatine Phosphokinase—J. Walker.
A Case of Lymphosarcoma—B. Bodger.
How Antibiotics Work—I. Orchard.

A taped account of a case of diphtheria by Mr H. C. W. Shott was also played.

Those who were not present may well conclude that this was a full programme. It commenced after morning tea, at 10.30 a.m., and concluded at 5.30 p.m., with most topics restricted to 10 minutes for presentation and a further few minutes for discussion. Because of these restrictions discussion was often incomplete and so it was suggested by the chairman that in future seminars topics be limited to occupy 5-10 minutes.

All the subjects were well presented with some use of slides, apparatus and other material to demonstrate the particular subject. The chairman expressed the gratitude of the audience to the speakers for their work in preparing their subjects.

Thanks are also due to the Timaru Hospital Board for the use of the nurses' lecture room for the Seminar and for providing morning and afternoon teas as well as a buffet lunch. After the seminar a few members retired to a local hotel for a dinner and other refreshments.

B.N.S.
E.P.S.N.

CROSSWORD SOLUTION

Across: 2 Paracelsus, 8 onset, 9 Candida, 10 haematin, 11 Beer, 13 merits, 14 peptic, 16 salt, 18 adherent, 21 Rothera, 22 ramus, 23 neutrophil.

Down: 1 Biochemistry, 2 Pasteur, 3 retracts, 4 cocci, 5 lung, 6 urine, 7 macrocytosis, 12 research, 15 thermal, 17 litre, 19 diazo, 20 heat.

Letters to the Editor

RETURNING TO LABORATORY WORK

Sir,

Concerning the letter to the Editor written by Mr P. H. Curtis in your March 1967 issue, I quote this statement from his letter:—

"Finally, I feel that even those who do become qualified under our present system can hardly expect to return to laboratory work as competent technologists after, say, ten years of raising a family—they wouldn't even recognise the laboratory, let alone the work!"

I wish to challenge Mr Curtis's statement, as I have returned to laboratory work, two children later, to a discipline — Haematology — from which I had been absent for thirteen years, and I was able "competently" to recognise both the laboratory *and* the work.

P. B. HOLLAND (*nee* Scott),
Central Laboratory,
Auckland Hospital.

A BOUQUET FOR THE JOURNAL

Sir,

I feel this latest issue of the *Journal* (March 1967) calls for a very hearty vote of congratulations to you all. The new cover and colour work are both first class and the Institute now has a publication it can be proud of. There is certainly no excuse for members using any other journals for the publication of articles in the future.

We are also all very impressed with the world cover it now has, as evidenced by the requests for reprints of articles we have received from the most unlikely corners of the globe.

You certainly deserve all the support we can give you in the future and I hope all members will realise its great potential as a means of communication between all our laboratories.

I can assure you that I look forward to its arrival every four months.

P. H. CURTIS,
Chief Technologist,
Medical Laboratory, Auckland.

"A" LEVEL EXAMINATIONS

I qualified in 1965 under the old comprehensive C.O.P. and since then I have specialised in Haematology. Wishing to show a higher degree of proficiency in this subject, I wrote to the Medical Laboratory Technologists Board to ask if I was eligible to sit the new "A" level examination in Haematology. Your readers may be interested in the following abstracts from the reply:—

". . . the sitting of an "A" level examination by a qualified technologist will not equate to an extra qualification. This means that persons so doing will not be eligible for higher salaries or grading by virtue of a pass in such an examination.

"The Board will issue no certificate to qualified candidates who obtain a pass in such an "A" level subject.

". . . no official notification of acceptance to "A" level examinations would be issued until final figures for trainee candidates are available."

It would appear from this that:—

1. The continuance of study and the attainment of further examination passes is not encouraged. Indeed, it would seem that this is being strenuously discouraged.

2. No acknowledgement of having sat and passed extra examinations will be given.

3. The candidate will not be informed of his acceptance to the examination until the last possible minute.

4. Further examinations and experience will not be eligible for consideration by the Grading Committee.

This decision makes it appear that the Medical Laboratory Technologists Board is hand in glove with the Grading Committee in deciding salaries and gradings.

Is the Institute actively fostering the cult of mediocrity? No wonder our qualified technologists are going overseas.

N. A. MacGIBBON,
Nelson Hospital.

When invited to comment, the Secretary to the Medical Laboratory Technologists Board replied as follows:—

1. The Board's Circular No. 1967/2, dated 10 March 1967, over my signature, unfortunately included in the duplicated copies a typographical error. I refer to the second condition, page 2 of the circular. This should read: "The Board will issue *a* certificate to qualified candidates who obtain a pass in such an 'A' level subject."

   The Board decided at the last meeting to recommend the Director-General of Health to issue to all such successful candidates a "Certificate of Attainment" in the appropriate subject at 'A' level standard.

   The mistake occurring in this circular was corrected by a circular letter despatched to all training laboratories on April 14, 1967.

2. The Board is definitely of the opinion that the sitting and passing of an 'A' level examination by a Technologist already qualified should not automatically equate to an extra qualification, with entitlement to a higher salary. The Board realises that possession of a "Certificate of Attainment" in a particular 'A' level subject *may* influence the Grading Committee in an applicant's favour when considering future grading but the Grading Committee and the Board are entirely unrelated bodies and the question of grading is outside the Board's terms of reference.

   The Board, however, has in mind the introduction of a post-graduate certificate in all of the four disciplines at present offered for the Certificate of Proficiency examination. Such an examination would be open to all qualified technologists who have reached a satisfactory post-graduate theoretical and practical standard in the discipline of their choice.

3. The proviso that "no official notification of acceptance to 'A' level examinations would be issued until final figures for trainee candidates are available" was incorporated into the circular to impress upon qualified technologists who are contemplating sitting 'A' level examinations, that C.O.P. trainees must, of necessity, be allotted a place in the examinations.

   The Board regrets that extra examination places (if required), exceeding the limit of the facilities for practical and oral examinations available to the Board at the present time, cannot be allotted.

   It can however be assured that the Board will endeavour to accept all applications from trainee candidates and qualified technologists to the limit of facilities and examiners available for the examinations.

   I can assure you that the Board is anxious to encourage a continuance of study and attainment of higher standards by all laboratory staff by such measures as are found practically possible.

RON. PILGRIM.
Secretary to the Medical Laboratory Technologists Board.

VOTING WITHIN THE INSTITUTE

The more one takes the trouble to look into this matter and the more people one speaks to about it, the more I realise that this is not an easy problem to overcome. It is our intended aim to try and regularise a situation that does exist but I must admit that there are many hypothetical possibilities which could well bear investigation at the same time in order that a more lasting solution may be formulated. Let us not, then, be hasty.

For instance, if we say now that the right to vote within the Institute will only be accorded to those with the Intermediate qualification or better —this now takes three years during which time the individual has the time to assimilate the information necessary to vote with knowledgeable sincerity —how will we relate this to the person who completes the Intermediate training under any new scheme which may evolve? During such a full-time course, at local or national level, the candidate would have virtually no knowledge of, or contact with, branch or Institute affairs but, because they had attained the equivalent of the present Intermediate—possibly in only two years— they would be allowed to vote. Perhaps we should say that the vote will only be extended to those who have been members of the Institute for a minimum of three years and that this includes graduates who wish to join? This then would allow everyone, regardless of their qualifications, to enjoy the right to vote after three years' continuous financial membership, during which time they should have had ample time to become aware of the aims and ideals of the Institute and therefore to vote intelligently.

In the event of the voting rights being restricted, we must be very careful when it comes to setting the annual subscription payable by non-voting members. Regardless of what we may say or think, the majority of younger members weigh their subscription against what they get out of it. Therefore, to make the non-voting subscription greater than the annual subscription to the *Journal*—now set at 10/- or $1 per annum—would be to court the loss of a large proportion of our younger members.

Another danger which could appear as a consequence of setting the non-voting subscription too high, would be a decline in branch membership, because branch membership, now, is dependent entirely on financial membership of the parent body. However, if students were encouraged to join the Institute at a reasonable fee, they could have some say in Institute voting by influencing the feeling of the branch through branch meetings. Voting members of the branch going to the Annual General Meeting would then feel obligated to take into serious consideration the arguments of these younger members—and a good thing this could prove to be. We tend to assume these younger people suffer from apathy in Institute affairs, but there are some who are far more alive and aware than many of their seniors, and much of the apparent apathy is due to the failure of senior members to take the time and trouble to acquaint the younger folk with the affairs, aims and objects of the Institute.

A more complex problem to consider, which ever way we may decide to restrict the voting, is that of the smaller country laboratories who possess a charge technologist and who are only permitted to train up to the Intermediate level. They could rarely hope to have more than one voting member. However, if we make the criterion three years' membership, then these people could be considered for voting membership in the event of their failing to pass the examination. This would encourage students to join the Institute from the start of their training and not wait until they have gained a pass at the Intermediate level.

To summarise: I consider that the criterion of three years' continuous financial membership has a lot more to offer by way of intelligent voting within the Institute than setting the voting rights by qualification alone. This continuous membership for a non-voting member should be at the

rate of $1 or 10/-, per year with an automatic increase to $6 once the right to vote has been attained, irrespective of the qualifications of the member at that time. Qualifications do not necessarily imply an intelligent interest in Institute affairs, nor does the lack of qualifications imply a lack of intelligent interest. Therefore, by setting a time criterion as specified, I consider more sincere and knowledgable voting will result.

I. C. KING.

[A thing that deserves to be challenged is this correspondent's assertion regarding the return that non-voting members will get for their annual subscription. It must be remembered that besides their subscription to the *Journal*, all members, regardless of their voting status, enjoy various other privileges of membership, including the right to participate in branch activities and the security of knowing that their interests are being protected by the Council and by the Institute's representatives on the Medical Laboratory Technologists Board and the Combined Committee of Hospital Employee Organisations. If the *Journal* subscription is calculated as being worth $1, surely these other privileges must be worth at least another $1. —Ed.]

COMPARISON OF QUALIFICATIONS

Sir,

As a New Zealand qualified technologist who has been residing in the United Kingdom for the past 18 months, it is stimulating to read in the *Journal* of the general desire for higher academic standards. Although English-born, I greatly value my New Zealand qualification, a training which has given me at least a comparable standing with English technicians at the several hospital laboratories I have worked at during my stay in this country.

In a highly industrialised state such as Britain, the choice of careers open to school-leavers is considerable, and the profession here may well be suffering because of failure to attract the right calibre of trainee. New Zealand readers may not be aware, for example, that a student in this country only requires "O" levels (equivalent to New Zealand School Certificate) before commencing training, and that few who possess "A" levels (equivalent to New Zealand University Entrance) bother to take up medical laboratory technology as a career.

From a perusal of past I.M.L.T. examination papers the questions indeed, appear comparable, but one can hardly describe them as being of a higher standard than those set in New Zealand. A trainee over here may sit his final examination after one year and if successful qualifies in one subject, whereas his counterpart in New Zealand (at least up until 1966) could qualify in three subjects.

It was surprising therefore to read of two motions carried at the last New Zealand Annual Meeting, one concerned with bringing the New Zealand qualifying system into line with the I.M.L.T. system, the other with negotiating for reciprocity with other overseas Institutes (presumably including the I.M.L.T.).

If the present healthy desire to raise the academic standard is fully realised, surely the day cannot be far off when it will be the turn of "other Institutes of medical laboratory technology overseas" to clamour for New Zealand recognition!

One wonders if the terms "English-trained" or "I.M.L.T.-qualified" are not fast becoming mere status symbols, because speaking from this hemisphere, I'm afraid the ol' grey mare ain't quite what she used to be.

M. R. FORD,
60 Bath Road,
Bedford Park,
London, W.4.

VALUE FOR YOUR MONEY

Sir,

It is a matter of real regret that, of the successful candidates at the last Intermediate examination, less than half are currently financial members of the Institute.

From a value-for-money point of view alone Institute membership at £1 1s must be one of today's best buys.

The *Journal* alone is undervalued at ten shillings and, for Hospital Board and Government employees, the facilities of the Public Service Investment Society is worth many times that amount. Further advantages of belonging to a professional society should be self-evident.

The duty of all Institute members, not only Charge Technologists, must be to approach all their laboratory colleagues, and in the event of their being non-members, point out clearly and precisely where their obligation and advantage lies, in membership.

May I appeal to all your reader members to act, having asked themselves, "Am I currently financial; Is my bench workmate a member?"

If everybody does this, the Institute can continue to progress in the best interests of its members, present and future, and you will have played a positive part in that progress.

JOHN MORGAN,
Honorary Secretary, N.Z.I.M.L.T. (Inc.)

Royal Perth Hospital
Western Australia.

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ADMINISTRATOR.

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2. Hicks, N. D., and Pitney, W. R.: **Brit. J. Haem.** 3:277, 1957.
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