MARCH, 1974

THE NEW ZEALAND JOURNAL OF

medical laboratory technology

An Official Publication of the New Zealand Institute of Medical Laboratory Technology Incorporated

VOLUME 28, No. 1

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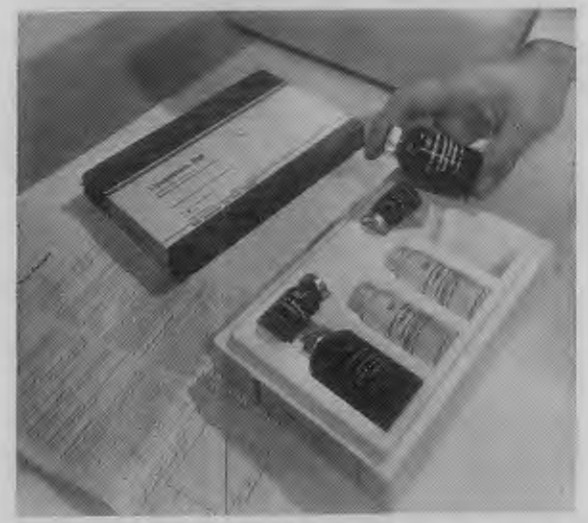

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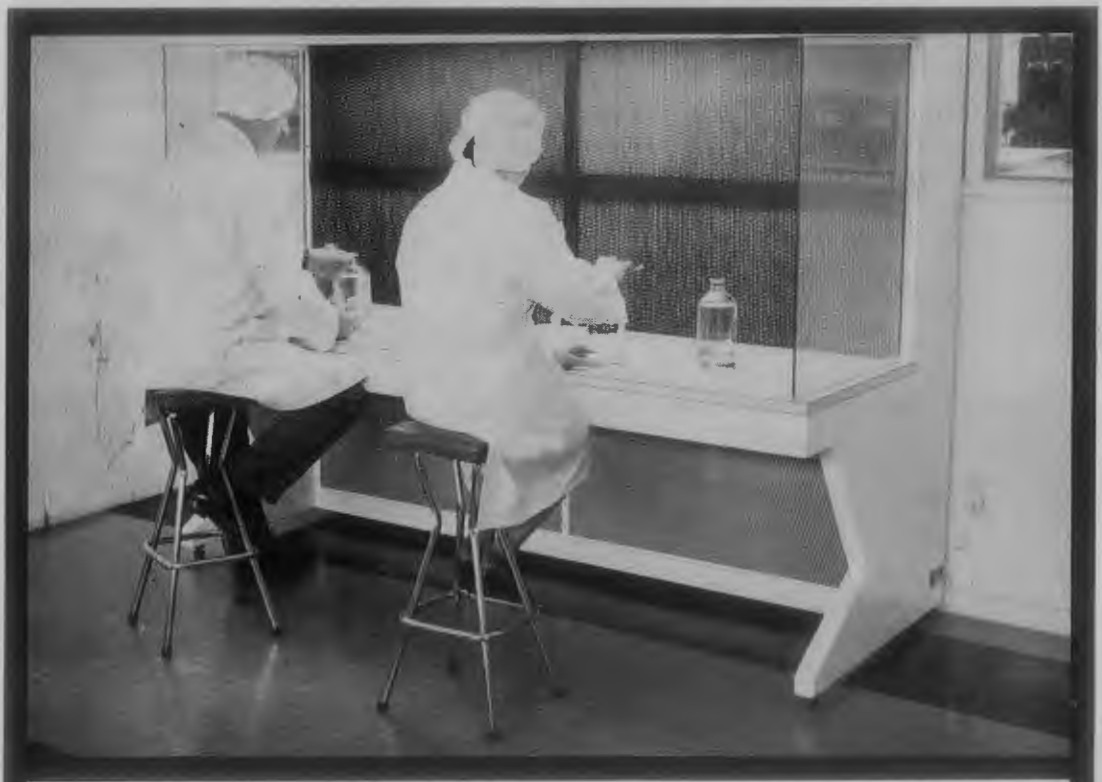

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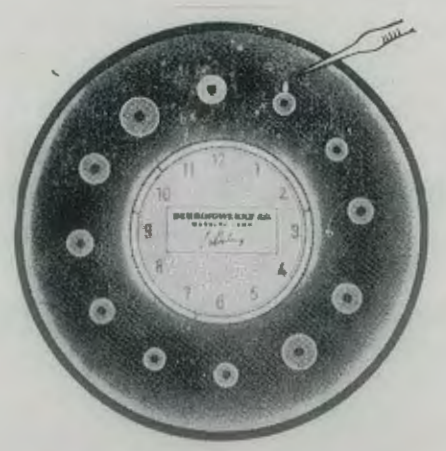

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The New Zealand Journal of Medical Laboratory Technology

Volume 28, No. 1 March, 1974

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Subscription to the JOURNAL for non-members is THREE DOLLARS per year or ONE DOLLAR FIFTY CENTS per single issue, postage paid. Overseas subscription rates on application.

Intending contributors should submit their material to the Editor, Diagnostic Laboratories, Dunedin Hospital. Acceptance is at the discretion of the Editor, and no undertaking is given that any article will be published in a particular issue. The copy deadline for each issue is the first of the month prior to the month of publication.

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A Microdilution Technique for Minimum Inhibitory Concentrations

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Received for Publication, November 1973

Summary

The results of measuring minimum inhibitory concentrations (MIC) by the tube method were compared with those obtained by using a micro method. An agreement of 95% was achieved with ±1 twofold dilution, as the criterion of satisfactory comparison. Its high reproducibility, convenience and speed make the microdilution technique worthy of consideration for routine use in diagnostic laboratories.

Introduction

It is accepted that tube MIC's are of more value than disc sensitivity tests in the management of infections with antibiotics. Because tube MIC's are time-consuming and cumbersome, requiring large numbers of test tubes and pipettes, disc sensitivity testing has become the method of choice. However, tube MIC's are still used for reference work and in cases where greater accuracy is required in relation to antibiotic dosage against a specific microorganism.

The "Microtiter" apparatus (produced by the Cooke Engineering Co.) which is used routinely in many serological laboratories can be used quickly and easily to perform serial twofold dilutions of antibiotics suitable for MIC's in approximately one-twentieth of the time required for tube dilutions.

Materials

The "Microtiter" appartus consists of disposable, transparent, rigid, styrene U plates; polypropylene and stainless steel pipette droppers and stainless steel microdiluters. Although the U plates are disposable they may be reused if cleaned by placing in a solution of 5% formaldehyde for four hours, rinsing thoroughly in running water, soaking in pyroneg or similar detergent for four hours, brushing and rinsing thoroughly, rinsing with ethyl alcohol, inverting and draining overnight and then placing under an ultra-violet light before use [1 and 2].

All solutions and dilutions used in this study were made with nutrient broth. The penicillin standard was prepared each day. The gentamicin standard was prepared every two weeks at a concentration of 1000 micrograms per millilitre (μg/ml), divided into aliquots, sealed in individual vials and frozen at —10°C until used.

Methods

The tube dilution method was carried out as described by Stokes[6].

For the micro method a sterile "Microtiter" plate consisting of eight rows of 12 holes was used. Fifty microlitres (μl) of sterile nutrient broth were dropped into each well and dilutions were made by dipping a sterile 50μl diluter in the stock antibiotic solution, then placing it vertically in the first well of the titration series and rotating backwards and forwards six times between the thumb and forefinger to mix the antibiotic and nutrient broth, transferring to the second well, mixing and continuing along the row. Each row was similarly treated.

An inoculum of 50μl of a suitable dilution of a nutrient broth culture was then added to each well and the plates were then sealed by covering with a sterile "Microtiter" plate and incubated for 16-20 hours at 37°C.

For the plate method growth was indicated either by a generalised turbidity of the medium or a button 0.5mm in diameter or larger in the bottom of each well[6] and the MIC was read as that dilution in which there was inhibition of growth. With experience no difficulty was found in deciding the end point but triphenyl tetrazolium chloride (TTC) can be used to improve visibility as described by Goss *et al*[4].

Using the materials and techniques described three investigations were made.

1. The first investigation was to check the accuracy of the microdiluters and the micropipettes as described in the "Microtiter" Instruction Manual[2].

2. The second investigation was to find an inoculum that would give MIC values comparable with those obtained by the Stokes method. Three inocula were used in the investigation; A, an inoculum consisting of a 1/1000 dilution of 6-10 colonies from an overnight primary plate culture suspended in 2ml of nutrient broth[5]; B, an inoculum prepared by inoculat-

ing 5ml of nutrient broth with five or six well separated colonies from an overnight plate culture, incubating seven hours at 37°C and then diluting one drop of culture to 5ml of nutrient broth; and C, an inoculum prepared as inoculum B but diluted one part of seven-hour culture to 1000 parts of nutrient broth. Inoculum C was prepared according to the criterion laid down by Ericsson and Sherris[3]—a suitable dilution from a broth culture in the logarithmic or early stationary phase of growth.

3. The third investigation was to confirm the final choice of inoculum by a series of MIC's. The micro-organisms tested were six *Escherichia coli* and six *Staphylococcus aureus* strains isolated in this laboratory. MIC's for each micro-organism were estimated twice by the tube method and 16 times by the microdilution method.

Results

1. Microdiluters designed to hold 50μl ± 1.0μl were found to pick up volumes differing from this value (Table I). The mean capacity of microdiluters was 47.8μl with a standard deviation of 0.80μl. Micropipettes designed to deliver 50μl ± 1.0μl were found to deliver volumes differing from the value (Table II). The maen volume of the micropipettes was 47.20μl with a standard deviation of 0.83μl.

For the purpose of making doubling dilutions these volumes were satisfactory. They differed by more than ± 1μl from 50μl but their variation from each other was negligible.

2. The inoculum was found to be the most critical factor.

When inoculum A was used microdilution MIC's were found to have an agreement of 24% with tube MIC's taking ±1 dilution as the criterion of comparison. Because the microdilution MIC values were lower than the tube MIC values it was concluded that inoculum A was either too light or not sufficiently viable.

Similarly inoculum B gave an agreement of 40% with tube MIC's taking ±1 dilution as the criterion of comparison. Because the microdilution MIC values were much higher than the tube MIC values it was concluded that inoculum B was too heavy.

Inoculum C gave a correlation of 92%. Results of a more extensive investigation to confirm the suitability of this inoculum are described in the next section.

3. The MIC results are shown in Table III.

Table I

Volume of Physiological Saline Picked up by a 50 μl Microdiluter

Pipette	Vol. in μl	
1	47.88	
	47.35	
	47.42	
2	47.45	
	47.79	Mean ± 2 SD 47.78 ± 1.60
	47.91	
3	47.44	
	46.97	
	47.81	

Table II

Volume of Drops of Physiological Saline From the 50 μl Micropipette

Pipette	Vol. in μl	
1	47.22	
	46.80	
	47.89	
2	48.77	
	46.64	
	46.49	
3	46.20	Mean ± 2 SD 47.20 ± 1.66
	47.32	
	46.27	
4	46.95	
	48.07	
	47.79	

When a microdilution MIC within ±1 dilution of a tube MIC was taken as the criterion of reproducibility the overall 95% correlation obtained here was most satisfactory.

Discussion

A comparison of the standard tube dilution method for MIC's with the micro-technique using Cooke's "Microtiter" appartus was undertaken because the microdilution method appeared to have the advantages of requiring less time, space and apparatus and was claimed to be as reliable as the tube method. The method made savings on time. Manually diluting one row at a time, "Microtiter" dilutions took approximately 1/20 the time of tube dilutions. The "Microtiter" apparatus requires less storage, incubator and bench space

Table III
Microdilution MIC Compared with Tube MIC

E. coli	1. gentamicin	0.75	0	0	6.25	43.75	50.0	0	0
	2. (μg/ml)	1.5	0	0	6.25	50.0	37.5	6.25	0
	3.	1.5	0	0	12.5	50.0	25.0	12.5	0
	4.	0.56	0	6.25	6.25	18.75	62.5	6.25	0
	5.	2.3	0	0	12.5	62.5	25.0	0	0
	6.	0.75	0	12.5	37.5	31.25	18.75	0	0
S. aureus	1. penicillin	0.04	0	0	25.0	50.0	25.0	0	0
	2. (units/ml)	0.04	0	0	6.25	31.25	62.5	0	0
	3.	0.09	0	0	6.25	12.50	81.25	0	0
	4.	31.0	0	0	6.25	87.5	6.25	0	0
	5.	0.18	0	0	6.25	18.75	56.25	18.75	0
	6.	125.0	0	0	0	93.75	6.25	0	0
	Mean for 192 replicates		0	1.56	10.94	45.83	38.02	3.65	0
							94.79		

Agreement of Microdilution MIC with the tub e MIC taking $\pm$ one twofold dilution interval as the criterion of comparison = 95%.

and the flaming of autodiluters was a saving on sterile glass pipettes. However, sterile glass pipettes and time were still needed for making up initial working solutions.

For a laboratory that does or was intending to do many MIC's an investigation of the automatic pipetting and diluting devices available with the "Microtiter" apparatus would be well worth while as the time saved would be considerable.

There were two disadvantages with the microtechnique, one, that it was limited to doubling dilutions and the other, that it was dependent on the skill and reliability of the operator. In the tube dilution method carry-over and accuracy of measurement were easier to estimate than with the "Microtiter" apparatus where drop size could be varied by an unsteady hand and the autodiluters lost fluid if accidentally touched against the side of a well. Chitwood[1] found a change in correlation from 75% to 94% after the micro-technique had become firmly established.

In conclusion, once experience has been gained in diluting and reading end points the "Microtiter" MIC appears to be a possible substitute for tube MIC's with the advantage of speed but the disadvantage of being restricted to doubling dilutions.

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Evaluation of 'Precipitest', A Screening Test for Hyperlipaemia

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Received for Publication, August 1973

Introduction

As testing for hyperlipaemia becomes more significant in light of the relation between serum lipids and coronary artery disease and relatedatherosclerotic diseases, methods for mass population screening are constantly being proposed. One of these, a microcapillary method of precipitating serum lipoproteins with a density of less than 1.063, was compared with serum cholesterol, triglycerides and lipoprotein analysis, to evaluate its usefulness as a screening method for hyperlipaemia.

Materials and Methods

The screening test is available as a kit set containing a precipitating reagent, capillary tubes, sealing clay and a measuring scale. The precipitant contains 0.3% sodium amylosulphate (a sodium salt of sulphated potato amylopectin) in 0.85 m magnesium sulphate. The capillary tubes are 75 mm in length with 1.0 mm inside diameter and precalibrated at 70 mm. The measuring scale has calibration lines 2.5 mm apart.

Serum cholesterol was estimated by the direct Liebermann-Burchard reaction with a correction made for icteric sera. Serum triglycerides were estimated using a Dade kit set (Dole extraction and Hantzch condensation reaction). Lipoprotein electrophoresis was performed on cellogel 200 (17 by 3 cm) in a Shandon universal tank containing High Resolution buffer (Helena), pH 8.6 and ironic strength 0.05. A mixture of Oil Red Om (Helena) and 1 m NaOH in a ratio of 5 : 1 was used for staining the strips.

Blood was collected from patients who presented themselves to our laboratory for Fredrickson's classification after 12 to 16 hours fasting; and all tests were done on the same day of collection.

Serum, 0.2 ml, was mixed with 0.1 ml of the precipitating reagent and mixed well on a "Vortex" mixer. The mixture was then drawn up into the capillary tube to a precalibrated height of 70 mm, sealed with clay and centrifuged for 10 min in a microhematocrit centrifuge. The capillary tube was then inspected for the presence of precipitate and interpreted by the quantity and centrifugal behaviour of the precipitate (Table I).

Results

Usually the precipitate sediments to the bottom or floats to the top of the tube. Generally if the ratio of triglycerides to cholesterol is greater than one, a floating precipitate forms, otherwise a sedimented precipitate forms. Occasionally the precipitate is dispersed, and mixed forms can occur. A sedimented precipitate of greater than 2.5 mm or a floating precipitate is deemed to be indicative of hyperlipaemia, and quantitative assays for serum cholesterol and triglycerides are indicated. Table II shows serum cholesterol and triglycerides levels with their probable pheno-type (from lipoprotein electrophoresis) of specimens which showed a sedimented precipitate of 2.5 mm or greater. Likewise Table III for floating to the described method, and after centriprecipitates.

Two sera (A and B) were treated accordingly to the described method, and after centrifugation the precipitate free supernatants were analysed for cholesterol and triglyceride levels (Table V), and subjected to lipoprotein electrophoresis.

Discussion

Searcy **et al** (1972), demonstrated the specificity of the relationship between sodium amylosulphate and lipoproteins with a density of less than 1.063 (beta—and prebeta—lipoproteins) and showed that the lipids remaining in the precipitate free supernatant was most likely that of alpha-lipoproteins. In a later paper they compared the Precipitest results with pheno-

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Table I

Precipitate Characteristics	Interpretation
Precipitate Sinks	Precipitate column non-existent or shorter than 2.5mm: normal cholesterol and triglycerides. Precipitate column longer than 2.5mm: cholesterol greater than 300mg/100ml and normal or moderately elevated triglycerides.*
Precipitate Floats	Precipitate column located at top of liquid column: triglycerides greater than 250mg/100ml.*
Precipitate Dispersed	Precipitate remains suspended throughout capillary: triglycerdies and cholesterol levels are similar and either or both are elevated.*

*Quantitative assays for serum cholesterol and triglycerides are indicated.

Table III

Cholesterol	Triglycerides	Probable Type	Additional Findings
194	368	1V	—
226	248	1V	Dispersed ppt.
264	509	1V	—
372	434	1V	—
428	722	11 (B)	—
270	164	Mixed	Dispersed ppt.
320	250	11 (B)	—
337	536	1V	Dispersed ppt.
334	770	1V	—
248	255	Mixed	—
297	331	1V	Dispersed ppt.
268	265	1V	Dispersed ppt.

Specimens showing a floating percipitate.

Table IV

Cholesterol	Triglycerides	Probable Type	Additional Findings
276	214	11 (B)	—
230	192	1V	—
284	288	1V	—
275	310	1V	—
348	406	11 (B)	—
248	207	1V	—
242	164	Non-fasting	—
254	260	1V	—
249	331	1V	—
311	300	11 (B)	—

Specimens showing a dispersed precipitate only.

Table V

	Before Precipitation / After Precipitation / % Reduction			
Specimen A	Triglycerides:	122mg	23mg	81%
	Cholesterol:	310mg	35mg	89%
Specimen B	Cholesterol:	212mg	18mg	91%
	Triglycerides:	536mg	12mg	98%

Changes in lipids after treatment with precipitating solution.

Table II

Cholesterol	Triglycerides	Probable Type	Additional Findings
239	180	1V	—
291	136	11 (A)	—
281	275	1V	—
315	198	11 (B)	—
284	97	11 (A)	—
265	102	Normal	—
320	247	11 (B)	Dispersed ppt.
305	111	11 (A)	—
314	139	11 (A)	—
242	163	Mixed	—
251	215	1V	—
278	207	1V	—
362	160	11 (A)	—
301	202	11 (B)	—
362	310	11 (B)	Dispersed ppt.
344	287	11 (B)	—
358	97	11 (A)	—
321	120	11 (A)	—
282	122	11 (A)	—
414	68	11 (A)	—
287	161	11 (A)	—
227	225	1V	—
268	163	Mixed	—
324	102	11 (A)	—
317	55	11 (A)	—
282	98	11 (A)	—
295	208	11 (B)	—
315	53	11 (A)	—
316	73	11 (A)	—
291	179	11 (B)	—
303	129	11 (A)	—
272	197	1V	—
337	113	11 (A)	—
283	153	11 (A)	—
314	176	11 (B)	—
325	230	11 (B)	—
291	200	11 (B)	—
245	220	1V	Dispersed ppt.
275	240	1V	—
274	216	1V	—
263	162	Mixed	—
264	254	1V	—
308	152	11 (A)	—
327	236	11 (B)	—
295	243	1V	—
305	145	11 (A)	—
250	232	1V	—
294	181	11 (B)	—
211	214	1V	—
278	117	Mixed	—
300	295	1V	Dispersed ppt.
350	114	11 (A)	—
240	256	1V	—
317	138	11 (A)	—
300	330	1V	Floating ppt.
295	63	11 (A)	—
267	103	Mixed	—
287	91	11 (A)	—
260	105	Mixed	—
252	97	Normal	—
291	108	11 (A)	—

Specimens showing a precipitate of 2.5mm or greater.

types and concluded that it is possible to establish the phenotype with a reasonable degree of certainty on the basis of precipitest results and the appearance of serum after overnight refrigeration. They concluded that all type II patients yielded sedimented precipitates of 2.5 mm or more, while types I IV and V showed floating precipitates. From our results we concude that this is so in the majority of cases, but not always true. In the 13 sera showing a floating precipitate, only eight turned out to be type IV, the rest being type II (B), no type II (A). Of 62 sera showing a sedimented precipitate of 2.5 mm or more, 38 turned out to be type II. Out of these 62 sera, 12 had a precipitate of exactly 2.5 mm and four of these showed normal lipoprotein patterns.

An abnormal precipitest result was usually accompanied by an abnormal serum lipid result. Although the height of the precipitate was not proportional to serum cholesterol or triglyceride levels, a sedimented precipitate of greater than 2.5 mm usually showed serum cholesterol values of 260 mg/100 ml or more, while floating precipitates usually showed serum triglyceride values of 250 mg/100 ml or more. A few cases of sedimented precipitates of greater than 2.5 mm showed lower cholesterol levels but had corresponding abnormal triglyceride levels. Dispersed precipitates generally showed similar cholesterol and triglyceride level, and one or both were abnormal. Searcy recommends repeating the test if the precipitate is dispersed, but this was not done in our laboratory as all showed abnormal lipid levels and if used as a screening test, full lipid studies are indicated.

In one instance a normal precipitest result was accompanied by a serum cholesterol of 187 mg/100 ml and trigycerides of 233 mg/ 100 ml. Pre-beta lipoproteins were increased ad beta lipoproteins were slightly decreased. As the precipitate is composed of both fractions, the decrease in the beta fraction compensated for the increase in the prebeta fraction, thus producing a normal precipitest result. This could be a problem occasionally as a floating precipitate usually does not occur until the serum triglycerides are greater than 250 mg/ 100ml. In another instance a normal precipitest result was accompanied by a serum cholesterol of 292 mg/ 100 ml. Lipoprotein electrophoresis showed an increase in alpha lipoproteins, while the beta fraction was normal. This is often observed in women taking oral contraceptives.

Table V shows the results of two hyperlipaemic sera before and after treatment with precipitating solution, and shows that most of the cholesterol and triglycerides are removed by this treatment. Only alpha lipoproteins could be demonstrated by electrophoresis in the precipitate free supernatant.

Five sera showing a sedimented precipitate of exactly 2.5 mm were repeated after some time as identical results might change to normal and thus abnormal results could be missed. The test showed good reproducibility in this aspect.

Three samples, one normal, one with a floating precipitate and one with a sedimented precipitate of 2.5 mm were reassayed after three days at room temperature. No significant difference in centrifugal behaviour was observed.

Searcy never encountered the presence of both a floating and sedimented precipitate in fresh serum, but experienced this with frozen and lyophilised sera. One of our sera, which was fresh, showed this characteristic and had a serum cholesterol of 300 mg/100 ml and tryglycerides of 330 mg/100 ml. The test was repeated two more times and identical results were obtained.

Conclusion

Although we found some differences in results and interpretation, all but one abnormal precipitest result had a lidid abnormality. No false negatives were found. Precipitest is a rapid and precise screening test for hyperlipaemia and if abnormal results are obtained, full serum lipid analysis is indicated. The test should be well suited for mass population screening which is popular overseas, but with some exceptions, seems not to have caught on yet in New Zealand. Many cases of hyperlipaemia remain undetected until a chance abnormal laboratory result occurs, or clinical symptoms become apparent. Although it has not been conclusively shown that reduction in serum lipids will prevent coronary artery disease and related disorders, many workers agree that this probably will be the case and other benefits, such as weight reduction, have been beneficial to the patient. Lastly, the classification of the hyperlipoproteinaemias using precipitest results and observance of the serum after overnight refrigeration, does not seem to

be satisfactory according to our results. Full lipid studies must be performed on all abnormal precipitest results.

Table VI
Normal Ranges Employed

Age	*Chloesterol*	*Triglycerides*
Up to 19 years	150 - 230	40 - 140
20 - 29	150 - 240	40 - 150
30 - 39	160 - 250	40 - 160
40 - 49	160 - 260	50 - 170
Over 50	170 - 270	50 - 180

N.B.: All normal precipitest results, except one, showed normal cholesterol and triglyceride levels according to this criteria.

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Recurrent Infections and Immunoglobulin Deficiencies in Childhood

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A paper read at the 29th Annual Conference of the NZIMLT, Christchurch, August 1973

Introduction

Since Bruton in 1952 first described a clinical condition of severe infections in a patient who lacked gamma globulin there have been many reported findings of specific immunoglobulin deficiencies. These can be briefly summarised as follows:

1. Hypogammaglobulinaemia from defective synthesis of one or all five major classes of immunoglobulin. Included in this category are Swiss-type agammaglobulinaemia, sex linked agammaglobulinaemia, sporadic congenital agammaglobulinaemia, hypogammaglobulinaemia, either idiopathic or secondary to neoplasms, dysgammaglobulinaemia and Ataxia telangiectasia.
2. Excessive catabolism of one or more classes of immunoglobulin. This category includes familial idiopathic hypercatabolic hypoproteinaemia, myotonic dystrophy and abnormal immunoglobulin interactions.
3. Excessive loss of immunoglobulins as a result of nephrotic syndrome and protein losing gastroenteropathy.
4. A transient hypogammaglobulinaemia in infancy where children aged between three months and three years have repeated infections with low immunoglobulin levels which do not respond following antigenic stimulus. This is classified as a form of humoral deficiency.
5. Secondary immunological deficiencies caused by viral infection, particularly rubella and cytomegalic inclusion virus. When the causative agent (i.e.. virus) is removed the immune function returns to normal.

Immunoglobulin abnormalities have now been classified under the broad headings of humoral immune deficiencies and cell mediated immune deficiencies. The salient features of the humoral immune deficiency are as follows: Recurrent severe bacteria infections. hypoplasia of the lymph nodes and tonsils, low or absent immunoglobulins, absent or low isohaemagglutinins, poor ability to respond to antigenic stimulation and an absence of plasma cells in the bone marrow. Cell mediated immune deficiency shows severe viral infections, severe fungal infections, infection with a typical acid fast bacteria (generalised BCG reaction), lymph nodes and tonsils underdeveloped, depletion of the lymphoid elements in the deep cortical areas of lymph nodes and other thymic dependent regions, lymphopenia and negative skin tests to candida, 2-4 dinitro-chloro benzene (D.N.C.B.) and other antigens.

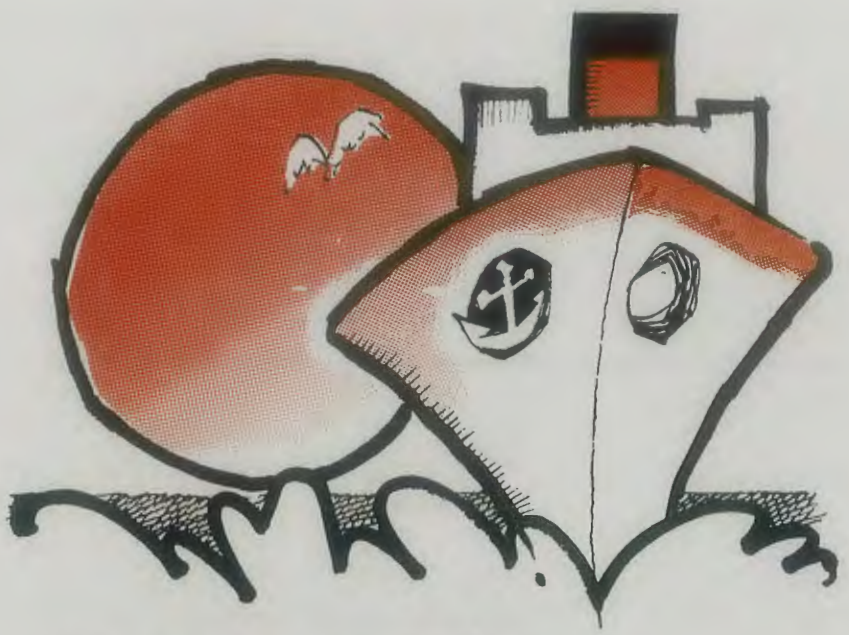

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TABLE I

Age	No. of Individuals	IgG mg/100ml	IgA mg/100ml	IgM mg/100ml
Newborn	20	800 (600-1000)	<5 (0-<5)	13 (6-20)
1-3 months	10	450 (300- 600)	35 (20-50)	30 (10-50)
4-9 months	20	425 (250- 600)	43 (25-60)	43 (25-60)
10-24 months	20	700 (400-1000)	90 (30-150)	65 (30-100)
2 years	18	775 (450-1100)	120 (40-200)	65 (30-100)
3 years	15	900 (500-1300)	120 (40-200)	70 (30-110)
4 years	10	950 (600-1300)	150 (50-250)	70 (30-110)
5 years	15	1000 (600-1400)	150 (50-250)	75 (30-120)
6-10 years	20	1050 (600-1500)	280 (60-500)	75 (30-120)
Over 10 years	30	1100 (600-1600)	290 (80-500)	90 (30-150)

NORMAL RANGE FOR SERUM IMMUNOGLOBULINS

It might be advisable at this stage to look briefly at the basic function of the immunological apparatus.

Experiments have made it clear that the thymus plays a key role in the establishment and functioning of the immunological system. The proliferation of lymphocytes is primarily influenced by the thymus and stimulation of committed lymphocytes in the lymphoid tissue only occurs when there is an antigenic challenge. It has been shown that the lymphocyte within the lymph node, when activated by an antigen, is transferred into an intermediate cell producing IgM and then into a plasma cell producing IgG. The amount of IgM produced is small but is liberated at the site of antigenic stimulus and is more avid and type specific than the more classical IgG antibodies in the serum. IgA, IgD and IgE are also produced by the plasma cells. IgA is of special interest as it is the main immunoglobulin of human colostrum; it is secreted in the saliva, in the lachrymal glands and the mucus glands of the intestines.

Normal values for immunoglobulins have been quoted in many papers but I would suggest that it is very worth while compiling your own list of normals. The results obtained should be fairly close to the ranges obtained elsewhere but some environment factors may have an influence on the final result. The levels obtained at Geelong compare favourably with other published values and in general adult levels are reached at 10 years (Table I.)

Investigation

It has been noted over the past three years that there are many children presenting with recurrent infections of many types and showing only mild or no immunoglobulin response. The ages varied from a few weeks to teenage and the clinical history and immunoglobulin levels did nothing to help classify these children into any of the known immunological deficiency diseases. In most instances it was not possible to undertake more definitive immunological function tests but in view of the clinical history supplied and immunoglobulin levels obtained some indication of immune function could be determined. Follow up medical treatment was also helpful in assessing a patient's susceptibility and immune response to infection. In each case the following information was obtained.

1. Type of infection:
   (a) Proven or presumed bacterial;
   (b) Proven or presumed viral;
   (c) Fungal;
   (d) Other.
2. Source of infection:
   (a) Upper respiratory tract infection (throat and tonsils);
   (b) Lower respiratory tract infection (bronchitis and pneumonia);
   (c) Eye;
   (d) Ear;
   (e) Nasal;
   (f) Urinary tract;
   (g) Gastrointestinal.
3. Recurrence of infection.
4. Immunoglobulin levels.

It was then possible to clarify this information into several distinct categories. These are as follows;

1. Abnormalities of one or more immunoglobulins.
2. Failure to activate primary or secondary immunoglobulin response even though immunglobulin levels appear normal.
3. Increased susceptibility to infection even though primary and secondary immunoglobulin response is within normal limits.

During the course of a pilot study done at Geelong Hospital a total of 51 patients had immunoglobulin levels measured following recurrent infections: 25 had been hospitalised at some stage of their illness and the other 28 were managed privately as outpatients by two paediatricians. The ages of the patients ranged from six weeks to 10 years and the results obtained were placed in the following broad classifications:

(a) Primary response to infection.
(b) Secondary response to infection.
(c) No response to infection.
  (i) Normal immunoglobulin levels.
  (ii) Immunoglobulin abnormalities.

(a) Primary response to infection:

Primary immune response to infection is mainly IgM dependent and estimations of this immunoglobulin give an indication of a satisfactory primary immune response to antigenic stimulus. Of the 51 patients presented in this study 18 showed a satisfactory response.

(b) Secondary Response to infection:

Secondary immune response to infection is mainly IgG dependent and estimations of this immunoglobulin gives an indication of a satisfactory secondary immune response to antigenic stimulus. Of the 51 patients presented in this study 31 showed a satisfactory secondary response.

(c) No response to infection:

(i) In this category we must consider the patient who has normal immunoglobulin levels but apparently no immunological response to infection. Of the 51 presented in this study 13 were placed in the classification.

(ii) Immunoglobin abnormalities of either one or all three immunoglobulins occurred in 10 patients of the total 51 patients studied.

Results and discussion

From the figures obtained it was shown that an adequate IgM response to antigenic stimulus was in the range of 150% to 250% of the mean IgM value for age and where it was possible to obtain serial levels, the IgM value showed an increase of between 50% to 100% for that individual.

Similarly an adequate IgG response was shown to be in the range of 50% to 120% of the mean IgG value for age and where serial levels were available the IgG value showed an increase of between 100% and 150% for that individual.

From these observations it is clearly indicated that the most successful way of determining immunoglobulin response to antigenic stimulus is to do at least two immunoglobulin estimations. The first at 2-7 days and the second at 14-21 days following that stimulus. With such a wide individual range of normal levels, it is far better to assess each individual's immunoglobulin response as a percentage increase for serial values rather than a percentage increase of a fairly wide normal range.

The most common immunoglobulin abnormality noted was an IgA deficiency (Table II). Eight of the ten children had a mild to moderate IgA deficiency usually associated with recurrent respiratory tract infections. One patient had an IgG deficiency associated with recurrent tonsillitis and one patient had an IgM deficiency associated with recurrent bronchitis.

From experience I have noted that an immunoglobulin level between plus or minus 10 percent of the lower limit of normal should be considered as indicative of a deficiency and further assessments and other immune function tests carried out.

Whilst it is too early to follow up the immunoglobulin development of the children in this survey it would appear that while these deficiencies should be classed in the category of humoral immune deficiencies they do not necessarily conform to all the criteria set down for this group. I tend to think of these deficiencies as a primary impairment of immunoglobulin synthesis which appears to improve as the patient grows older.

From a very limited trial, patients with IgA deficiencies have responded well to treatment with IgA concentrate and similarly IgG and IgM deficiencies show a good response to gamma globulin concentrate.

In conclusion, I would like to present a fairly representative case history for this type of deficiency:—

TABLE II

Age	Sex	Clinical History	IgG level	IgA level	IgM level
5/12	F	Recurrent URTI and chest infections	500 (250- 600)	<5 (25-60)	50 (25-60)
		8.9.1971			
		13.10.1971	320 (250- 600)	<5 (25-60)	32 (25-60)
		31.7.1972	430 (400-1000)	<10 (30-150)	29 (30-100)
15/12	M	Recurrent Tonsilitis	650 (400-1000)	45 (30-150)	50 (30-100)
21/12	M	Recurrent URTI	240 (400-1000)	21 (40-150)	36 (30-100)
2½ yr	M	Recurrent bronchitis	400 (450-1100)	33 (40-200)	100 (30-100)
3 yr	M	Recurrent URTI (Asthmatic)	800 (500-1300)	36 (40-200)	38 (30-100)
3½ yr	M	Recurrent bronchitis	430 (500-1300)	27 (40-200)	75 (30-110)
5 yr	F	Pneumonia and recurrent URTI	1300 (600-1400)	33 (50-250)	57 (30-120)
8 yr	M	Recurrent bronchitis	1200 (600-1500)	93 (60-500)	50 (30-120)
7/12	M	Recurrent Otitis media	700 (250- 600)	25 (25-60)	100 (25-60)
15/12	M	Chronic diarrhoea	1200 (400-1000)	28 (30-15)	35 (30-100)

The value shown in brackets indicates the normal range of that immunoglobulin for the age group.

IMMUNOGLOBULIN ABNORMALITIES

Child A:

First seen by doctor when three months' old, eight occasions of upper respiratory tract infections prior to this.

Immunoglobulins done at six months—Marked IgA deficiency.—Mild IgM deficiency.

Gamma globulin given but not much clinical improvement.

Mother very anxious and—Child not progressing, very poor health.

October, 1972, IgA concentrate given—36mls from 12/10/72 to 30/4/73.

Clinical improvement quite remarkable. IgA leves improved.

18/5/73—No more IgA available and child has begun to revert to original poor health.

The Laboratory Diagnosis of Respiratory Infections other than T.B.

H. C. W. Shott, F.I.M.L.T.

Microbiology Department, Diagnostic Laboratories, Dunedin Hospital.

Received for Publication, December 1973

Introduction

In general, the traditional method of culturing sputum has been by spreading a loopful of material over the surface of suitable medium before incubating the plates. The choice of inoculum may be taken from the purulent part of the sputum in the hope that such an area supports the majority of pathogens likely to be present. Technically this method has two inherent disadvantages, not only are organisms known to be scattered irregularly throughout a speciment but owing to the superficial layer of saliva and mucus direct contact with the actual pus becomes difficult. Mulder (1952)[4], to some extent overcame such problems by washing selected flecks of pus in buffered saline before seeding the culture medium. Having proved the irregular dispersal of organisms throughout sputa, May (1953)[2], predicted that cultural techniques would only become worthwhile if specimens could become homogenised by a means consistent with the isolation of the most fastidious organisms likely to be found in fresh samples. During the same year, Rawlins (1953)[5], demonstrated successfully the liquification of sputum by the action of pancreatin. This method proved unharmful to bacteria and white cells.

More recently, Webb (1962)[6], reported on the clinical use of N-acetyl-L-cysteine for the liquification of mucoid and purulent pulmonary secretions. Subsequently, Kubica (1963)[1], and Mead and Woodham (1964) exploited N.A.C. as a method of liquification of sputa for the culture of mycobacteria and other organisms. Technical difficulties have meant that this alternative technique has not met with universal acceptance. May (1969)[3], has concluded that the pancreatin method devised by Rawlins, is at the moment, the reagent of choice.

Methods

Effective chemotherapy in relation to lower respiratory tract infections can only be achieved if more accurate evaluation of the etiological agents is possible. Formerly sputum cultures proved to be of a grossly mixed bacterial flora, more often than not a mixture of mouth, throat and to a lesser degree, bacteria actually recovered from the sputum itself.

The purpose of this paper is therefore to assess the value of a technique designed to make a quantitative study of potential pathogens found in digested sputum. Additionally an attempt is made to compare the degree and significance of mouth and throat contamination in patients who were, on the basis of laboratory findings, thought to have a genuine respiratory infection.

In the first instance, an equal measured volume of fresh sputum and pancreatin solution were mixed together and the specimen homogenised by the digestive process for a maximum of 40 minutes at 37°C. Frequent agitation enhanced the breakdown of sputum and mucus. From the digested sputum a series of ten-fold dilutions were prepared in half strength Ringer's solution by the conventional technique of Miles and Misra. A standard volume, 0.02ml was then plated on to the appropriate medium from all dilutions within the range of 10^{-1} to 10^{-7}. Such inoculum was then spread across the surface of the plates: chocolate agar, blood agar and MacConkey agar. As a control measure neat digested material was plated by loop in the normal manner. Where clinical evidence prescribed, anaerobic culture was undertaken. With such an exception all other plates were incubated overnight in the CO_2 incubator.

During the sampling trial period, some 25 cases were studied where, on direct smear and by clinical indication an acute respiratory infection was apparent. The same number of specimens were also examined from patients known to produce sputum but not manifesting other symptoms consistent with a current infection. From these initial studies it was agreed to proceed on the following basis of classification.

Table I

CULTURAL FINDINGS

Growth Classification	Not Significant	Doubtful Significance	Diagnostically Significant	Total
No Specimens	130	40	80	250
% of Total	52	16	32	100

Definitions—

Not Significant = No growth x 10^{-3} dilution

Doubtful Significance = Up to 10 colonies x 10^{-3}

Diagnostically Significant = 10 or more colonies x 10^{-4}

Table II

CLASSIFICATION AND DISTRIBUTION, CULTURES OF DIAGNOSTIC SIGNIFICANCE

Organisms	No.	% Total Specimens
Staph. aureus	5	2
H. influenzae	17	7
S. aureus + *H. inf.*	6	2.5
Pneumococci	20	8.0
Pneumococci + *Staph. aureus*	3	1.5
Klebsiella sp.	17	7.0
E. coli	5	2.0
Pseudomonas sp.	3	1.5
C. albicans	1	0.5
Serratia	2	0.7

Growth not significant occurred when all organisms were eliminated by the 10^{-3} dilution of the cultured sputum. This report was made irrespective of the occasional colony of potential pathogens appearing after direct plating in the conventional manner. Such growth on direct plating proved consistent with mouth and throat contamination.

Growth of doubtful significance represented the all too familiar area of diagnostic uncertainty and warranted a special comparative study. Although impressive numbers of potentially pathogenic organisms grew on the primary plates they were elminated by the 10^{-4} dilution of digested sputum but not completely by the lower 10^{-3} plate. As many as ten colonies at such a level would frequently occur. Saline mouthwashings processed in the same manner as the sputum samples commonly indicated a source of mouth contamination and will be referred to in the tables and summary.

Growth of diagnostic significance occurred when during the initial study, the Gram smear findings, the clinical picture and cultural results agreed. In particular without exception, the pathogens were readily seen growing on the 10^{-3} and 10^{-4} sectors of the culture plates, whereas they were eliminated in cases not meeting those criteria.

Figure 1 illustrates the direct plating of homogenised sputum from an acute respiratory infection showing a profuse *mixed* flora. Commensals have been eliminated in Figure 2 showing a pure growth of *H. influenzae* growing on the 10^{-3} and 10^{-4} plate dilutions.

After the analysis of the trial series it was agreed on a basis of classification, growth not significant, of doubtful significance, and that

Table III

COMPARATIVE STUDY, GROWTH, DOUBTFUL SIGNIFICANCE WITH PAIRED MOUTH WASH

	Klebsiella sp.	*E. coli*	*H. inf.*	*C. albicans*	Serratia	Total*
Sputa 10^{-3}	20	5	4	8	3	40
Mouth Wash 10^{-3}	17	2	nil	6**	2	27

* 13 mouth washings negative

** 2 cases oral thrush

of diagnostic significance, to proceed with a survey covering specimens obtained from 250 different patients, not selected on strictly clinical grounds but rather in keeping with normal laboratory requests. Additionally, when a presumptive report claimed growth of doubtful significance a saline mouthwash was examined to provide a comparative study.

Results

The results from 250 treated specimens obtained in three groups are shown in Table I. The 32 percent of diagnostically significant tests have been further studied in Table II.

Those of doubtful significance promoted a comparative study using mouthwash cultures obtained at the same time. Table III.

Of the 250 survey specimens examined rather more than half, 52 percent, were not diagnostically significant. Some 32 percent proved helpful and consistent with a diagnosis of respiratory infection. Sixteen percent, a total of 40 specimens, gave a bacterial yield which was difficult to interpret. Of course, no less than 17 out of 20 had identical mouthwash cultures, significant in numbers and classified as presumptive Klebsiella.

Summary

A method of quantitative culture of homogenised sputa is presented which appears helpful in the diagnostic sense. There are doubtless limitations to the value of all techniques related to the diagnosis of respiratory infections. In particular, ordinary direct plating may over-emphasis the presence of those potential pathogens which would be otherwise eliminated as contaminants by the dilution technique. By the same token the retention of direct plating as a control measure ensures that limited numbers of 'persistors' would not be ignored. In this regard clinical liaison is essential.

When results appear in doubt duplicate specimens of sputum and mouthwashings may be worthwhile. There is a strong case for such control measures to become a matter of routine. Clinically it was a significant fact that when a heavy mouth flora was apparent, invariably the patient's saliva production was diminished. Limited space does not allow analysis to be shown of Gram stain findings versus urgent culture results, it should nevertheless be stressed that there is very much a place for microscopic findings when acute infection is not a common feature of laboratory diagnosis and calls for a more accurate interpretation of cultural findings.

Acknowledgments

I wish to express my thanks to Professor N. P. Markham and Professor T. O'Donnell for their interest in this work. (To Miss M. Johnstone I am indebted for much meticulous work at the bench.)

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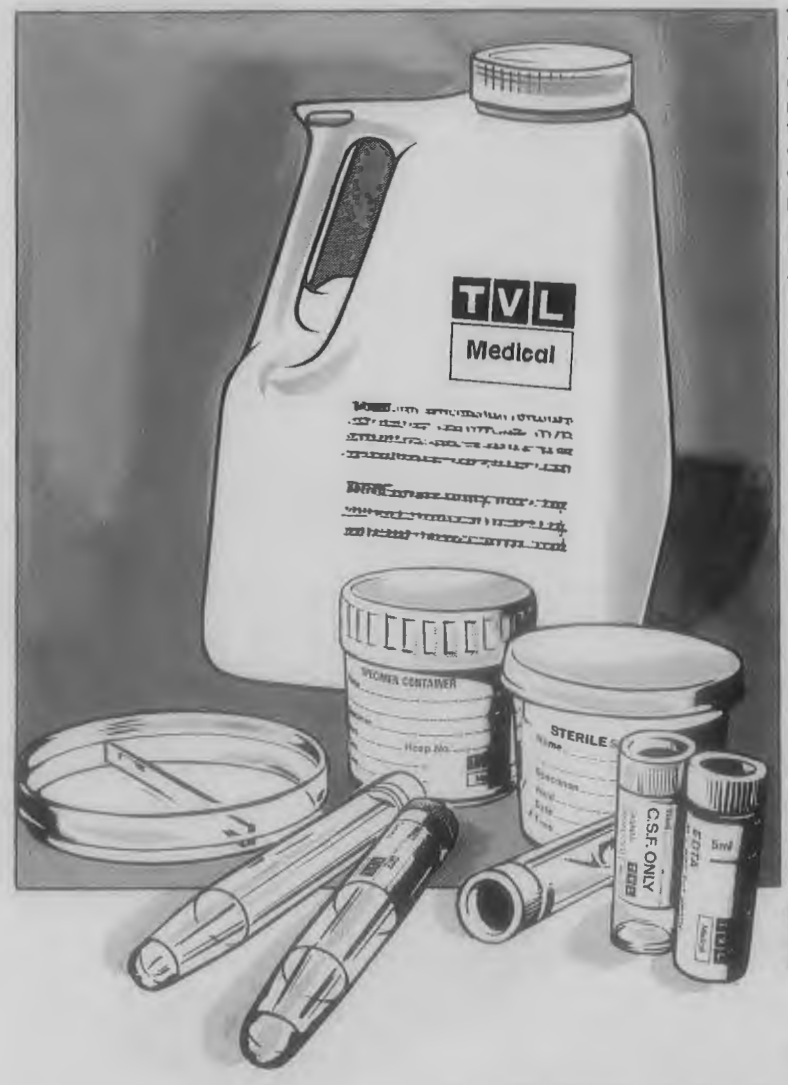

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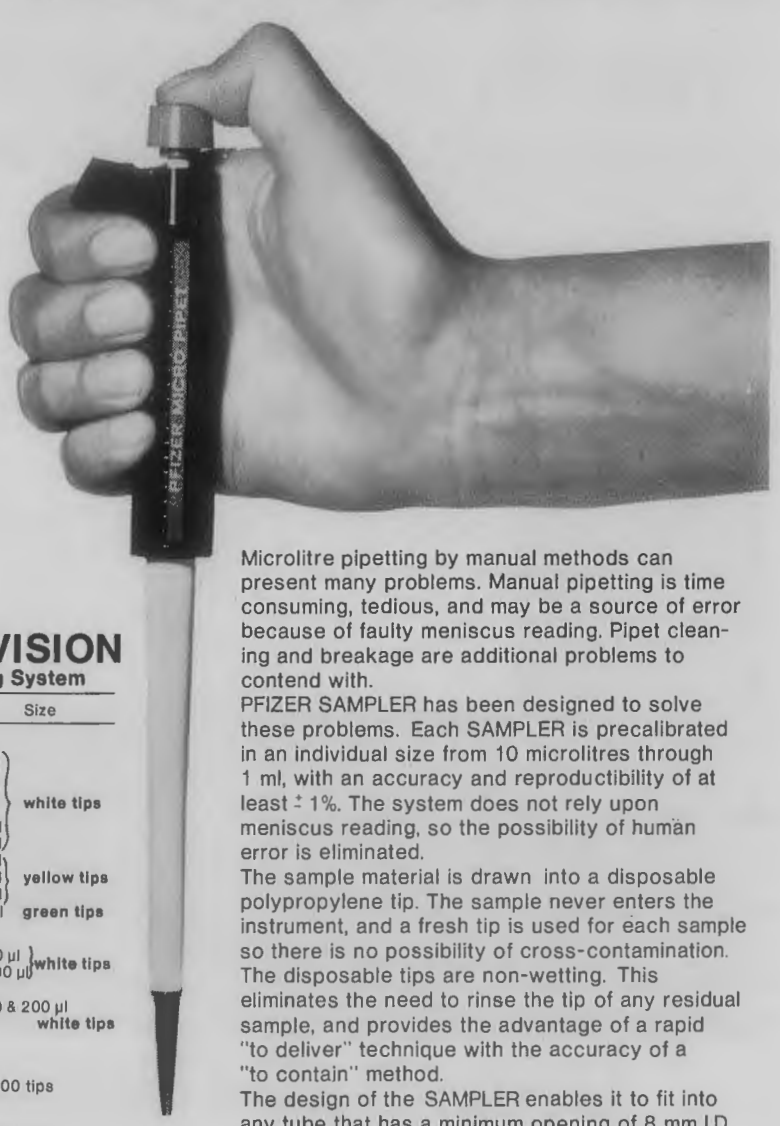

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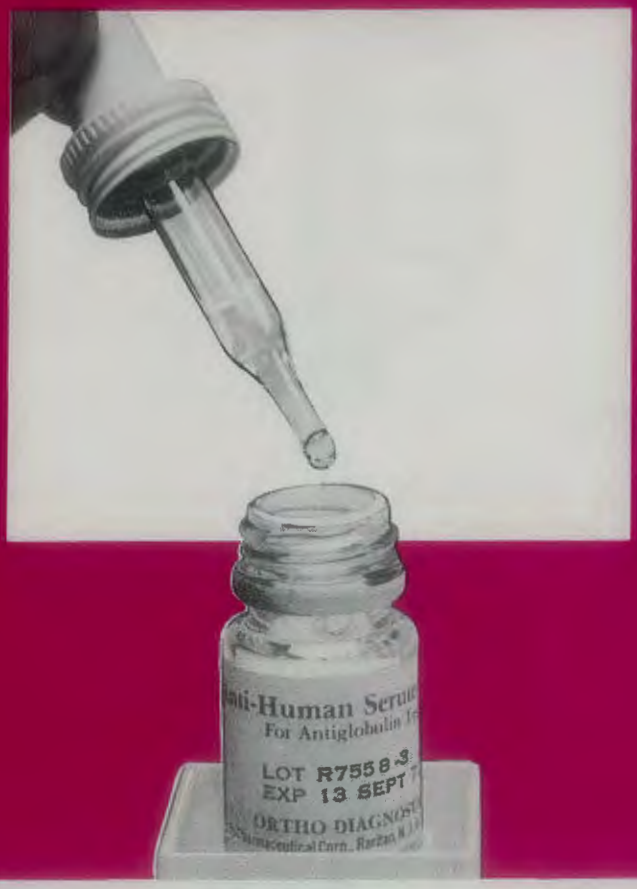

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Correspondence

Sir,

In the direct Liebermann-Burchard cholesterol method a correction should be made for bilirubin interference. In a recent quality control programme our cholesterol values were lower than our method mean results when applying a correction value of 5 mg cholesterol per 1 mg of bilirubin. By not correcting, our values were close to the method mean and this made us wonder whether other laboratories using the Liebermann-Burchard reaction were applying a correction value or performing a serum blank.

We approached the company, who then ran a bilirubin correction survey. Out of a total of 240 laboratories using the Leibermann-Burchard reaction, 178 replies were received and the results obtained were as follows:—

66 Automated 41 Manual	No correction or extraction.
24 Automated 13 Manual	Made a correction. Usually 5 mg/100 ml.
16 Automated 8 Manual	Extracted the serum and made no correction.
2 Automated 8 Manual	Performed a blank.

From the results obtained it appears that the majority of laboratories do not apply a correction. Although the error involved is generally small compared with the accuracy required it could give a false representation on a quality control chart.

I wish to thank Mr M. A. Cresswell of the Wellcome Research Laboratories for carrying out the survey and for permission to publish the results.

November 7, 1973. R. W. L. Siebers.

LABORATORY, MEMORIAL HOSPITAL, HASTINGS

Sir

I read with interest the comments of R.D.A., 'Laboratory Standards', N.Z. J. med. Lab. Tech. 1973, **27,** 3.

The penultimate paragraph of R.D.A.'s paper expressed the hope that smaller laboratories would avoid unnecessary duplication of expensive test facilities and would submit specimens for rarely performed non-urgent tests to larger metropolitan centres for examination. The writer stressed the obvious advantages in better quality control with a larger throughput and the attendant economic advantages of centralisation. It is implied in the paragraph that parochialism in provincial laboratories is the reason for their persisting in carrying out complex procedures with inadequate control measures.

Let me hasten to disabuse the writer. Many in secondary centres would be only too happy to send such specimens to a larger centre, if a centre offering an adequate service could be found. Two recent experiences may draw to the attention of the laboratory managers of larger Boards the shortcoming of their organisations as seen by the provincial user.

If one uses a large metropolitan centre's hospital laboratory for P.B.I. and T3 estimations, it is necessary for an outside user to submit separate specimens to two separate departments as it is not possible to have a single specimen passed from one department to another. In a second large non-metropolitan hospital the laboratory service charges a higher fee than allowed by the S.S. Schedule for T3 examinations. When challenged, the department concerned alleged that this was the true cost and they were not prepared to accept the Schedule fee only. As a result of these experiences this hospital has been forced to use a metropolitan private laboratory to carry out these tests and is of course (parochially) contemplating setting up to carry out the tests.

I invite the laboratory managers of major centres to consider how they can offer a better service if they wish to see 'parochialism' eliminated and avoid much needless duplication of equipment and test facilities at the taxpayers' expense. The service currently provided by them to outside users is in the main unacceptable with some laudable exceptions, including R.D.A.'s own laboratory service. 'Parochialism' is not primarily the fault of peripheral laboratories.

D. G. Bolitho,
Cook Hospital,
Gisborne.
December 7, 1973.

Chapter 9, 'Immunological Methods of Pregnancy Testing', describes the history of pregnancy testing and the development of immunological methods. The effect of various drugs and physiological states on the tests are described. Quantitative estimation of Gonadotrophin levels provides valuable information in the diagnosis of abnormal pregnancies and neoplasms and testicular tumours in the male. Data relative to these various conditions is provided.

Chapter 4 covers the examination of semen and as it has been estimated that 12 percent of all marriages in the U.K. are sterile, it might be expected that this test would be required rather frequently. All the essential details of the technique and the criteria for assessing the various parameters are simply and lucidly explained. The need to collect the specimen into a warm container to avoid 'cold shock', is particularly stressed. A technique for extracting spermatozoa from cloth and identification by a staining method is described. This is sometimes required for forensic purposes.

In summary, there is a lot of useful information on the somewhat arbitrary selection of topics gathered together in this book.

—R.D.A., L.D.G., H.C.W.S. and A.G.W.

Medical Technology Examination Review. Volume 2. Second Edition. Alter, Dittmar, Higgins, Johnson, Kaminsky, Kim, Robinson, Schwartz and Wisch. 245 pages. Medical Examination Publishing Company Inc. New York (1971). Price $7. N. M. Peryer Ltd., Christchurch.

This book really only serves to marginally up-date the first edition printed in 1966 as the majority of the questions are the same and the only noticeable difference is the use of more recent reference books plus several additional references. However as the authors state, this is merely a review book, and as such should prove useful to candidates sitting examinations in any medical technology discipline as they are all covered in ten chapters.

One particularly good aspect is that virtually all types of questions are used, ranging from true false, matching statement with fact, number with letter, complete the sentence, and match one of four or five answers with the question. As multiple choice questions are now being used extensively in our Institute examinations this gives prospective candidates experience with the possible variations of such questions. Also each of the 2,000 questions contained in the book is provided with a reference for further research should the reader be unsure of the subject. This I think is an excellent means of getting straight to a topic without recourse to endless indexes.

As stated in the preface the object of the book is to help the reader in checking areas of weakness and to provide a source for further reading. A book which does this must surely be an asset to any training laboratory.

—D.A.H.

Methods and Techniques in Clinical Chemistry. P. L. Wolf, M.D., Dorothy Williams, M.T., Tashiko Tsuduka, M.T., and Leticia Acosta, M.S. (1972). 417 pages. Illustrated. Wiley Interscience, a division of John Wiley and Sons Inc., New York. Price in New Zealand $14.50. N. M. Peryer Ltd., Christchurch.

This book has been written by medical technologists for use by pathologists and technologists. The authors have produced this book with emphasis on the practical aspects of clinical chemistry and have covered the methods used in their laboratory at the Stanford University Medical Center.

The methods are listed alphabetically and in all 78 determinations are covered usually by more than one method. The presentation of each method is described followed by a well set-out worksheet covering reagent preparation and procedure. Where calculations are involved these are shown in detail. Methods using autoanalysis have flow diagrams and useful comments on trouble-shooting and maintenance. A pleasing aspect of this book is that the methods used are up-to-date and widely used. For example, enzymes such as CPK and gamma glutamyl transpeptidase are described using commercial kits although details of principle and calculation are still given.

Some of the more esoteric methods described are barbiturates and sedatives by gas liquid chromatography and by UV spectrophotometry. LDH enzymes by electrophoresis, (conventional LDH and HBD assay by UV spectrophotometry are also covered).

Chapters on measurement of serum osmolality using a Fiske freezing point osmometer and protein lipoprotein and immuno-electrophoresis are well detailed.

One disadvantage of the book is the ab-

sence of an index which means that some methods are difficult to locate, for example lipoproteins appear in the section on proteins.

Another disappointment is that the authors have not acknowledged S.I. units and the expression mg % is used throughout the book.

Despite this the book would be a useful addition to any chemical pathology laboratory and would be especially useful to candidates preparing for Part II and Part III examinations in chemical pathology.

—A.G.W.

Abstracts

Contributors: D. G. Bolitho, Lexie Friend, Lynette R. Gazeley, J. Hannan, B. McDonald, W. Stead, A. G. Wilson.

CHEMICAL PATHOLOGY

A Solid State Fixed Wavelength Colorimeter for Continuous Flow Analyses. Walker L., and Amador E. (1973), *Clin. Chim. Acta.* **46**, 181.

A completely solid state colorimeter has been built using a subminiature light emitting diode (L E D) as the fixed wavelength source and a PIN silicon photodiode as the detector. Because light emitting diodes are very stable and emit narrow bandwidth light, a reference circuit and monochromator is not required. The photodetector is a photodiode whose output current drives an F E T input operational amplifier coupled to a logarithmic operator whose output voltage is rectilinear with sample absorbance. The output can drive Technicon recorders. The sample cuvette can be flow cell or discrete cuvette. This type of circuit is very robust, consumes little power, emits scant heat and is of low cost.

—A.G.W.

Curve Regeneration in Practice in Continuous Flow Analysis. Carlyle J. E., McLelland A. S., and Fleck A. (1973), *Clin. Chim. Acta.* **46**, 235.

The results of a commercially produced analogue device (Weir curve regenerator) which permits increased throughput with continuous flow systems are presented. The precision with this device is improved and the disadvantages are few.

—A.G.W.

Plasma or Whole Blood Glucose? Barbara Morrison and Fleck A. (1973), *Clin. Chim. Acta.* **45**, 293.

A comparison of plasma and whole blood glucose determinations indicated that no conversion factor from one to the other can be derived and the difference between the two can be much greater than the figures 10-15% usually quoted. The authors regard the contention that "blood" is a single compartment as misleading and suggest that levels should be followed in a single system and suggest the measurement of plasma glucose.

—A.G.W.

Estimation of Gold in Urine by Atomic Absorption Spectroscopy. Dunkley J. V. (1973), *Clin. Chem.* **19**, 1081.

An atomic absorption spectrophotometric method is described for estimating gold in urine of patients undergoing chelation therapy. Variable absorption at 242.8nm not due to gold is avoided by wet ashing and extraction of gold into methyl isobutyl ketone. The technique will not detect gold in normal urine.

—A.G.W.

Evaluation of Rapid Latex Agglutination Test for Detection of α^1 Fetoprotein. Alpert E. and Costor R. L. (1973), *Clin. Chem.* **19**, 1069.

A latex agglutination assay for α fetoprotein (A.F.P.) has been evaluated and compared to the results obtained by counter immuno-electrophoresis (CIEP). The control latex, coated with normal rabbit gamma globulin agglutinated with 8% of sera from hepatoma patients and 18% of sera of patients with other liver disease makes the test inconclusive in these patients. There was 88% agreement with CIEP in the detection of A.F.P. in a series of 50 hepatoma patients and no false positives from 98 patients with other diseases, or from normal sera. The authors conclude that the method would be suitable as a screening method, positive results being confirmed by CIEP.

—A.G.W.

Evaluation of the Harleco apparatus for Determining Carbon Dioxide in whole Blood. Strever B. C., Johnson C. A., and Gadsden R. H. (1973), *Clin. Chem.* **19**, 1075.

A simplified method for determining the total CO_2 content of whole blood is evaluated. A high degree of correlation was found with Instrumentation Laboratories Blood gas analyser. The device consists of a small reaction chamber which is plugged after introduction of the sample. Lactic acid is added through the cap by a special calibrated syringe, after mixing, the liberated CO_2 forces the plunger to rise and CO_2 content is obtained by comparison with a standard.

—A.G.W.

Direct Measurement of Chloride in Sweat with an Ion Selective Electrode. Szabo L., Kenny, Margaret A., and Lee, Winnie. (1973), *Clin. Chem.* **19**, 727.

The technique using the Orion sweat chloride instrument has been modified to obtain more sweat for determination. The authors used felt pads saturated with pilocarpine nitrate and $NaHCO_3$ to replace the pads supplied by the Orion Co. After iontophoresis, sweat is collected for 10 minutes under a plastic cup held over the induction area, the chloride concentration is then measured with the electrode. Good precision is claimed.

—A.G.W.

Serum Sodium Measurement by Manual and On Line Dilutions. (1973), Haven G. T., and Haven, May. Letter to the Editor *Clin. Chem.* **19**, 791.

In this letter the authors describe the errors which can be obtained when Instrumentation Laboratories' Model 144 dilutor is used on line with IL 143 Flame Photometer. False low results are obtained with sera when the aqueous standard they supply is used. This is attributed to a viscosity effect and the authors found that a standard containing protein raised the sodium content by 3m mol/l of patient's sera.

—A.G.W.

An Evaluation of Kits for Determining Urea Nitrogen. Wenk R.E., Lustgarten J. A., and Byrd C. (1973), *Amer. J. clin. Pathol.* **59,** 542.

The authors have studied 14 commercial kits available for urea nitrogen determination and have assessed their accuracy, precision, recovery, speed, cost and convenience.

Kits using diacetyl monoxime rated high and were apparently as specific as those using urease. Kits using the Berthelot reaction also rated favourably.

Performance of the kits was good except at very low (10mg per dl) or high (50mg per dl) urea concentrations.

—L.R.F.

A Previously Unrecognised Laboratory Hazard — Hepatitis B Antigen Positive Control and Diagnostic Sera. Wetli C. V., Heal A. V., and Miale J. B. (1973), *Amer. J. clin. Pathol.* **59,** 684.

A wide range of Control and Diagnostic sera were tested using a radioimmunoassay technique and it would found that 33% contained demonstrable HBAg.

This survey included Chemistry control sera, pooled plasma and serum, Quality Control survey sera, coagulation controls, blood grouping reagents, Immunohaematology reagents, bacteriologic typing sera, febrile agglutinin reagents and serology test control sera.

The product names, manufacturers and lot numbers can be obtained by writing to the authors.

—L.R.F.

An Improved Automated Method for Determination of Serum Albumin Using Bromcresol Green. Beng C. G., and Lim K. L. (1973), *Amer. J. clin. Pathol.* **59,** 14.

An improved AutoAnalyser manifold for albumin determination using bromcresol green (BCG) is described. The improved manifold makes a one stage sample dilution instead of the usual two stage sample dilution previously described.

The article also recommends the addition of at least 3 ml of 50% Brij 35 per litre of BCG reagent to eliminate unstable baselines and falsely high albumin peaks.

Since human and bovine albumin have different degrees of binding BCG, the authors recommend that standards and samples used, be as similar as possible. Thus human serum is recommended as the standard for this method and bovine-type commercial control sera is not suitable unless recalibrated.

—L.R.F.

Changes in Lipid Values and Lipoprotein Patterns of Serum Samples Contaminated with Bacteria. Schwertner H.A., and Friedman H. S. (1973), *Amer. J. clin. Pathol.* **59,** 829.

Certain bacteria can cause an apparent increase in serum triglyceride and chylomicron levels and a decrease in alpha, beta and prebeta lipoproteins. This article could be of particular interest to laboratories who receive specimens through the post with consequent delay in the processing of the specimens.

Ethylenediamine tetra-acetic acid was found to be an effective anticoagulant for lipid sample analysis.

—L.R.F.

Aldomet Interference with Melanogen Tests. Langelaan D. E. (1973), *Assoc. clin. biochem. Newssheet, September.*

False positive interference by Aldomet with the ferric chloride and Thormahlen tests for melanogen in urine is described.

A urine was observed to darken from the top on standing. This was from a patient with hypertension on large doses of Aldomet. It was positive by these two tests but negative for homogentisic acid.

Experiments showed that Aldomet gave positive screening tests at a level of 0.2mg/ml in alkaline solution. Only alkaline urines (pH 8) gave the false positive tests as well.

In view of the widespread use of this drug the possibility of false positive tests for melanuria should be borne in mind.

—R.D.A.

Alpha-1-Antitrypsin Deficiency and Pulmonary Emphysema: The Role of Proteolytic Enzymes and Their Inhibitors. Hutchison, D. C. S. (1973), *Br. J. dis. Chest,* **67**, 171.

Much of the emphasis of present-day research into the aetiology of pulmonary emphysema stems from the discovery of an abnormality in the electrophoretic protein pattern of a number of sera undergoing routine examination. The subjects concerned were found to have a marked deficiency of α_1-antitrypsin (α_1-at), the main component of the α_1-globulin fraction. There is a high incidence of pulmonary emphysema among those with this abnormality.

Some of the serologic methods in common use for the estimation of α_1-at are outlined.

It now appears that the destruction of the lung substance, which is the principal feature of emphysema, is brought about by the excessive release of proteolytic enzymes within the lung. The lysomal granules of both polymorphonuclear leucocytes and alveolar macrophages contain potent elastolytic enzymes. —J.H.

Unusual Glucose Tolerance Curves as Variants of Normal. Danowski, T. S. (1973), *Cur. med. Dial.,* **40**, 627.

Entirely normal glucose tolerance curves and those which are clearly diabetic are readily identifiable. There remain, however, a number of glucose tolerance curves with somewhat unusual patterns. These include curves with reactive hypoglycaemia in

the later hours of the test, those which are flat and tests with two or more glucose peaks.

Two-peak curves occur in the normal range of glucose tolerance values but the initial hyperglycaemic peak is followed by a return toward or to the starting glucose value and in turn by a second glucose peak. The author suggests that the second peak should exceed the antecedent blood sugar level by about 20mg/dl or more.

When insulin levels are measured during such tolerance tests, an increase in insulin of 10 microunits/ml or more is present with 40 percent of the second peaks. This suggests that such second peaks are not laboratory artifacts. They are variants of normal which could result from intermittent passage and absorption of glucose from the stomach; however, other possible explanations include sudden rises in blood glucose as a consequence of sudden increases in growth hormone, cortisol, glucagon, or catecholamines, or sudden release of glucose from the liver. —J.H.

CYTOLOGY

Hydrogen Peroxide Bleach Technique in the Diagnosis of Malignant Melanoma. Lefer L. R., and Johnston W. W. (1972), *Acta Cytologica* 16, 505.

A brief resume is made of the classical method for the detection of melanin, only it is used both histologically and cytologically, with the necessary control slides.

—W.J.S.

D.N.A. Content of Dysplastic Cells of the Uterine Cervix. Wagner D, Sprenger E., and Blank, M. H. (1972), *Acta Cytologica* **16,** 517.

Papanicolaou-stained smears of various dysplastic lesions in the cervix uteri were reviewed with regard to different cell types found in these lesions. The smears were then subject to Feulgen-hydrolysis and re-dyed by acriflavine—SO_2 for fluorescent—cytophotometry.

The resulting histograms suggested that quantitative cytochemistry might be used to differentiate between the various forms dysplastic and in-situ lesions. in ways not yet established by cytomorphology.

—W.J.S.

Human Ovum-Like Structures in Cervical Smears. Benson P. A. (1972), *Acta Cytologica* **16**, 527.

Structures found in cervical-vaginal smears and believed to be human ova have been described in four cases

Subsequent observations provide evidence that none of the reported "ova" are in fact ova. Presented in this paper is an egg-like structure which can be shown to be in consistent with an ovum.

While the nature of these ovum-like structures is not known, it is now apparent that they are not human ova.

They could be artefacts which are rendered spherical on the smear because of surface tension phenomena.

—W.J.S.

HAEMATOLOGY

Screening for Abnormal Haemoglobins. A Pilot Study. Stuart, J., Schwartz, F. C. M., Little, A. J., and Rain, D. N. (1973), *Brit. med. J.*, **4**, 284.

A pilot study has been made of the implications of screening for abnormal haemoglobins in immigrant school children. An abnormality was detected by capillary blood electrophoresis in 8.4 percent of 6,835 children and a haemoglobinopathy Outpatient Clinic had to be established to deal with the heavy work load which resulted.

—L.R.G.

Drug-induced Thrombocytopenia. Meishen, P. A. (1973), *Seminars in Haem.*, **X**, 311.

This review gives a summary of the current information related to drug-induced thrombocytopenia, with a critical evaluation of the mechanisms involved as well as the presently available serological techniques for the detection of the causative agent.

—L.R.G.

Cytogenic Analysis as a Diagnostic Aid in Leukaemia. Meisner, L. F., Inhorn, S. L., and Chuprevich, T. W. (1973), *Amer., J. clin. Path.*, **60**, 435.

Results of cytogenetic studies of 100 cases of suspected or proven leukaemia showed that significant spontaneous division in 24h peripheral blood cultures is indicative of an abnormal state. If viraemia can be ruled out clinically and the spontaeous division is persistent, a leukaemic process is suggested, even before the development of overt leukaemia. —L.R.G.

A Capillary Blood Micro-method for Coagulation Screening. Stuart, J., Breeze, G. R., Picken, A. M., and Wood, B. S. B. (1973), *Lancet*, **973**, 1467.

A capillary blood micro-method has been developed to allow serial screening of newborn infants for coagulation defects. The resulting coagulation profile consists of six tests; fibrinogen factor V. prothrombin and proconvertin, platelets. PCV and F.D.P.s. —L.R.G.

A Method for Preservation of Papainized and Rh-sensitized Red Cells. Dale, I. (1973). *Transfusion*, **13**, 135.

Papainised red blood cells and red blood cells sensitised with Rh antiboides were stored at 4°C in Alsever's solution containing inosine. The papainised cells were used for detection of Rh antibodies. The cells sensitised with anti-D were used for the positive control of the antiglobulin reaction. When stored for no more than three weeks, the preserved cells gave reactions almost identical to those obtained with freshly prepared cells. (Author's abstract.)

—B.M.

Mechanism of Red Cell Agglutination by IgG Antibodies. Romano, E. L., and Mollison, P. L. (1973). *Vox. Sang.*, **25**, 28.

It has been shown by others that agglutination of red cells by IgG antibodies depends on the density of antigen sites at the red cell surface, suggesting the possibility that agglutination by IgG antibodies might possibly be due to the cells being rendered 'sticky' rather than to specific cross-linking. However, the present experiments show that, when a mixture of purified IgG anti-A and anti-B is incubated with a mixture of A and B red cells,

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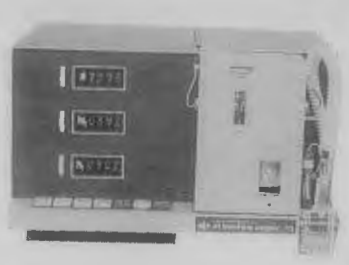

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the clumps contain only A cells or only B cells, indicating that agglutination by these antibodies is, in fact, due to specific cross-linking. (Author's abstract.) —B.M.

Australia Antigen and Viral Hepatitis, 6th Symposium on Basic Progress in Blood Transfusion, Brussels, 1972. Edited by Desmyer, J., Thomas W., and Verylen, C. (1973). *Vox. Sang.*, **24** (Suppl.), 1.

The entire Supplement is devoted to various aspects of Australia Antigen and its relation to disease. The papers cover a wide range of topics, from the genetics of the Australia Antigen to methods for its detection. —B.M

MICROBIOLOGY

Nosocomial Urinary Tract Infection with *Serratia marcescens*: An Epidemiologic Study. Maki, D. G., Hennekens, C. G., Phillips, Constance W., Shaw, W. V., and Bennett, J. V. (1973), *J. infect. Dis.*, **128**, 579.

Frequent nosocomial urinary tract infection (—10^5 organisms/ml) with nonpigmented *Serratia marcescens* was found in patients in the urology ward of a large Veterans Hospital. Serotyping and antibiograms suggested a single endemic strain. Each of 10 patients who became infected in a 27-day study had an indwelling Foley catheter and was receiving one or more antimicrobial agents.

In some hospitals in the U.S.A., strains of Serratia constitute >10 percent of nosocomial urinary isolates. —J.H.

Giardiasis in Young Adults. Chretien, Jane H., Esswein, J. C., and Garagusi, V. F. (1973), *Med. ann. Dist. Columbia*, **42**, 375.

The protozoan, *Giardia lamblia*, once thought to be an insignificant finding in the faecal flora, has been increasingly incriminated as a cause of acute and chronic gastrointestinal illness. Using a direct smear-iodine method, *G. lamblia* cysts were identified in five students over an 8-month period in a student health service.

In chronic giardiasis, cysts may be shed only periodically, and numerous stool examinations may be negative. In such cases, examination of duodenal aspirates for the trophozoite form or touch preparations of duodenal biopsy specimens may be necessary.

G. lamblia may occasionally be found in the stool of asymptomatic carriers. —J.H.

A New Tissue-Culture System for Clinical Virology. Rossier, E., and Bourgaux-Ramoisy, Danielle (1973), *Pathologia, Microbiol.*, **39**, 81.

The system consists of an optically clear, nontoxic plastic, eight-chamber container, mounted on a microscope slide. The completely separate chambers allow testing and control cultures to be maintained, using several cell strains. Direct observation with an inverted microscope is readily and accurately made. The excellent optical conditions allow for the detection of early cytopathic effects that would probably go unnoticed in tubes.

By removing the chambers from the slide, a permanent record can be made after staining, or immunofluorescent studies can be undertaken. Illustrations show normal monolayers of cells and cytopathic effects of Coxsackie B_5 virus, parainfluenza 2 virus and adenovirus, type 2.

—J.H.

Clinical Evaluation of Testing Immediate Antibiotic Disk Sensitivities in Bacteriuria. Perez, J. R., and Gillenwater, J. Y. (1973), *J. Urol.*, **110**, 452.

Results are presented on 2,500 urine samples tested with a preliminary disk sensitivity test. A sterile cotton swab was soaked in urine and generously streaked over the surface of a Mueller-Hinton agar plate. Sensitivity disks were added and the reading taken after 6 to 16 hours of incubation. There was 96.8 percent accuracy when compared with the final sensitivity results as described by Kirby and Bauer. The incidence of falsely positive results was 0.5 percent. —J.H.

Nitroblue Tetrazolium (NBT) Reduction by Neutrophils. Rao, K. V. (1973), *Am. fam. Phys.*, **8**, 157.

The NBT test is a valuable diagnostic aid in differentiating bacterial and mycotic infections from viral infections. It is becoming a routine test in many laboratories because of its relative simplicity and the rapidity with which it can be performed.

Equal amounts of freshly drawn, heparinised blood and 0.2 percent NBT in 0.15 M phosphate-buffered saline are incubated for 15 min at 37°C and for another 15 min at room temperature. Films of the mixture are air-dried and Wright-stained. The percentage of neutrophils showing a heavy deposit of formazan is determined under oil immersion. Total and differential WBC counts are done at the same time. The absolute number of NBT-positive cells is computed from these data and the result read from a nomogram which classifies patients into four groups: A Normal; B Viral infections, non-infectious febrile illness and partially treated bacterial infections; C Untreated bacterial infections; and D Ineffectively treated bacterial infections.

Although the NBT test yields valuable information, it should be interpreted with the history, physical examination and other laboratory data. It is useful for monitoring patients at high risk of infection —J.H.

Epstein-Barr Virus in Human Disease. Langenhuysen, M. M. A. C., and The, T. H. (1973), *Neth. J. Med.*, **16**, 85.

A review is presented of the clinical implications of recent work done on the subject of Epstein-Barr virus (EBV), a member of the herpes virus group. This virus may have an oncogenic potential, as evidenced by its ability to convert lymphocytes into lymphoblastoid cell lines with an unlimited life span. Other herpes viruses have been held responsible for malignant diseases in animals. A strong relationship between the virus and Burkitt lymphoma and nasopharyngeal carcinoma has been demonstrated by serologic and virologic means. The exact nature of this relationship remains a controversial matter. If the virus is more than a passenger, it could have a co-carcinogenic or carcinogenic capacity. The most convincing arguments for a causative role of EBV have been put forward in infectious mononucleosis. The prevalence of anti-EBV antibodies in a variety of diseases with a disturbed immunologic surveillance like sarcoidosis, systemic lupus erythematosus, lepromatous leprosy and Hodgkin's disease could possibly be explained by a compensatory humoral hyper-reactivity.

—J.H.

Rapid Determination of Antimicrobial Susceptibility for Urgent Clinical Situations. Barry A. L., Joyce L. J., Benner E. J. (1973), *Amer. J. clin. Pathol.* **59**, 693.

This paper discusses methods of speeding up the reporting of antimicrobial sensitivity tests using a modification of the Kirby Bauer technique. The authors present evidence to show that sensitivity tests carried out by the direct plating of specimens which are liable to contain a significant number of non-pathogenic flora will lead to gross errors in the interpretation of susceptibility tests, thus confirming the work of several earlier workers. The authors also found that direct sensitivity tests on urines were unsatisfactory due to the impossibility of standardizing the inoculum. In view of these factors they concentrated on rapid methods of testing pure cultures once obtained. It was found that reliable results could be obtained after incubation for only 5-6 hours compared to the more conventional 16-18 hours of incubation. It should thus be possible to read sensitivity results during a normal working day. This technique may well be useful.

—D.G.B.

Drug Resistant *Escherichia coli* Isolated from Patients with Urinary Tract Infection. Bissell P. S. (1973), *Med. lab. Technol.* **30**, 281.

The author demonstrates the rise, over the period 1969-1971, in the incidence of *Escherichia coli* strains resistant to Chloramphenicol, Sulphafurazole and Tetracycline. This is tied in with the prescribing pattern of antibiotics in the area from which the patients were drawn.

—D.G.B.

Quality Control Recording Methods in Microbiology. Woods D., Biers J. F. (1973), *Amer. J. Med. Technol.* **39**, 79.

This paper presents an outline of a quality control system for use in microbiology. Some of the recording methods described have advantages over those previously described. Others described by these authors appear to be unnecessarily tedious. Unfortunately the paper is rather brief and though good illustrations are given of the recording charts a more comprehensive description of their methods of use would have made this a more valuable contribution to medical laboratory technology.

—D.G.B.

Evaluation of a New Preservative for Urine Cultures. Wasilauskas B. L., and Stallings R. A. (1973), *Amer. J. Med. Technol.* **39**, 689.

A trial of the urine preservative suggested by Amis and Colpas is presented. Amis and Colpas preservative consists of Sodium Chloride, Polyvinylpyrolidone and water. The mixture can be autoclaved. This preservative has been claimed to stabilize the bacterial population and prevent significant multiplication up to 48 hours, this being particularly useful in laboratories where large numbers of postal specimen are received. Unfortunately the preservative did not live up to its reputation in the opinion of these investigators but it did show advantages over untreated urine.

—D.G.B.

The Effect of Temperature of the Culture Medium on the Outcome of Blood Culture. Model D. G., and Peel R. M. (1973), *J. clin. Path.* 26, 529.

A report of an investigation as to whether pre-warming blood culture bottles to 37°C before collecting blood will improve the isolation rate of pathogens. This entirely in-vitro investigation demonstrates that no significant advantage is to be gained by pre-warming blood culture bottles.

—D.G.B.

A Clinical Trial of a Chemical Test for Bacteruria. Patterson L., and Miller A. W. F. (1973), *J. clin. Path.* **26**, 375.

The results of a trial of the chemical test for bacteruria 'Uriglox' is presented. 351 specimens of urine collected by suprapubic aspiration were examined and a comparison of the Uriglox method and routine culture made. The Uriglox method relies on the detection of minute amounts of glucose in the urine. It has been found that small but detectable amounts of glucose are found in the urine of healthy persons. The range varies from 2-20mgm/100ml. In the presence of significant bacteruria urinary glucose is reduced as a result of the metabolic activities of the multiplying organisms, therefore a reduction in urine glucose can theoretically act as an indication of urinary tract infection. The Uriglox strips are an ingenious device consisting of a paper strip, the lower portion of which is impregnated with an ion exchange resin and the upper portion with a glucose test substance. If the level of the glucose in the urine is above 10mgm/100ml the strip turns blue and at lower levels it remains colourless. The authors found a correlation of 96.9% between this method and culture methods but both false positives and false negatives were found.

—D.G.B.

A Taxonomic Scheme for Aerobic Diptheroids from Human Skin. Sommerville D. A. (1973). *J. Med. Microbiol.* **6**, 215.

More than 1,500 strains of aerobic cutaneous diphtheroids were examined to determine if a simple series of tests could be devised for the characterization of strains from the skin. Morphological, Biochemical and Nutritional tests were used. It was found that the results of nine tests: lipophilic character, lipolytic ability, production of prophyrin, reduction of nitrate, and the ability to decompose glucose, maltose, sucrose, fructose or galactose that strains could be divided into 15 groups. Seven fluorescent and eight non-fluorescent strains. The author discusses the distribution of the various groups over the body surfaces. This scheme of identification could well be useful for work on those odd isolates from wound swabs and other sites where one is in doubt as to the pathogenicity of the organism.

—D.G.B.

Coagulase Negative Staphylococci Causing Endocarditis after Cardiac Surgery. Speller D. C., and Mitchell R. G. (1973), *J. clin. Path.* **26**, 51.

Seven cases of bacterial endocarditis due to coagulase negative Staphylococci are discussed. Details of biochemical biotyping, phage typing and antibiotic sensitivity patterns are given. It appears that no set pattern of antibiotic sensitivity or resistance can be associated with any particular biotype or phage type.

—D.G.B.

What's New?

NEW HOSPITAL DATA ACQUISITION AND COMMUNICATIONS SYSTEM WILL IMPROVE PATIENT SERVICE, CUT HOSPITAL'S COSTS AND REDUCE LOST CHARGES

CORPUS CHRISTI, Texas.—A hospital data acquisition and communications system, designed by Automated System Corporation and featuring a Varian 620/L and NCR century 200 computers, has completed a successful shakedown period and is now being pushed into active service throughout the 500-bed Memorial Medical Center here.

A similar system will soon be installed in a Tulsa, Oklahoma, hospital.

Because the system will significantly reduce the volume of paperwork and improve communications in the hospitals, it is expected to have three major benefits for patients and for the hospital.

The first benefit will be improved patient care, which will be possible because nurses will have more time to spend with patients and because of the increased speed and accuracy of hospital communications regarding patient needs.

The second benefit will be a reduction in hospital costs, due to the elimination of considerable amounts of paperwork and keypunching.

Third, the hospital will capture thousands of dollars per month in charges, previously lost through errors, omissions or delays in paperwork.

The hospital data acquisition and communications system could be described as "two" systems, tied together by a data transfer unit. The data transfer unit allows both systems to work asynchronously and independently, and then to transfer data at channel speeds when one is ready to send and the other is ready to receive.

One of the systems is the central computer system, which processes patient accounting, general accounting, medical records, accounts receivable, accounts payable, inventory management, medical audit and payroll. Heart of the central computer system is an NCR century 200 computer, with a minimum of 32K bytes memory. NCR peripherals include a card reader, I/O writer, line printer, discs and tapes.

The other system is the data acquisition and communications system itself, consisting of a network of 70 terminals throughout the hospital, linked to (and controlled by) a data communications controller system. Brains of this controller system is a Varian 620/L computer, with 24K of core. Also included in the controller system are a data communication multiplexer, a display terminal multiplexer, an I/O writer and a 2.34 million word disc.

The data acquisition and communications system will employ five different types of terminals throughout the hospital, including thermal printers, card readers, and CRT displays. The type of terminals present in each area will vary with the specific needs of each area.

To describe the day-to-day functioning of the hospital data acquisition and communication system, it might be useful to follow a representative patient through his stay at the hospital. When the patient arrives at the hospital, the admissions office will enter him, via CRT, into an on-line doctor/patient/room file. The file will include the patient's name, room and bed, and doctors, including consultants. The file will remain in the computer to be continually updated or inquired upon, as long as the patient is in the hospital.

Also, at the time of admission, a patient card will be keypunched and delivered to the patient's nursing station.

When the patient's doctor arrives at the hospital, he will be able to punch his doctor number into the doctor's register and receive a printout of the doctor/patient/room file for all his patients in the hospital. Noting the location of his newest arrival, the doctor calls on him.

After visiting his patient, the physician decides that certain drugs, lab tests and X-rays will be required. All three can be ordered from the nursing station, by means of a card reader and the appropriate cards from a file at each nursing station.

In the case of the pharmacy order, for example, a patient card is put into the card reader, followed by the employee card of the employee approving the order. Next, a card describing the drug by brand or generic name and number, indicating the drug strength and containing a destination code indicating the pharmacy is inserted, followed by cards indicating the amount ordered and the frequency of dosage.

As the order is being received on the pharmacy printer in label form, *it is also being automatically charged to the patient's account!* (Should drugs be administered from floor stock, they may also be charged to the patient's account from the terminal at the nurses' station. At the same time, a copy of the order may appear on the pharmacy printer, so that the pharmacy can automatically replenish floor stocks.)

In the case of lab tests and X-rays, ordering will follow much the same procedures, substituting laboratory test cards or X-ray cards for the drug cards. As these services may require action by more than one department, all appropriate departments will be notified automatically, routed by destination codes on the test or X-ray cards. Of course, a record of the charges for these tests will also be automatically posted to the patient's account.

Laboratory and radiology test forms and pharmacy labels can be automatically printed in their respective areas, to adhere to proper test result forms or dosage information. Ancillary departments will also have the ability to transmit test results back to the nursing station from keyboards located in the labs.

ASPIRIN—ONE OF THE OLDEST DRUGS KNOWN TO MAN

Aspirin is one of the most common and most widely used drugs in the world today. It is the universal remedy for a score of ailments from a hangover to painful and crippling arthritis.

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and pain—this bark is very similar to Aspirin in its substances.

Needless to say Hippocrates' aspirin was not greatly popular because of its bitter taste and was not much used until the 19th century. Patients suffering agonies from rheumatism had the choice of a foul tasting medicine to quell the pain or just putting up with their rheumatism instead.

The various scientists began studying ways of producing salicylic acid, firstly from the willow tree, then by chemical synthesis.

Felix Hoffman, a Bayer chemist, whose father suffered from rheumatism (and also from the vile tasting medicine) decided to try and produce a better tolerated pain reliever. In 1897 he succeeded in producing acetylsalicylic acid in a pure and stable form which was quite palatable.

Bayer quickly realised the sales potential of the new pharmaceutical discovery. Because its full chemical name was a tongue twister, they named it "Aspirin". The "A" was derived from acetyl and the rest came from *Spiriea ulmaria*, a small plant in the meadowsweet group.

With the outbreak of the 1914-18 war Bayer lost their short monopoly of Aspirin and during the next 30 years various firms launched aspirin on a world-wide basis.

Unfortunately many pharmacists were sceptical of the claims being made on behalf of aspirin, some claims were far fetched, and they preferred to stock older forms of the drug. The Aspro company who were then the leading sellers of aspirin, decided to try and persuade grocers to stock the drug. The result was a long-term loss of valuable revenue for the pharmacist.

Today aspirin is known and widely used under a dozen different trade names. Dispirin, Solprin, Codis, Aspro and many others are merely brand names for the basic drug aspirin.

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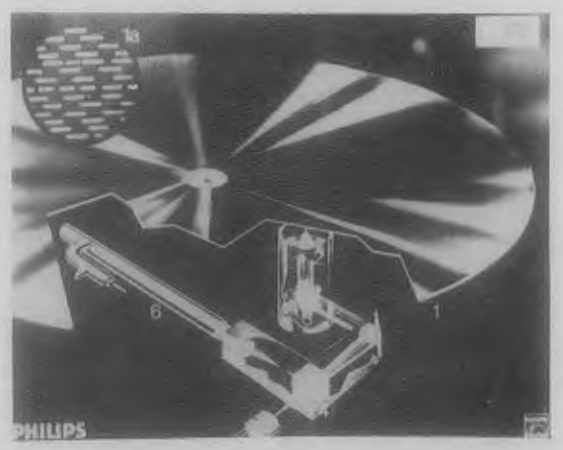
COLOUR PROGRAMMES THROUGH A STANDARD PHILIPS K9 COLOUR TELEVISION RECEIVER FROM THE VIDEO LONG PLAYING RECORD

VIDEO LONG PLAYING RECORD WITH CONTACTLESS SCANNING LIGHT BEAM REPLACING STYLUS

The Philips VLP-system (Video Long Play) is a method for the reproduction of colour programmes stored on video long playing 30cm records made of transparent material with a very thin reflecting metal layer on one side. For the VLP-record a playback speed of 1,500 r.p.m. has been chosen, corresponding to 25 r.p.s.

The video and audio information is contained in encoded form in a track which is only 0.8 μm wide and consists of a sequence of pits so small that they are visible only under a microscope. The tract starts near the centre of the record and continues in a spiral towards the outer edge. The play-back time of a VLP-record ies 45 minutes.

By means of a novel contactless optical light beam scanning system which is therefore absolutely



free of any wear, the specially designed VLP record player converts the pitted track pattern into signals suitable for electronic processing. The VLP-video and audio information is reproduced by ordinary television sets to which the signal is fed via a cable connected to the aerial sockets. In addition to normal reproduction, provision is made for stills, slow motion, speeded-up reproduction, stepwise reproduction and reverse.

Other advantages of the Philips VLP-system are immediate random access to any part of the stored information, the multi-channel sound reproduction in HiFi-quality and the fact that the video long play record is almost completely indifferent to soiling, scratching and dust.

Most advanced technology: Opto-electronics

The light source chosen is a small helium-neon laser generating red light of a wavelength of approximately 600nm (1nm = 1 millionth of a millimeter). Its output power is only about 1nW = 1/1000W. For the purpose of "reading out" the track, the laser generates a tiny light spot with a diameter of one thousandth of a millimeter. The laser beam is projected on to the track via a system of lenses and mirrors and will be reflected according to the track pattern. A photodiode then converts the light variations into a proportional electric signal which is supplied to the subsequent electronic stages of the VLP-record player.

Because the pattern elements and the track width are so extremely small (1mm of record radio contains 500 track windings), the optical system with the read-out laser beam must be controlled by electronic systems which among other things, provide for precise tracking and focussing of the light spot, for the special features such as slow motion, etc., and for constant speed of the drive unit.

Introduction

For several decades the gramophone record with its musical information pressed in grooves has been one of the everyday things of life. "Canned music" on the black disc can be produced relatively simply and quickly in large quantities, is cheap and offers a high level of quality. Therefore the question inevitably arose whether it would not be possible to store and reproduce visual information as required in

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(1) Klein, G. C. and Jones, W. L. : Applied Microbiol. 21 : 257, 1971.
(2) Janeff, J., Janeff, D., Taranta, A., & Cohen, H. : Lab. Med., 1971 (in press).

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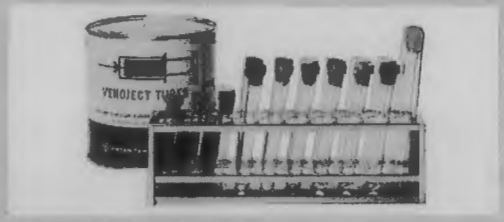

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the same way.

The great demand for more communication, the possibility of individual information independent of a time schedule, the possibility of quickly copying the video information and its immediate availability were arguments so covincing that examination of these problems started years ago in the Philips Research Laboratories. Provisional results of this work were presented in September, 1972, at an international trade press conference: The Philips VLP-system consisting of the information carrier "Video Long Play record" and the VLP-record player with a novel contactless light beam scanning system had entered the field of the new audio-visual media. One year later on the occasion of the Internationale Funkausstellung, 1973, the press in general was also acquainted with the many advantages of the Philips VLP-system.

Video long playing record

The 30cm diameter video long playing record is the same size as an audio LP, and is manufactured by a similar pressing operation. The VLP-record consists, however, of a transparent polyvinyl material coated on one side with a very thin reflecting metal layer. The transparent material protects the information track from contamination so that handling of the record is easy.

The method of storing the information and the playback speed also differ from those of an audio record. The video and audio information is not stored in a continuous groove but encoded in a sequence of longish pits forming a spiral-shaped track. The track starts near the centre of the record —during playback the scanning device moves from the smallest to the largest diameter.

The pits, of micron size, are all 0.16 μm deep and 0.8 μm wide (1 μm = 1/1000mm), but the intervals between them and their individual lengths vary according to the encoded picture/sound information. The pitch of the track is also extremely small, amounting to 2 μm (centre to centre distance of two adjacent tracks). One millimetre of the record diameter thus contains 500 spiral track windings.

A video long playing record playback speed of 1.500 r.p.m. was chosen, corresponding to 25 r.p.s. This was done to comply with our television standard allowing 25 pictures per second to be transmitted. As the VLP-track contains one complete television picture per revolution, the chosen speed and playback via the television set results in a steady, flicker-free screen image. Furthermore, this method facilitates various special operation modes (trick techniques).

Trick techniques

The standardized television picture consists of two fields one after the other, the first containing all the even, the second all the odd lines. This arrangement was also used for the video long playing record. The VLP-TV-image stored in one track winding thus consists of two fields. The information contained in these fields is separated by two synchronization signals. (These signals are not visible in the TV-picture because they fall outside the picture tube screen.) The electron beams of the colour picture tube are shaded by the synchronizing signals during this time so that the image screen does not light up.

To realize the various trick techniques one can, during such a dark period make the read-out light spot on the video long playing record jump from one track winding to the other without its being visible on the TV screen . This is effected by an opto-electronic system which controls the desired functions. The Philips VLP-record player connected up to a television set is equipped for the following operation modes: Normal picture reproduction forward, fast forward (reproduction at twice the normal speed), reverse, continuous still picture, frame by frame reproduction, slow motion forward and reverse (adjustable from 4 seconds to 1/25 second per image). Immediate random access is also important.

An example of the numerous possibilities: As a completely different image can be stored per revolution a 30-minute video long playing record can, for instance contain about 45,000 single frames which can be scanned one by one and selected manually via the remote control unit or by programme control.

Optical track reading

For "reading-out" the VLP-track an optical method is very suitable which, contrary to mechanical scanning, allows contactless information pickup. The traditional stylus is no longer used, thus wear of the record surface is completely eliminated. The quality of the image/sound information remains as good as ever even after the record has been played many hundred times.

The VLP-record player employs a light spot for reading-out the track which is projected on the track via a lens/mirror system. The diameter of the spot of light is approximately 1 μm. If this spot falls on the metal-coated level surface of the record between the pits, nearly all the light is reflected back into the optical system. If however, the light strikes a pit, diffraction occurs and as a result the major part of the light by-passes the optical system because of the changed path of the diffracted light. Thus the intensity of the reflected light is controlled by the pattern of the subsequent pits and then converted into a proportional electric signal by a photodiode.

Laser light source

For several reasons the reflected light must be as bright as possible. Therefore a small helium-neon laser with a wevelength of about 600nm (1 nanometer = 1/1,000.000mm) and red light emission was chosen as light source. Its output power is only about 1mW = 1/1,000W. A special procedure developed by Philips allows mass production of the laser.

The read-out of the information in the track by the laser beam is not affected by the transparent protective layer on the video long playing record. This is because of the optical design of the lens and an electronic control which ensure that the light spot is always focussed on the plane of the pits so that contaminations on the surface of the protective layer, which is on a higher plane, have practically no influence on the photodiode signal.

The master plate which is used for the production of the pressing dies is also "cut" with the aid of the laser which in this case, however, has a much higher output power. Specially prepared glass plates are used for master plates. These plates are cut at the playback speed of 25 r.p.s. (real time procedure). This means that a scene which has, for instance, been recorded by a television camera can be transfered directly to the master plate by means of the cutting laser.

Technical Communications

Avoiding Clots in Auto Analyser Manifolds

One of the most aggravating mishaps afflicting continuous flow systems is blocking of the sample tube line. This is usually due to a fibrin clot from sera separated in a hurry, or post-clotting of plasma, particularly when heated. This can necessitate changing tubes or membranes repeatedly.

Other less common causes are precipitates from the reagents and these are more easily dealt with. Solutions can be filtered or filtering devices attached to the end of the tubes aspirating the solutions. A membrane type of filter is produced by Technicon and a porous filter by Scientific Products.

Clot Detectors have been described. For example Melley **et ali** (1972) [1] described a system whereby an abnormal bubble pattern changed the audible signal from a photodiode and (with luck), the machine could be stopped in time to remove the clot.

A simple alternative on the principle that prevention is better than cure, is to use the standard twin screw plastic mixer normally used to keep suspensions of blood mixed for blood sugars, at all times. This gathers up small fragments of clots and substantially reduces the number of blockages.

A discussion on this subject at the Chemical Pathology Forum at the last conference also brought forth the idea of using a dilute heparin solution in the wash reservoir to prevent plasma reclotting in the sample line.

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—R. D. Allan, Dunedin Hospital.

INDEX TO ADVERTISERS

analytical bio chemistry

For the solution of a variety of considerably complex questions, many analytical methods are employed by the biochemist. An almost classical method of analytical biochemistry came into existence from biochemistry itself, viz. the enzymatic analysis.

Enzymatic analysis means determination of metabolite concentration with the aid of enzymes, measurement of activities and study of the characteristics of enzymes in vivo and in vitro, and analysis of the control and regulatory functions within the cell and in organ metabolism.

molecular biology

The research fields of protein and nucleic acid biosynthesis are nowadays lumped together – unfortunately – under the term "molecular biology." In our program we offer enzymes, substrates, templates, primer, inhibitors and auxiliary reagents, all of which are indispensable for the study of molecular action mechanisms.

enzymology

Enzymologic research revolutionizes biology not only because of the mushrooming number of newly-discovered enzymes. To the extent to which the biologist advances into cellular regions, he will become an enzymologist; to the extent to which the enzymologist interprets biological functions of cell components enzymatically, he will become a biologist. Thus, enzymology has become one of the main pillars of all biological disciplines.

food analysis

The first knowledge of biochemical processes, of the role enzymes play in them and the first experiences with enzymatic analytical methods were gained with foods. Analyses with the aid of enzymes have many advantages in food analysis: enzymatic methods are fast, safe and reproducible. The measurement of enzyme activities as a criterion of the condition of foods is supplemented more and more by enzymatic analysis of the components of foods.

clinical chemistry

This program is noted for its search for ever more specific and predictable test methods for medical research and routine diagnosis while adhering to the rising requirements for precision and accuracy of laboratory data. On the other hand, such a program must also take into consideration the requirements for simplification and rationalization. The ideal requirement is reached when simplification of actual labour allows, at the same time, for an increase in precision.

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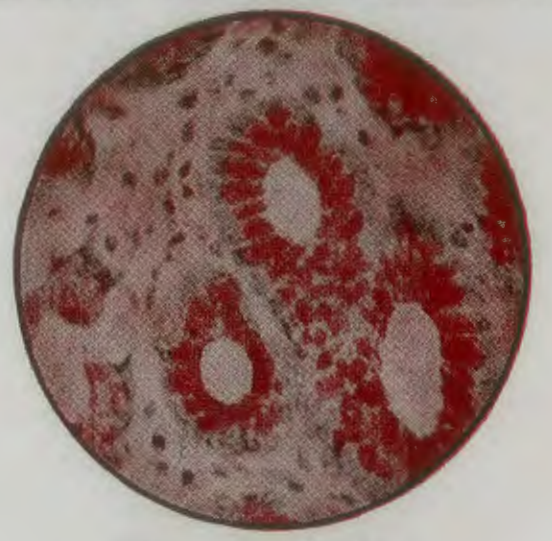

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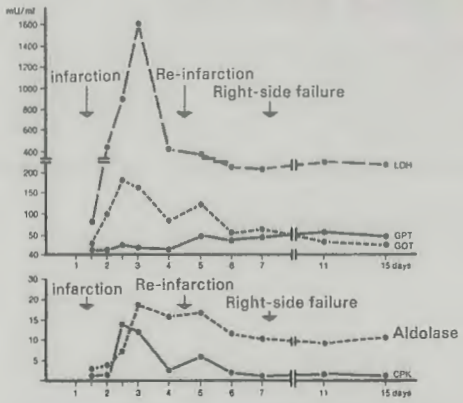
CPK
GOT
In the diagnosis of myocardial infarction, activities of both enzymes rise significantly within 6 hours. If both remain normal, angina pectoris is suspected

Revealing:
The Complications

GPT
Determined along with CPK and GOT to elucidate possible right ventricular failure with liver involvement, noted in a sharp rise of liver-specific GPT activity

Serum Enzyme Activities in Myocardial Infarction



Schmidt, E and Schmidt, F.W: Guide to Practical Enzyme Diagnosis Mannheim, Boehringer Mannheim, GmbH, 1967

The Sensitive Six – The Security of BM Enzymes

In the diagnosis, prognosis and monitoring of myocardial infarction, you can rely on BM enzyme assays to maintain linearity throughout the higher ranges of activity.
Each reagent is manufactured with the meticulous research-oriented care pioneered by Boehringer Mannheim in the production of pure biochemicals. And that means the utmost in specificity, sensitivity, stability and reproducibility.

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