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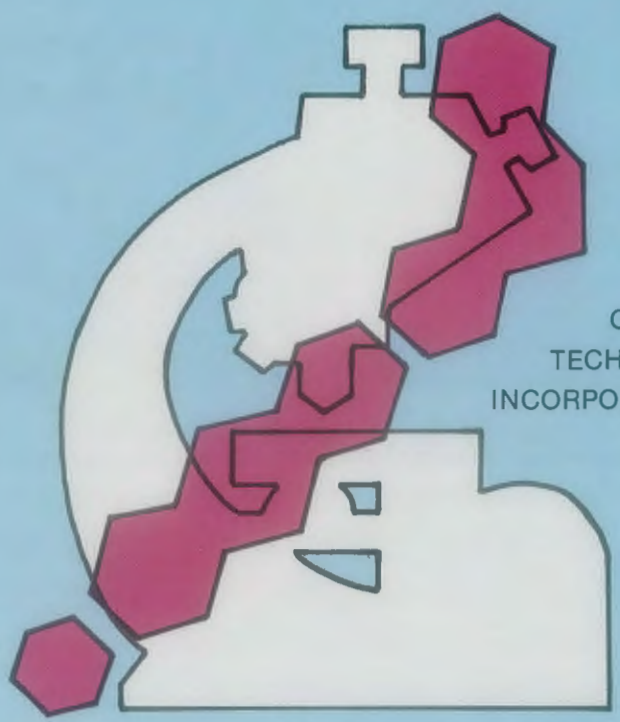

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An Automated Data Management System for Blood Gas Quality Control*

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*Parts of this report were originally presented at the South Pacific Congress in Medical Laboratory Technology, Christchurch, New Zealand, August, 1982.

Abstract

This report describes the development and operation of an automated blood gas quality control data management system that has been recently field tested in 110 blood gas laboratories. The software is designed for centralized data processing and review, but it also enables participating laboratories to monitor and analyze their routine quality control performance individually or as a group. This system utilizes readily available computer hardware components located at a central facility. Simplified data management forms minimize time spent by participating laboratories and facilities rapid turnaround of reports. By emphasizing data management simplicity, as opposed to being tied to large amounts of paperwork, the system aids the laboratory staff, so that they may take appropriate corrective action when a quality control problem is indicated. An additional benefit of this system, is that quality control value comparisons are based on frequent measurements of quality control materials, as opposed to the single samples that may be sent on a monthly or quarterly basis in some QC schemes. This is useful to participating laboratories as it is a more realistic approach to performance assessment. A program that utilizes this approach and is sponsored by a professional association or regulatory group, may provide the basis for improved inter-laboratory standardization.

Introduction

The objective of a blood gas quality control program is not only to produce statistically reliable data, but also to monitor the continued accuracy and precision of quality control measurements and to facilitate interlaboratory comparison and standardization. To this end, an automated data management system has been developed that enables a central processing facility to accept and analyze blood gas quality control data from groups of laboratories. This system allows the individual laboratories to assess their quality control performance relative to the group as a whole, as well as to components of that group (such as all instruments of a particular model).

To accomplish this, a central facility is established (possibly under the auspices of a local Bioanalyst or Technologist Society). This central group has a dual role, one is to set-up and oversee logistics of Program Management, and the other is to actually process the data and report on the technical aspect of the resultant figures.

Once participants and central groups are set-up, the participating laboratory collects data and records them on specialized data forms, and then submits the forms to the central facility for processing. This central facility, using software designed to run on readily available mini-computer hardware components, then processes the data as received, reviews them and submits reports to the participants.

The system, originally developed to test the in-laboratory performance of a new multi-sample control material*, was later expanded to be of general use in quality control data assembly and analysis. The interactive data management program used in the central facility is menu driven for all functions, including data entry, editing and report generation. The system has been refined into an informative and systematic method highly suitable for tracking and presenting blood gas quality control data, whether it is used primarily as a part of a field trial program or as a stand alone system.

*Confirm^{TM}pH/Blood Gas Quality Control (Corning Medical; Medfield, MA 02052, USA).

Materials and Methods

In order to make this system applicable to the general requirements of blood gas quality control as well as to the particular needs of individual labs, it was necessary to select system components according to worldwide availability and serviceability, cost, flexibility, ease of data access, and overall ease of operation. It was also necessary to develop specialized support documents that simplify data input tasks of the participating labs.

HARDWARE

The system hardware requirements were met with an Apple II (Apple Computer, Inc., Cupertino, CA, USA) processing unit coupled to three disk drives that utilize DOS 3.3 and 16 sector diskettes. This basic arrangement is combined with a video display terminal (Sony Trinitron Model KV8000, Sony Corp., Tokyo Japan), an IDS 460 printer (Integral Data Systems, Milford, NH, USA) and an Apple keyboard with a separate numeric keypad (California Micro Products, San Jose, CA, USA) that facilitates data input. Alternative hardware components can be used, but may require system modifications to run the QC software.

SOFTWARE

The data management is programmed in Applesoft Basic, and requires three types of information on three diskettes. First, there is the main program disk, which contains all files for group cumulative statistics and backup files of laboratory demographic information. Finally, each lab is assigned a unique disk that contains customer identification and quality control information. These disks are completely interactive, as shown in Figure 1.

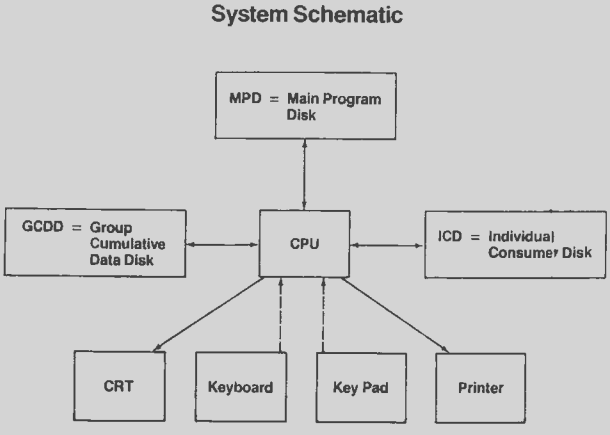

Fig. 1. A schematic diagram of the interrelationships between the Central Processing Unit (CPU), the disk drives for each major function and the input/output devices. The CRT (Cathode Ray Tube) or VDU (Video Display Unit) is actively used during data input, review and editing while the printer is used primarily for final reporting.

Support Documentation

The support materials package includes four basic components: the file assignment cards; the laboratory start-up kit; the data input records; and the laboratory data/troubleshooting record.

The file assignment cards contain spaces for all pertinent laboratory demographic data, and when completed and returned to the central facility establish the user's file in the main system.

The laboratory start-up kit contains instructions and a letter introducing the user to the system. Data input forms enable customers to report their quality control data to the central facility. In addition, an optional form for use within each laboratory, which combines a "Weekly Data Record" and a Troubleshooting Log, enables blood gas operators to record on one document all information regarding quality control and troubleshooting activities.

Initiation

The data management system is put in place by the member laboratories, who submit file assignment cards to the central processing facility. The cards establish hospital or lab identification, address, data volume information, and blood gas instrument identification. If several instruments are used in an institution, they may be entered under one file number.

Once the file assignment cards have been processed, the laboratory is then sent a start-up kit and the required data submission forms. Upon receiving the start-up kit, the member laboratory begins routine QC data submission to the central facility.

Laboratory Data Management

The participating laboratory's role consists of filling out the various records and carrying out basic data management functions. The data management functions include filling out the Weekly Data Record, performing statistical calculations, and entering "reduced" data in Summarized Input Forms. The Weekly Data Record facilitates proper data entry, and may also be used to record error conditions and troubleshooting actions taken to correct a problem. Once data have been collected for an appropriate interval, the participating lab must derive the mean and other basic statistical values. Using this system, it is recommended that QC data reduction is done in four steps with a standard hand-held calculator.

The first step is the elimination of "analytical" outliers, values that are known to have occurred during a period of instrumental malfunction. The mean, standard deviation, and number of data points are then calculated. The next step is the elimination of appropriate "statistical" outliers. Finally, the mean, standard deviation, and data points are recalculated and recorded on the Summarized Data Form, which has entries for each level and constituent. The form is then submitted to the central computer service.

Upon receiving the Summarized Data Form from a participating lab, the central computer service enters the data onto a customer disk, determines individual lab and group summaries, and makes appropriate comparisons of the results. The technical staff of the central service then reviews the information to determine if there are either individual or group patterns showing sub-optimal performance. The staff then contacts the individual labs to assist in any required troubleshooting actions. If no performance anomalies exist, the data are reported without further follow-up.

Results

The quality control data management system described in this article was successfully used to collect, enter, and report data back to some 110 participating labs within three working days after cut-off date for the receipt of the data forms. In general, a major limitation of using a central computer service is its dependency on the initial receipt of data from participants. As expected, this factor accounted for about 75 percent of time interval between the end of a month and laboratory receipt of data; nevertheless, the total interval between the end of the month and the return of analyzed data from the central facility was normally less than 12 days. Once data forms were returned, data were en route to participating labs within three working days.

Discussion

The QC data comparisons that the system provides enable useful inter-lab performance evaluation either by participating labs alone or by integration of the system with programs sponsored by professional societies or regulatory groups. The experience cited, with approximately 110 labs included, demonstrates the system's usefulness with small, relatively homogeneous groups of participants. When these factors are coupled with hardware availability and relatively low cost, the advantages of such a system as part of the development of a comprehensive quality control scheme become significant. The system appears to be well-suited for groups of hospitals, or a single hospital that has satellite facilities. This could be particularly useful in isolated or remote areas, as it would enable labs there to monitor their QC information on a real-time basis.

Several modifications currently under consideration would further enhance the utility of the system. One modification would enable the user to directly enter his lab's identification number and data via a modem. This communication link would provide the advantages of simplifying the data reporting phase, and offering virtually instant turnaround time. The communications link would also indirectly lower the initial cost of the system for the central facility. With the current system, the central facility must maintain an individual diskette for each participating lab. The communication link would enable the central facility to drop the individual diskettes and the corresponding hardware.

A final system enhancement—a graphics software package—would provide histograms and other basic visual displays for the QC data. This would allow for easier interpretation, and thus help lab personnel to more quickly recognize problem patterns within the group.

While the software package that we used is not currently marketed, we believe that our experience has demonstrated the feasibility of the basic concept of using a mini computer for this type of application. Where the enhancements described are combined with the basic features of the current system, a participating lab as well as the central facility should find a similar package to be an extremely useful component of their overall quality assurance program.

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A Simplified Method for the Demonstration of the Phospholipid Profile of Amniotic Fluid using Plastic Backed Thin Layer Chromatography Plates

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Abstract

A rapid simplified method for the determination of the phospholipid profile of amniotic fluid is described. The method uses plastic backed thin layer chromatography sheets and the phospholipid spots are identified by staining with cupric acetate solution and heating. The rapid, precise identification of the individual phospholipid spots and easy handling and storage of the sheets make the method especially suitable for the estimation of fetal lung maturity.

Key Words

Thin layer chromatography, Phosphatidylglycerol, Respiratory distress syndrome.

Introduction

Phospholipid analysis of amniotic fluid by two dimensional thin layer chromatography is now widely used to predict fetal lung maturity. It has been shown by Whittle[1] that with a lecithin sphingomyelin ratio of two or more and phosphatidylglycerol present in amniotic fluid, pulmonary distress in the newborn infant is virtually nonexistent. Thin layer chromatography methods including our own[2] have commonly used glass plates coated with silica gel H and added ammonium sulphate. When these plates are heated to high temperatures (160°C) the sulphuric acid produced chars the phospholipid spots. There are however several major drawbacks to the use of glass plates; these include cracking, the time required for the chromatogram to run in two dimensions and the inability to produce long term records. Plastic backed thin layer chromatography sheets and cupric acetate stain used in one method of producing the lecithin: sphingomyelin ratio have recently been employed in our laboratory to simplify the production of the lung phospholipid profile. The extraction methods described are based on the work of Kulovich et al[3] with the inclusion of an acetone precipitation step suggested by Gluck et al[4-6] to separate surface active phospholipids from total lipid extracts. A 5 ml sample of amniotic fluid allows duplicates to be run, but analysis may also be performed on 2 ml of fluid. The amniotic fluid obtained by amniocentesis was centrifuged at 900 g for 10 minutes and the supernatant was stored at 2-3°C if not being processed immediately and frozen if kept longer than several hours. 5 ml of the supernatant was pipetted into a small conical flask and 5 ml of methanol added and shaken. 12 ml of chloroform was then added and the mixture was stirred vigorously for five minutes. The resulting emulsion was poured into a 30 ml corex tube and placed in ice for two minutes. On centrifuging at 2000 g for five minutes the emulsion separated into two layers, the upper one of which was aspirated off and discarded. 5 ml of the bottom chloroform layer was pipetted into a tapered centrifuge tube and evaporated with a stream of dry nitrogen at 60°C. The sides of the tube were washed down with a small amount of chloroform which was evaporated to dryness as before. Two drops of cold acetone were added to the tube which was agitated slightly. A white precipitate formed, after which 0.75 ml of cold acetone were added and the tube was placed in an ice bath for 15-30 minutes. The tube was then centrifuged for two minutes at 2000 g, the supernatant decanted and the precipitate dried in a stream of dry nitrogen. If 2 ml only of amniotic fluid was available, the supernatant was pipetted into a stoppered tube and shaken with 2 ml methanol after centrifugation. 6 ml of chloroform were then added and the tube was vortexed for 1-2 minutes. The resulting emulsion was then centrifuged for five minutes at 2000 g, the chloroform layer transferred to a tapered tube and treated as for a 5 ml sample. The dry precipitate was redissolved in 30 μl of chloroform: methanol (75:25) and 12 μl of this sample was spotted onto a 10 cm × 10 cm chromatography sheet (Merck silicagel 60 without fluorescent indicator) 1.5 cm from the bottom and 1.5 cm from the right hand side of the sheet. 5 μl of 2:1 lecithin: sphingomyelin standard (Sigma) and 5 μl of 0.2 mg/ml phosphatidylglycerol standard (Sigma) were spotted onto the sheet 1.5 cm from the bottom and 1.5 cm from the left hand side. 5 μl of the phosphatidylglycerol standard were also spotted 3 cm from the top and 1.5 cm from the right hand side of the sheet (Fig 1a). A further 12 μl of sample were spotted onto another sheet together with 5 μl of 0.2 mg/ml phosphatidylglycerol standard, 1.5 cm from the bottom and 1.5 cm from the right hand side. (Fig 1b). This sheet acted as a reference for the position of the phosphatidylglycerol spot and was run and developed at the same time as the sample sheet. The sheets were developed for a distance of 5 cm (approx 15 minutes) in solvent A, dried in an oven at 100°C for two minutes then cooled, turned through 90° and developed a similar distance in solvent B (approx 10 minutes). The sheets were dried in air for 10 minutes and stained standing vertically in cupric acetate stain for two minutes. The sheets were then drained and stood upright on paper tissue until the surface no longer appeared wet. After wiping the backs of the sheets free of excess stain, they were placed in an oven at 145-160°C for 10 minutes. The phospholipids appeared as black spots. After cooling, the L:S ratio could be measured by reflectance densitometry or with a ruler (l × w) and the presence or absence of phosphatidylglycerol noted. (Fig 2).

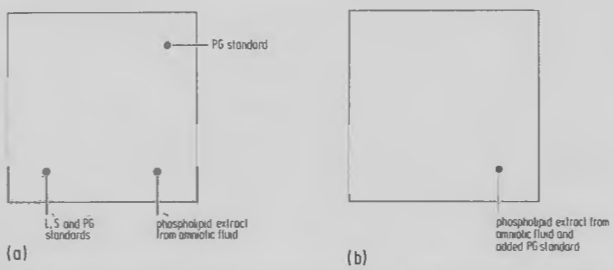

Fig. 1. Preparation of thin layer chromatography plates for analysis of amniotic fluid phospholipids. (a) Sample sheet. (b) Reference sheet with phosphatidylglycerol (PG) added to amniotic fluid extract.

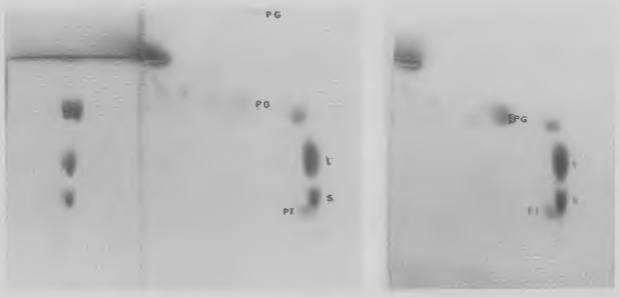

Fig. 2. Thin layer chromatograms showing the lung phospholipids lecithin (L) sphingomyelin (S) phosphatidylglycerol (PG) and phosphatidylinositol (PI). (a) Sample sheet. (b) Reference sheet with phosphatidylglycerol added to amniotic fluid extract.

Solutions and Reagents

SOLUTION A. Chloroform 40: methanol 20: conc. ammonia 3.

SOLUTION B. Tetrahydrofuran 40: methylal 28.5: methanol 7.8: 2N ammonium hyroxide 4.2.

STAIN. Cupric acetate. H_2O 22.5 g, orthophosphoric acid 8 percent 580 ml, ethanol 146 ml. Add the phosphoric acid and ethanol to the cupric acetate. Stir to dissolve. Stable at least one month.

Results and Discussion

Plastic backed thin layer chromatography sheets and cupric acetate stain used for the determination of the lecithin: sphingomyelin ratio have now been successfully employed to demonstrate the phospholipid profile of amniotic fluid. Suitably sized chromatography sheets are developed in two dimensions resulting in clearly recognisable phospholipid spots (Fig 2). Where the phosphatidylglycerol spot is barely visible, this is recorded as a trace only and is not regarded as sufficient evidence of a mature lung profile. Phosphatidylglycerol alone may be used as a test for fetal lung maturity, but we would agree with the view[7] that the lung phospholipid profile is a much more valuable result. The profile not only gives the L:S ratio but also shows the presence or absence of phosphatidylglycerol and other developmentally important phospholipids such as phosphatidylinositol[8]. The plastic sheets are suitable for storage as a permanent record of the analysis and are easily photographed.

The detection of phosphatidylglycerol (PG) as a diagnostic test for fetal lung maturity is now widely used. It overcomes the problems of ratio measurements, is unaffected by blood or meconium contamination of the amniotic fluid and is also readily detectable in vaginally draining amniotic fluid resulting from premature rupture of the membranes[9].

It is hoped that the presentation of this method will encourage more widespread local use of the lung profile.

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Crossreactivity in Immunoperoxidase—Human Placental Lactogen and the Pituitary

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Abstract

Commercially available antisera to human Placental Lactogen (hPL) was found to crossreact with an antigen present in the pituitary when used in an immunoperoxidase procedure. When applied to pituitary adenomas it was observed to stain similarly to human Growth hormone (hGH) and was not present in those adenomas exclusively positive for Prolactin (Pro). Its application to pituitary pathology lies in its use as a possible inexpensive alternative to hGH immunohistochemistry. Antisera to pituitary hormones though should be tested for crossreactivity and placental tissue is suggested as a useful substrate.

Introduction

It is well documented that antibodies raised against human Chorionic Gonadotrophin crossreact with Leutenizing hormone in radioimmunoassay procedures (1). Robertson et al (2) found human Chorionic Gonadotropin-like immunoreactive material to be present in the normal pituitary.

Recently a Granular Cell Myoblastoma of the posterior lobe of the pituitary (3) was submitted for immunoperoxidase studies. This tumour stained positively for carcinoembryonic antigen whilst it was negative for alpha fetoprotein, human Chorionic Gonadotrophin, human Placental Lactogen (hPL), and Pregnancy-specific beta-l-Glycoprotein. The uninvolved anterior pituitary associated with this tumour showed positive hPL stained cells as well as positive human Chorionic Gonadotrophin cells. This unexpected finding prompted us to attempt to identify the antigen present in the pituitary crossreacting with rabbit anti-hPL.

Methods

In order to determine the hPL crossreacting antigen, immunoperoxidase staining was done on four autopsy pituitaries, fourteen pituitary adenomas and placental tissue. Immunoperoxidase kits from two suppliers (Table 1) were used in the study. Antigens localised included: human Growth Hormone (hGH), Prolactin (Pro), Corticotrophin (ACTH), Thyroid

Table 1. Reagents

Reagent	Manufacturer	Code No.	Lot No.
Rabbit anti hPL sera	Dako	A137	128 C
hGH, Pro, ACTH PAP kit	Dako	K514	062/1
hGH histoset	Immulok	H136	031 018
Pro histoset	Immulok	H133	041 029
ACTH histoset	Immulok	H123	081 020
TSH histoset	Immulok	H132	051 046

Stimulating Hormone (TSH) and hPL. Immunoperoxidase techniques used were as suggested by the kit inserts. HPL localisation has been described previously (4).

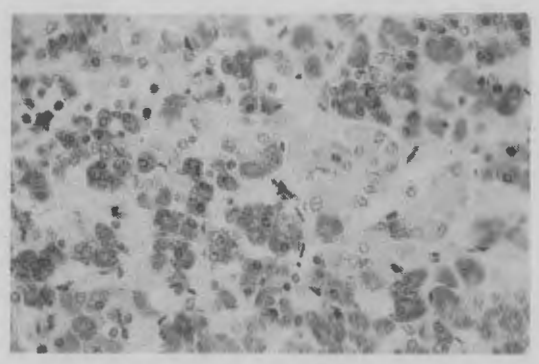
The optimum titre (4) of rabbit anti-hPL was done on placental tissue and pituitary. Each case included in this study was stained with HE and Periodic acid-Schiff-Orange G (PAS-OG) (5).

Absorption studies were not done due to the excessive cost of purified pituitary hormones.

Results

NORMAL PITUITARY STAINING AND KIT DIFFERENCES

Immunoperoxidase kits from two suppliers (Table 1) were used in parallel on autopsy pituitaries and placental tissue. For hGH and Pro the Dako kit have a stronger reaction than the Immulok kits but background staining was unacceptable. Therefore the Immulok kit was selected for use. The Immulok ACTH kit gave a less definite result than the Dako procedure so the Dako kit was preferred. These differences are believed to exist due to the differing optimum titre determinations. There was no detectable staining on placental tissue for any of these hormones with either of the manufacturers' kits. HPL was localised in all four pituitaries and placental tissue (see Figure 1). In each of the pituitaries approximately 50 percent of the cells stained for hPL. This distribution was similar to that obtained with hGH staining.



Normal pituitary stained with IPhPL. Note dark staining hPL immunoreactive cells (×250).

OPTIMUM TITRE RESULTS

Using two different substrates, namely pituitary and placental material a similar optimum titre of 1/300 was found for Rabbit anti-hPL (Table 2). A detectable reaction was noted up to a dilution of 1/6400 in both instances. This most probably infers total immunohistochemical crossreactivity of the hPL antisera with the hormones present in the pituitary and the placenta.

PITUITARY ADENOMA STAINING

Results of hormone localisation in 14 pituitary adenomas is given in Table 3. With the exception of one adenoma, all adenomas were chromophobic on HE and PAS-OG stains. The exception (case 2) was a mixed type consisting of acidophils interspersed with chromophobic cells. Two adenomas (cases 1 and 2), associated with clinical features of acromegaly, stained positively for hGH. These tumours were also strongly reactive for hPL. One of these adenomas was also positive for Prolactin (case 2). Pro was localised in a further four adenomas (cases 3 to 6). Staining for hGH, hPL, ACTH, and TSH were negative in these cases. A further eight adenomas (cases 7 to 14) were negative for all hormones studied.

Since positive hPL staining occurred in hGH positive adenomas and not in those adenomas exclusively positive for Pro, it is reasonable to strongly suspect that the hPL antisera used in this study crossreacts with hGH.

Table 2: Rabbit anti hPL optimum titre results

Slide Titre	A neat	B 200	C 400	D 800	E 1600	F 3200	G 6400	H 12800
Placenta								
specific	0	3	2	2	2	1	1	0
B. ground	0	1	0	0	0	0	0	0
Pituitary								
specific	0	3	3	3	2	1	1	0
B. ground	0	2	2	1	0	0	0	0

3 Heavy Staining
2 Moderate Staining
1 Weak Staining
0 No Staining

Table 3. Staining Results on Pituitary Adenomas

Case No.	Identity	Hormones hGH	hPL	Pro	ACTH	TSH	Tumour Type
1	A7963	+	+	–	–	–	Chromophobic
2	A6986	+	+	+	–	–	Mixed acidophil and Chromophobic
3	439681	–	–	+	–	–	Chromophobic
4	A5868	–	–	+	–	–	Chromophobic
5	73976	–	–	+	–	–	Chromophobic
6	27646	–	–	+	–	–	Chromophobic
7	A15669	–	–	–	–	–	Chromophobic
8	A16094	–	–	–	–	–	Chromophobic
9	106564	–	–	–	–	–	Chromophobic
10	37718	–	–	–	–	–	Chromophobic
11	78536	–	–	–	–	–	Chromophobic
12	A9069	–	–	–	–	–	Chromophobic
13	71136	–	–	–	–	–	Chromophobic
14	42298	–	–	–	–	–	Chromophobic

Discussion

Human Growth Hormone, Prolactin and human Placental Lactogen may have evolved by genetic reduplication from a smaller common ancestral peptide. All are single chain peptides with two or three intrachain disulphide bridges, about two hundred residues and no carbohydrate (6, 7). Whilst comparing hPL with hGH it was found that of 190 amino acid residues, 163 were identical with those of hGH. This may explain the close immunological similarity between the two hormones (6). It is interesting to note that the discovery of hPL depended on its antigenic similarity with hGH (8, 9). It has been established though that hGH and hPL are different antigens, each with its own distinctive antigenic determinants, but both sharing other common antigenic groups (9). The titre results using Rabbit anti hPL on sections of placenta and pituitary suggest that this commercial antiserum was raised against those common antigenic groups.

This crossreactivity is further emphasized by the results on the pituitary adenomas. HGH and hPL were both localised in tumours of acromegalics, whereas hPL was not present in tumours which stained positively for Pro only. HPL positive tumours did not have detectable ACTH or TSH present. Therefore it is reasonable to assume that anti-hPL sera crossreacts with hGH. But this should be accepted with reserve. Anti-hPL sera may in fact crossreact with a hormone or antigen not tested by this sytem. Beck et al (9) localised hPL in pituitary cells using immunofluorescence. They found that antisera prepared in their laboratory reacted similarly to anti-hGH serum.

There are several methods of decreasing antibody crossreactivity in immunoperoxidase techniques. By the method of Optimum Titre (4), one may dilute out low titre crossreacting antibodies but as shown by the results on placenta and pituitary, this method was not successful. The antiserum may be absorbed with the crossreacting antigen. Halmi and Duello (10) found that using heterologous staining with anti-rat Pro raised in rabbits on pituitary adenomas, the staining of Pro was often enhanced by absorption of the anti-Pro sera with GH. Absorption of anti-hPL

with the pituitary hormones was not attempted in this study due to the high cost of purified hormones. A third method of dealing with crossreactivity is to use monoclonal or hybridoma antibodies. These antibodies are raised against a single antigenic determinant. This circumvents the problem of polyclonal antibodies in that one can produce an antiserum that will bind to a single unique part of the molecule to be demonstrated. Wahlstrom et al (11) used a monoclonal antibody to human Chorionic Gonadotrophin in an immunoperoxidase technique on sections of placenta, pituitary gland and pituitary adenoma and found specific staining in the trophoblast of the placenta whereas no staining occurred in the pituitary or adenomas. Bundesen et al (12) raised monoclonal antibodies to hGH for use in a radioimmunoassay system. In general they found that monoclonal antibodies which displayed minimal hPL crossreactivity exhibited low sensitivity in radioimmunoassay for hGH while those with high hGH sensitivity showed equal reactivity with hPL (12).

Many immunohistochemical studies in the literature use antisera absorbed with the antigen used to produce it as a control. If the commercial hPL antisera used in this study was absorbed with hPL and tested on placenta and pituitary resulting in no detectable reaction, one could suppose that this antisera is specific for hPL. The results of this study suggest that hPL antisera should be absorbed with its crossreacting antigen, in this case hGH, so as to demonstrate specific hPL staining (13). Alternatively this crossreactivity can be used to demonstrate hGH producing cells in pituitaries and their adenomas. This application is useful in that antisera raised to hPL is cheaper to produce than to hGH, since large amounts of hPL are present in the placenta and the serum of pregnant women. HGH is less readily available.

Acknowledgements

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A Brief Evaluation of the Auto Carousel in the Emit[1] Lab 5000 System Using Emit Aminoglycoside Reagents

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Abstract

A brief evaluation of the autocarousel used with the EMIT[1]Lab 5000 system is reported.

The three aminoglycoside kits used with this instrument were gentamicin, tobramycin and netilimicin. Of these, the netilimicin assays did not have a set of method parameters stored in the CP 5000 and this showed the ease and simplicity of introducing new assays to the Lab 5000 system.

We used the EMIT controls to measure the within and between run precision and obtained excellent C.V.s.

Difficulties arose when trying to cut down reagent usage by decreasing volumes on the autocarousel and this causes it to be the most inflexible part of the Lab 5000 system.

Storing the calibration curve for the life of the reconstituted reagents considerably lowers the cost of the analysis with no loss of analytical performance. This makes this system ideal for stat analysis.

Introduction

The autocarousel is one of three components that is used in the EMIT Auto Lab 5000 system.

The Auto Lab 5000 is a clinical laboratory instrument system designed specifically for EMIT assays and consists of the following modular components.

1. Autocarousel.
2. Spectrophotometer.
3. CP 5000 processor.

1. The autocarousel monitors the biological fluid, dilutes it in buffer, adds the required amount of reagents and transfers it to the spectrophotometer.
2. The spectrophotometer measures the absorbance of the reaction mixture at 30 degrees centigrade at 340 NM.
3. The CP 5000 processor automatically monitors and synchronizes the operation of the other two components and reads the absorbance from the spectrophotometer at predetermined times. These data are used in calculating concentration from stored calibration curve and other assay parameters.

Materials and Methods

The EMIT kits and controls for Gentamicin, Tobramycin and Netilimicin were obtained from Syntex Laboratories (NZ) Ltd and reconstituted according to the manufacturers instructions. All assays were performed on the auto lab 5000 system.

Table I: Within run precision obtained with the EMIT control for the three aminoglycosides.

	Gentamicin	Tobramycin	Netilimicin
n	17	19	16
ug/ml	6.3	6.3	6.05
SD ug/ml	0.03	0.17	0.07
CV %	1.3	2.6	1.1

The setting up of the autocarousel has only a few simple steps taking approximately five minutes. This time includes priming the lines with the buffer and placing reagents and sample in the sampler. The spectrophotometer and CP 5000 were set up according to manufacturers specification.

When the run switch is pressed, the carousel rotates until the cup sensor detects the presence of the reaction cup and then automatically pipettes sample and reagents and transfers them to the spectrophotometer.

As the calibrators are assayed, the CP 5000 prints the calibrator level together with initial absorbence and rate.

After the calibrators have been assayed, the calibration curve is presented to the operator (Figure 1).

The curve can then be accepted or aberrant calibrators reassayed. After the calibrator curve is accepted, all samples are analysed and the results cup calculated from this curve and printed out in concentration units.

The curve can be stored for use in additional runs and can be used until the expiry time of the EMIT reagents.

Table 2: Within run precision obtained with the Gentamicin assay using EMIT calibrators.

	CALIBRATOR CONCENTRATION		
	2.0 ug/ml	8.0 ug/ml	16.0 ug/ml
n	7	7	7
mean ug/ml	2.03	8.2	15.6
SD ug/ml	0.05	0.22	0.85
CV %	2.3	2.2	5.4

Results

WITHIN RUN PRECISION

Table 1 summarizes the within run precision studies. The EMIT control with a value of 6.0 ug/ml is right in the centre of the calibration curve, which would give a false picture of its performance. Using Gentamicin as an example, three calibrators, one at each end of the curve and one in the centre, were assayed and the results summarized in Table 2. These results prove this point of the danger of running a single control at the centre of the range which is most precise for this type of assay. As not all specimen results fall here there is a need to control the whole curve.

STABILITY OF STORED CALIBRATORS FOR TOBRAMYCIN ASSAY

The five calibrators for tobramycin were run and the calibration curve stored in the CP 5000. Over the next five days, the tobramycin assay was performed using stored calibrator data to calculate the concentration of the control samples.

The between run precision with the emit control (6.0 ug/ml) on the five consecutive days gave a CV of ± 1.6 percent.

REDUCTION IN REAGENT VOLUME

In an endeavour to economize on reagent usage, reagent volumes were reduced by two ways:

1. Decrease volumes by changing syringe stops.
2. Dilution of reagents.

In trying to reduce reagent volumes, the relationship between the sample volume and total volume ratio and reagent volume to total volume ratio, were kept as close as possible to Syva's recommended amounts. The syringes and stops available made it impossible to reduce volume and maintain the recommended ratios. When the reagent was diluted, an increased measuring time was used to give sufficient sensitivity and place calibration curves on the graph supplied by Syva.

However, without altering graph constants, it was not possible to get a linear graph which the CP 5000 requires to calculate results.

FIG 1

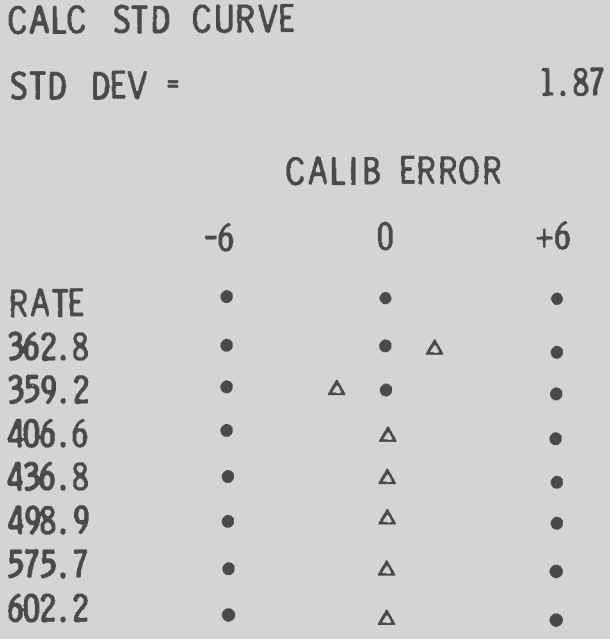

Fig 1. Each diamond (Δ) indicates the position of a calibrator absorbance rate in relation to the standard curve. The "+" and "–" columns indicate the maximum number of A units that a point may deviate from the standard curve and still remain within the tolerance limits recommended in the EMIT package insert.

Discussion

The autocarousel as part Auto Lab 5000 system, speeds up and makes emit assays simple, rapid and with a high degree of analytical performance. It processes large batches with minimal operator contact and stat samples with very little set up and run delay times.

The autocarousel showed excellent within run and between run precision throughout a wide range of concentration.

Although unable to decrease analysis costs by decreasing reagent volume, the ability to run stat tests on small batch setup using the started calibrator data reduces assay cost considerably.

Although the CP 5000 has only a few emit chemistries programmed into it, the flexibility of the unit enables new chemistries to be introduced very simply with minimal setup.

Reference

1. Emit is a registered trade mark of Syva Co., Palo Alto; CA 94304.

Environmental Sampling in Wellington Hospital

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Summary

At the end of 1979 a method was set up and used routinely throughout 1980 to check the environment in our hospital. The methods devised were to give us a reliable and replicable result and were carried out with the minimum of fuss and detail; they involved the use of the 'agar sausage' technique (1). As well as using an agar sausage impression, a swab was taken of each surface tested. A standard counting and grading system was

devised to give us a quick indication and guideline for each ward. The system indicates the level of bacterial contamination in a particular ward or area at the time.

Introduction

In a large hospital such as ours, we believe it is part of the responsibility of the Cross Infection laboratory to keep an environmental check on various areas of the hospital (2). We have found that our routine testing has enlivened an active interest by the staff, so as well as being informative to heads of staff, our tests are of educational interest to those concerned.

The routine testing of a hospital is a lengthy process so our objectives, before we started, were to devise a reliable but relatively simple testing procedure. Since the tests are of no value unless they are done throughout the hospital regularly, we have established a pattern of checking the wards six monthly and special care areas, for instance the milk room and the intensive care unit, more often, approximately three monthly.

In setting up this method for the testing we experimented with various types of impression plates. The most convenient to make, store and handle has been the agar sausage. We can count the colonies growing on the agar and convert them to organisms per metre squared. If we sample a range of at least ten areas it is possible to take a median count and give a grade for the whole ward. As well as using the agar sausage for an impression plate of the surface and consequently a bacterial count, a swab was taken of the area directly adjacent and this was plated out and used to identify any potential pathogenic bacteria. In some cases air counts have been done if there is some particular reason to be concerned over the ventilation process.

When we began routine testing we checked all the solutions and creams in the wards for sterility. However after several months of this and not growing anything, it was decided that we could drop this from our procedure.

Materials and Methods

AGAR SAUSAGES

These are made from 1.5 percent Letheen Agar. While the agar is still hot it is poured into a length of clean (brand new but not sterile) plastic sleeving (8 cm diameter) as used for packaging of needles etc. in theatre. The length is knotted tightly at the bottom and tied with string at the top. It is tied to an overhang and allowed to set. The sausages are stored in the fridge. Each sausage we use contains 300 mls agar—this seems a good size to handle in the ward.

SWABS

Ordinary throat swabs are used for swabbing surfaces. These are moistened in Letheen Broth before being used.

METHOD

In the wards every cubicle's floor and basin is tested, the utility rooms have benches, trolleys and shelves checked in addition. In the bathrooms the floor, bath, shower and one basin are tested and any other area in the ward which may be a harbourer of bacteria.

The sausage is pushed hard down onto the surface and a 3 mm slice is sliced off with a sharp knife into a sterile petri dish. The dish is labelled. A swab is then taken of a 9 cm^2 area adjacent to the sampled area, and labelled. Back in the laboratory the petri dishes are incubated for 24 hours at 37°C and the swabs are plated out onto blood agar and incubated under the same conditions.

Results

The results are read and recorded as a bacterial count and a description of what grew from the swab e.g. 24,000 orgs/m^2; mixed growth, predominantly *Staph aureus*.

Once all the plates have been looked at and the bacterial counts made of each cubicle, bathroom etc, the median is found and this is recorded and given a grade (A to E). This is the ward's grade.

The grade is a summary score, for example A $\leq$10,000 colonies/m^2, B = 10,000-19,999 colonies/m^2, C = 20,000-29,999 colonies/m^2, D = 30,000-39,999 colonies/m^2, E $\geq$ 40,000 colonies/m^2.

We interpret the grades to mean:

A = very good, B = good, C = satisfactory, D = not very good, E = unsatisfactory.

Following repeated testing, we have found that most of the ward areas generally have grades of C or D, while intensive care areas and theatres almost always get an A grade. Grades normally correspond to visual cleanliness but often a ward may look clean yet have high levels of bacterial contamination.

The swab cultures are used for identification of possible pathogens. The main organisms we find that we isolate and identify are *Staph aureus* and gram negative bacilli. We report these to the ward and usually mention it in the covering letter.

Discussion

The grading system is by no means a precise representation of the environment but it is a standard estimation which is easy to arrive at and as accurate as is necessary in this sort of work. We are now reporting these grades to the Charge Nurses and have found that it is easier for them to interpret as to whether or not their ward is up to par or not and consequently there has been more attention given to cleaning if the ward has had a D or E grade. Originally we did not release the grade because we thought people might think of our tests as a competition amongst themselves; however, even if this does occur, perhaps it might not be such a bad thing.

The grading system was devised by Doctor Stanhope (Epidemiology Unit, Wellington Hospital) after analysing results of our environmental tests. It was suggested the median value be chosen of at least 10 sites tested (10-20 representative sites is sufficient for statistical purposes). The median value was chosen as a measure of central tendency of bacterial counts because:

(a) the median can be chosen even when the upper boundary of the highest class is not defined, for example, $\geq$40,000 organisms/m^2 (whereas the mean cannot),

(b) the median is less influenced by extreme values than the mean,

(c) the median contains more information about the overall distribution than the mode.

"Grade" is actually a summary score and has been defined above.

We have found that our results are not always properly understood or are misinterpreted by the clinical staff. This is a problem as from time to time it is common to isolate a pathogen in a ward—this need not be anybody's 'fault' but inevitably it is misunderstood. We are now at a stage where we report the grades of the ward and anything of particular interest, otherwise we say nothing of isolating organisms. This practice may raise the question of 'is it worth doing the testing at all?' (3). We believe it is. We have frequently been asked by surgeons, nurses or administrators to go back through out files and follow grades through. We recently had a outbreak of a multiply-resistant organism in one of our wards and found the same organism on the mop that was being used to wash the floors. Mops in our hospital are now autoclaved once a week and daily in theatre.

The question of cost in a routine like this is often asked. In a big hospital we believe it is important to keep an eye on bacterial levels; apart from results it does keep nursing and cleaning staff interested in keeping their ward clean. We have a part-time technician who does all the testing and if the tests were not done routinely there would be no way of assessing the significance of results of the tests done during outbreak of an infection. While no claim is made that the results have any absolute significance the changes in values for the same areas at different times do represent real changes in the hygienic state.

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Micro Modification of a Syphilis Haemagglutination Test for Blood Donor Screening

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Summary

An inexpensive micromodification of a commercial Treponema Pallidum Haemagglutination assay kit is outlined. The method is suitable for use in mass screening of blood donor samples and is more specific and sensitive than other routinely used tests.

The screening of blood donors for the presence of *Treponema Pallidum* infection has long been an accepted and required part of routine blood donor testing. To date the methods used have relied on techniques such as the VDRL (Venereal Diseases Research Laboratory) and the RPR (Rapid Plasma Reagin) carbon antigen tests.

The Auckland Blood Transfusion Service has for many years used the RPR carbon antigen test[3]. This is a rapid and relatively inexpensive screening investigation costing (1981) 3.1 c for reagents. However false positive results do occur and more specific tests such as the Treponema Pallidum Haemagglutination Assay (TPHA) are required for confirmation.

Micromodification of haemagglutination tests has greatly reduced the costs of testing for hepatitis B markers[1, 2]. As a development of this type of cost effective procedure as well as to improve specificity of testing we have recently modified the TPHA technique for screening blood donors. We now report the technique and our experience with the method.

Materials and Methods

1. The Fuji-Zoki TPHA kit (Medic DDS Ltd.) was utilised for this project.
2. (a) Test cells were reconstituted with twice the recommended volume of absorbing diluent.
   (b) Test sera were diluted 1/20 in distilled water. Sera were not inactivated at 56°C.
   (c) 2μl of diluted serum was added to 2μl of previously dispensed test cells in the well of a Terasaki micro tissue culture tray (Nunc).
   (d) The tray was vibrated for 2 minutes (or mixed by gentle tapping) and incubated in the horizontal position for 30 minutes at room temperature (18-24°C).
   (e) The tray was tilted to an angle of 45° over a light box and the reaction patterns read after 10 minutes. Positive and negative control sera were tested with each tray. Reaction patterns were similar to those reported for the Micro-Hepatest[1] method.

Results

Titres of the positive control from the TPHA kit when used by this micromodification method revealed that there was a slight decrease in sensitivity when compared with the standard kit method. For instance, the micro-TPHA (mTPHA) usually had a titre of 1/1280 as compared to the stated titre of 1/2560 (absolute titre). This difference may be due to the more sensitive detection of haemagglutination by the "settling" pattern in a microtitre plate compared to the "drop" pattern of the Terasaki plate system.

By using a 1/20 dilution of test serum, instead of a 1/50 dilution, this compensated for the slight drop in sensitivity experienced with the change to Terasaki microplates.

Table 1 shows that all Carbon Antigen (RPR) positives, which were Fluorescent Treponemal Antibody (FTA) positive, were all detected by mTPHA. There were 7 sera which were RPR positive, and mTPHA and FTA negative, i.e. false positives. The RPR method also failed to detect 5 sera which were FTA and mTPHA positive. There were no RPR/FTA positives which were not detected by the mTPHA method. There were no mTPHA false positives detected in this initial study.

Table 1

Number of sera tested	9732
RPR+, mTPHA+, FTA+	20 (0.2%)
RPR+, mTPHA−, FTA+	0
RPR−, mTPHA+, FTA+	5
RPR−, mTPHA+, FTA−	0
RPR+, mTPHA−, FTA−	7

Panels obtained from both the Auckland Hospital Serology Department and the National Health Institute (Wellington) were tested by the mTPHA method and 100 percent correlation of results was obtained.

The method was easily learnt and the procedure was only marginally more time consuming to perform by technical staff than the RPR test.

Minimal batch to batch variation of test cells was noted. Storage of reconstituted cells at 4°C resulted in a one tube drop in the titre of the positive control. However the cells stabilise at 4°C after a few hours post reconstitution with a 1/1280 absolute titre and can be stored for up to 1 week.

Discussion

This technical variation of the TPHA has the advantages of improved sensitivity and specificity and is less expensive (1.25 c) than the RPR test. In addition the test is easy to perform and can be integrated into routine blood donor specimen testing. Micromodification of commercial kit sets is cost benefit effective and with careful attention to technique and controls, no substantial loss of sensitivity should be evident.

Acknowledgements

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Unexpected Leucocyte Antibody Production in Response to Transfusion Therapy: A Case Report

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A sixteen year old Polynesian male was admitted to the Dunedin Hospital having collapsed whilst shovelling sand. On admission, a routine blood screen performed gave the following haematological values:—

Haemaglobin 44g/l. Red Blood Cell count 1.5 × 10^{12}/1. Total Leucocyte count 45 × 10^{9}/1. Platelet count 19.9 × 10^{9}/1. Differential White Cell count 94 percent Blast cells.

A bone marrow examination was then performed and a diagnosis of Acute Lymphoblastic Leukaemia made.

The patient was commenced on a chemotherapy course comprising vincristine, prednisone and daunorubicin with supportive transfusion therapy of four units of packed cells and, seven days later, a platelet concentrate prepared from six units of fresh whole blood. Both transfusions were incident free.

On the twentieth day of treatment the patient had developed thrombocytopenia and severe granulocytopenia (platelets 62 × 10^{9}/1, white cell count 0.6 × 10^{9}/1, 21 percent neutrophils). As a result the chemotherapy was stopped. The patient also developed severe abdominal pain which was initially thought to be due to bowel perforation, but was later found to be a paralytic ileus due to vincristine. Because of the possibility of a bowel perforation a laparotomy was performed and granulocyte-platelet transfusions were arranged to cover the operative and post-operative period.

As the patient was a possible candidate for bone marrow transplantation, an HLA type was performed and the patients serum screened, against a leucocyte panel from forty randomly selected donors, for the presence of leucocyte antibodies. This screening was carried out prior to granulocyte transfusion therapy. No leucocyte antibodies were detected. Members of the patients family were approached as possible granulocyte donors. They were typed for HLA, ABO and Rhesus groups and a direct tissue type was performed. The only suitable family donor was the patient's father. Over a twelve day period, four granulocyte concentrates were prepared using an Haemonetics V50, three from the father and one from an unrelated HLA and Direct Match compatible donor. The cell separator collection procedure was performed with Hydroxyethyl starch as a red cell sedimenting agent, and followed steroid boosting of the donors' leucocytes.

During the course of the four granulocyte transfusions, the patient's sera was rechecked for the presence of leucocyte antibodies and a moderately high (60 percent) percentage of antibodies were detected. Follow-up haematological investigations revealed that there was a regeneration of the patient's bone marrow on the day after commencing cytotoxic therapy (12 days after surgery) and granulocyte transfusion therapy was concluded.

In view of the possibility of future leucocyte transfusions, it was decided that an attempt should be made to identify the specificity of the leucocyte antibodies. Since the HLA-A, B and C antigens are carried by leucocytes and platelets, each blood transfusion exposes the recipient to the hazards of immunisation to those antigens as well as to the red cell antigens. (1). No red cell antibodies were detected. The identification of the specific leucocyte antibodies was not straightforward as the antibodies formed appeared to be multispecific. The antibody specificities were determined by comparative analysis of familial investigations, twenty fully HLA typed staff members, the donors of the original blood and platelet transfusions and seventeen random HLA typed hospital patients. The pattern of reactivity that emerged revealed the following probable specificities:

Anti HLA-A3	Anti HLA-A11	Anti HLA-B7
Anti HLA-B14	Anti HLA-B17	Anti HLA-B35

The antibodies were stimulated by more than one of the original blood donors.

However, it was of interest to note that one of the antibodies, Anti HLA-B14, showed consistently stronger reactions, further the mother possesses this antigen and it is speculated that this resultant stronger reactivity could have been due to a secondary immune response. It is suggested that this may have followed a primary immunisation from a materno-foetal blood exchange at the birth of the patient.

The consequences of immunisation by HLA may be febrile, non-haemolytic transfusion reactions with a failure to respond to leucocyte and platelet transfusions. Thus a potentially life saving haematological support therapy may be of little value in those patients most desperately in need of it. If our patient requires further leucocyte or platelet transfusions, he will need support from donors who are HLA identical or at least partially matched.

This patient is, at the time of writing, on a second course of chemotherapeutic drugs, with a decision still to be made as to what further medical treatment will be adopted.

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Toward Self-Sufficiency in HLA Typing Sera

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The HLA-A and B antigens were discovered in 1958 where it was observed that the sera of multitransfused patients and multiparous women often contained antibodies directed against leucocyte antigens[12, 13, 16]. The antigens involved were called HLA-human leucocyte antigens—and they were found to belong to belong to the linked allelic series known as HLA-A and -B. By 1973 another allelic series, HLA-C was also discovered[1, 6]. The interest in the HLA system has justifiably begun to play a significant role in discussions and research concerning immunology, genetics, endocrinology, infectious diseases, inherited arthritis and renal transplantation. Locally, this polymorphic system is of major importance in research and routine disease association, paternity testing, renal transplantation, platelet and leucocyte infusions. It is, therefore, important to have monospecific or differentiating multispecific antisera to identify leucocyte antigens with confidence.

The aim of the local tissue typing laboratory is to provide an HLA typing service as outlined in 1978, [17]. The antigens of the HLA-A, -B, and -C loci are determined according to acceptable procedures using antisera that define the given specificities as demonstrated by their reactivity with an appropriately defined test cell panel. A minimum of two antisera from different donors are used to define each specificity.'

HLA antibodies induced by pregnancy are used in the typing

procedure and reports exist of antibodies produced by transfusion or tissue grafting also being used[2, 4, 5, 9, 11, 15, 18]. They are found by extensive screening programmes and constitute, at present, the only source of typing reagents, since attempts to obtain suitable antibodies by animal immunization and hybridomas have so far been unsuccessful. The antibodies used in HLA antigen typing, differ from those available for blood group serology, in that monospecific antibodies are rarely found and sera which contain a mixture of two or more antibodies are used.

Suitable reagents may be obtained by exchanging local sera with sera from other centres and commercial sources. Commercial reagents are expensive and the date of delivery is often unreliable. Because of the cost and other problems encountered, it became imperative to attempt to develop a self-sufficiency programme of antisera collection; incorporating intensive screening of post puerperal women immediately post delivery, and an efficient follow-up system whereby suitable donors are encouraged to undergo plasmapheresis or donate a volume of whole blood, the volume collected determined by their clinical condition.

Methods

1) Leucocyte Panel. A panel of leucocytes from unrelated donors was prepared for screening using differential gradient centrifugation (Ref 8);
2) (a) The method for screening for leucocyte antibodies described by Terasaki was used (Ref 7);
   (b) Where suitable antibody activity was detected, a fully typed panel was employed to determine the specificity or specificities present;
   (c) Specificities identified locally were confirmed in Sydney and Adelaide;
3) Organisation of the survey: the survey comprised serum samples collected at or within 24 hours of obstetric delivery from women admitted to the Queen Mary Hospital. Samples were collected from 553 women who had been pregnant on two or more occasions;
4) (a) Donations of leucocyte antibodies were collected when possible;
   (b) Recently a plasmapheresis procedure has been used to avoid red cell loss in the post partum women. A two unit manual plasmapheresis using 2000 units of preservative-free heparin (Ref 3) per donation has been performed at six weeks post partum and as required subsequently.

Results

The study reported here was performed as an additional but separate component, to a study on neonatal jaundice where maternal and neonatal samples were collected for investigation. The success of this study in obtaining a high rate of blood sample collection from multigravidae can be clearly linked to the involvement of paediatricians who actively sought the informed consent of individual mothers.

When this laboratory first commenced screening for leucocyte antibodies in 1975, a heavy reliance was placed on screening female blood donors for these antibodies (see Fig 1). The relative yield of useful antibodies from this source was small (see Fig 2) and involved considerable effort. Screening of multigravidae on the other hand has provided a higher proportional yield of leucocyte antibodies but has been associated with a poor donation rate: many women have not replied to an invitation to become blood donors.

During the period of study in the year 1981-1982 the reduced number of random blood donors and the increased number of post partum women screened (Fig 3), led to a very high proportion of positive antibody screening results (Fig 2A). This increased detection rate was unfortunately not matched by similar success in obtaining donations from many of the multigravidae (Fig 2B) and further changes in the procedure to obtain informed consent from potential antibody donors have been made.

The use of plasmapheresis using heparin anticoagulant has permitted much larger donations to be obtained without inducing iron loss in post partum women. The serum obtained following protamine sulphate induced clotting of heparinised plasma in glass, functioned quite satisfactorily in tissue typing procedures. This procedure permits 500 ml volumes of serum to be obtained from post partum women with a few weeks of obstetric delivery.

Table I
Numbers of sera screened for leucocyte antibodies each year:

May-April	1975-78	1978-79	1979-80	1980-81	1981-82	Total
Blood-donors	911	2983	3209	1629	94	7925
Post Partum Multi-gravidae	982	356	264	63	553	1236
Cardiacs***	*N/A	118	124	120	240	602
Transfusion Reaction Investigations	N/A	33	47	53	61	194
Miscel-laneous**	N/A	201	201	170	203	775
Total	1893	3691	3845	2035	1151	10732

* N/A = Not Available.
** Miscellaneous = red cell typing serum checks, referral from red cell antibody identification department, transplant list patients, requests from regional hospitals, unknown diagnosis requests from doctors.
*** Pre cardiac surgery work up.

Table 2A
Sera screened for leucocyte antibodies (Blood-donors and multigravidae only).

May-April	1975-78	1978-79	1979-80	1980-81	1981-82	1982 on
Number Tested	1893	3339	3473	1692	647	69
Number Positive	125	233	255	138	146	25
Number of Donations	83	48	189	101	72	23
Percentage Positive	66%	7%	5%	8%	23%	36%
Percentage of positives who were bled	66%	21%	74%	73%	49%	92%

Table 2B
Source of leucocyte antibody sera.

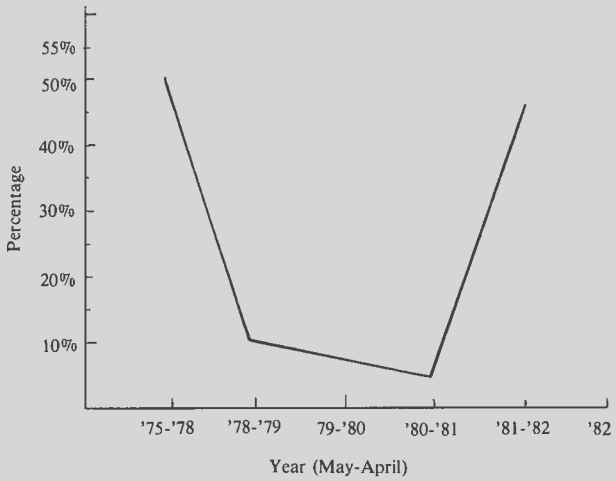
May-April	1975-78	1978-79	1979-80	1980-81	1881-82	1982 on
Post partum multi-gravidae	72	11	20	8	7	4
Blood-donors	11	37	169	93	65	19
Percentage from post partum multi-gravidae**	87%	23%	11%	8%	10%	17%
Percentage from blood-donors**	13%	77%	89%	92%	90%	82%

** A percentage of the total number of donations
NB after the first donation the post partum multigravidae are then included in the donor group.

Table 3
Test to Donation Collection ratio.

May 1975-1978	May 1978-1979	May 1979-1980	May 1980-1981	May 1981-1982	May 1982 on . .
23:1*	70:1	18:1	18:1	9:1	3:1

Table 4
Graph showing the percentage of post partum multigravidae : Total tested in each year.



Discussion

Van Rood et al observed the incidence of leucocyte antibodies in multigravidae post delivery women to be 20 percent. The frequency of leucocyte antibodies detected from May 1981 to May 1982 in our survey was 21 percent, showing remarkable agreement with Van Rood's findings.[9 10, 13, 14]

The results from our survey led us to revise our screening method and subsequent collection procedure. Screening is now almost exclusively confined to post partum women. The procedure is:

(1) On arrival at hospital, the prospective mother is now given details concerning the testing and the significance of leucocyte antibodies.
(2) If signed consent is obtained, the specimen is collected and screened within forty-eight hours.
(3) If leucocyte antibodies are detected, the prospective donor is personally informed while in hospital and given information about the donation procedure. Her general practitioner is sent relevant information; the general practitioner is requested to discuss a donation with his patient at the six week post natal check; the donor service makes contact with the woman and asks if she is able to give a donation by plasmapheresis.
(4) These donations are then stored and processed as required.

At present, this system is still undergoing evaluation. If the local tissue typing laboratory intends keeping pace with new developments, yet minimise expense, an aggressive, yet practical approach must be adopted in the continuing search for HLA typing serum. A major concern in implementing this program has been to avoid using excessive pressure on women to donate leucocyte antibodies. In the past, however, it is clear that many women have not responded to a mailed request to become an antibody donor, probably because of ignorance of the procedure involved and the obvious human desire not to become involved in procedures which may be uncomfortable. A direct, but low key, approach which provides clear, matter of fact information and indicates the value of leucocyte antibodies in medical care, appears to be providing more cost effective results.

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Technical communication

A simple economical Platelet Rotator. A Modification

Les Milligan, Alan Knight, Heather Kerr and Ralph Harvey

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Dunedin

Received 20/5/83

A modification to the original design of the platelet rotator was introduced in order to prevent frothing of the plasma during rotation and to reduce the overall rotation rate.

A second-hand windscreen wiper motor was adapted and the revolutions were reduced from 30 RPM to 1 RPM. The power supply was a 12 V. 4 Amp. R.M.S. transformer, bridge rectified, to give an unfiltered D.C. output to supply a 12 V windscreen wiper motor. A wormwheel drive gear reduced the revolutions from 60 RPM to 1 RPM. The output shaft was lengthened to accommodate the wormwheel and the motor shaft was extended to take a light weight alloy fan to cool the motor. The torque produced by the windscreen wiper motor was greater than of the original 240 V motor.

This modification has been introduced into routine use at a relatively low cost and has been totally successful.

Reference

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Technical Communication

An Enzyme Control

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Dunedin Public Hospital

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Introduction

The use of controls in an Immunohaematology Laboratory is extensive, yet one of the most commonly used procedures, the one stage enzyme test, is not routinely controlled to the extent it should be. Each batch of enzyme is controlled, and a known positive control is included with all tests, but we have no way of knowing that the enzyme is working in the tests or, in fact, if it has been added.

Methods

Control antiserum reagent was prepared by pooling absorbed anti-c plus anti-E. A local anti-D was diluted 1:4 in the mixture and titred in AB serum. The final pooled product gave the following results by one stage enzyme technique.

Cell Type and Titre, rr 4, r'r 2, r''r 8, R_2R_2 8. This reagent was frozen in 2 ml aliquots ready for use.

A one stage Low's Papain technique was employed in all procedures using glass tubes 70 x 11 mm as follows: one drop serum, one drop Low's Papain, one drop 3 percent suspension of red blood cells in Low Ionic Strength Solution incubated 15 minutes at 37°C, centrifuged 2,500 r.p.m. 20 seconds and examined macroscopically.

Procedures Assessed

(1) In all routine cross-matches, one drop of the control reagent was added to tubes which showed a negative reaction after the enzyme phase had been read. The tubes were then mixed and immediately centrifuged and re-examined macroscopically.
(2) In 25 cross-match procedures the enzyme test was put up in duplicate, but with saline being used in place of enzyme for one tube of each pair. After incubation and reading, the control reagent was added, the tubes were centrifuged and then re-examined. All negative results were reincubated for a further 15 minutes at 37°C and an Indirect Coombs Test (ICT) performed.
(3) Using two Dade Data Cyte antibody identification panels (Lot No. 272 and 274) one stage enzyme tests were performed using inert AB serum as test reagent. After these tests were read and found to be negative, control reagent was added to all tubes which were then centrifuged and re-examined.
(4) Using the same panels as procedure (3) above, one stage enzyme tests were set up substituting saline for the activated enzyme. All negative results were reincubated and an Indirect Coombs Test performed.

Results

PROCEDURE ONE:

In a series of 4000 cross-match procedures and 1000 cross-match auto-control procedures which were negative in enzyme tests, the tubes showed positive agglutination following the addition of the control reagent.

PROCEDURE TWO:

From a series of 25 random cross-matches where the effect of performing the enzyme test either normally, or as a mock procedure without activated enzyme being added, the following results were obtained:

(1) Tests with normal enzyme present: All positive on the addition of control reagent (Negative prior to adding control reagent).
(2) Tests without activated enzyme present: All negative on the addition of control reagent but positive by ICT after a further 15 minutes incubation.

The results show that the control reagent is able to induce agglutination in appropriately enzyme-treated cell but not where the enzyme is omitted. In the latter case binding of the anti-cDE to the red cells was confirmed by the Indirect Coombs Test.

PROCEDURE THREE:
DC272/274
(1) 15' Low's Papain 37°C: All tests Negative
(2) On addition of enzyme control all tests gave a strongly Positive reaction.

PROCEDURE FOUR:
DC272/274
(1) 15' Saline 37°C: All tests Negative
(2) On addition of enzyme control, all tests Negative
(3) On reincubation for a further 15' all tests then gave a Positive Indirect Coombs Test.

Discussion

The enzyme procedures used routinely in Blood Bank Serology have not been subject to the same degree of quality control which is usually required in other grouping and cross-matching techniques. In particular, the activity of the enzyme in being able to modify the red cell surface, is not controlled and use of inactive enzyme or omission of the enzyme therefore, is potentially able to lead to false negative results. The use of a control reagent such as the anti-cDE described above provides a means of excluding inadequate enzyme activity as a cause of spurious results. It is recommended that the titres of the individual antibodies in the pool should be kept low e.g. 1:4 to 1:8, to ensure that the control procedure detects an adequate degree of enzyme modification of the red cells.

Use of anti-cDE reagent for enzyme control will produce negative results with cells of the rare Rhesus phenotypes e.g. Rh Null, CCddee (r'r') etc. As these are rare, it is unlikely that addition of anti-C to the reagent pool would be of real value.

In our routine cross-match laboratory, employing both senior and junior staff, no anomalous results were detected which would have indicated the presence of a factor which blocked serological reactions with enzyme treated cells. (1) Therefore we are at present, unable to confirm the presence of a factor in the serum of some patients that may block serological reactions with these cells. We are however, continuing to evaluate the reagent as a broad enzyme control in our routine cross-match laboratory.

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Technical Communication

Carefree Cryotomy

R. J. Jarrett
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This article is for the benefit of those who are using a cryostat and have no histological background, such as Immunologists, and are producing unsatisfactory results. It is based on the use of an I.E.C. cryostat which has a rotary microtome.

Preparation of the Specimen

Pre-frozen specimens should not be allowed to thaw before being attached to the object holder, fresh specimens should be snap frozen onto the object holder. Object holders should be stored in the cryostat and a substance such as Cryoform pre-frozen on to them. This serves to maintain a safe working distance between the object holder and knife edge. The specimen should be placed on the top of this and one or two drops of water used to bond the tissue to the substance. This method of attachment removes the possibility of pre-frozen tissue being thawed by a vast amount of Cryoform and also facilitates quick removal of the tissue from the holder.

The specimen is then trimmed by cutting in about 20 micron increments, until the desired section profile has been reached. At this point, if urgency is not involved, close the lid for 5-10 minutes to allow the chamber temperature to stabilise.

Section Cutting

Turn the handle slowly and steadily with the anti-roll plate away from the knife and observe the sections. They should come off in loose rolls. If they crinkle up on the knife edge, they are too warm: if instead, they are segmented by fractures, they are too cold. Different tissues have different optimum cutting temperatures.

When the sections appear to be cutting well, lower the anti-roll plate and cut. When picking up the sections, allow them to fly onto the slide, do not *press* the slide onto the section.

Care of the Knife

At least two knives are needed, one for current use and the other for sharpening and storing at -20°C. Knives must be machine sharpened to maintain a straight edge and must be treated with the utmost respect. *Never* put them down on a bench. Never walk around with them out of their box. When brushing debris from the edge, use only a soft brush and always brush towards the edge. The most likely cause of poor sections is a blunt or damaged knife edge.

Maintenance

Most cryostats have automatic defrosting. This means that a cryostat may be left for a very long time without being cleaned and lubricated with the result that moving parts will wear more quickly and micro-organisms and viruses may accumulate.

Periodically the cryostat should be turned off, the microtome removed, after removing the knife of course, and all the inside walls and the machinery be washed with 70 percent alcohol and allowed to dry. All moving parts should be examined for wear and if insignificant, a low temperature oil applied. The instrument is then replaced and set up for use.

Remember

* Do not cut yourself on the knife, it blunts the edge.
* Clean the knife and anti-roll plate during use with alcohol using a brush only.
* Oiling will prevent ice forming on moving parts.
* Do not leave unfixed slides lying around for long periods and then wonder why nuclear detail is lost.
* Do not squash the slide onto the section when picking it up.

CORRESPONDENCE

Serum and Red Cell Folate Assays:

The Editor

Sir,

The measurement of serum and red cell folate plays a central role in the establishment of the aetiology of megaloblastic anaemias and assessing nutritional status. The Department of Haematology at Auckland Hospital carries out folate assays using a commercial radioisotopic method for the Auckland Hospital Board area and several other centres in the North Island. The majority of requests (80 percent) are for serum folate only.

There have been many reported observations which indicate a high percentage of sub-normal serum folate levels in hospital patients when related to a reference range derived from a normal population. This situation means that the assessment of the folate status of a hospital patient using a serum assay is spurious indeed. Furthermore, our laboratory has observed the reverse situation where the serum level is normal with a low red cell folate ie: the patient may have a normal serum folate whilst being folate deficient.

Results of 51 consecutively low red cell folate values have been collected and the serum levels collated on assays carried out over the last six months in our laboratory. The findings may be summarised as follows: for the whole group the mean red cell folate was 294 nmol/l (normal range 440-1750 nmol/l) with a mean serum level of 6.4 nmol/l (normal in our laboratory is 5.4-18.3 nmol/l). In this group 30 (58.8 percent) had low serum folate where the mean serum value was 3.5 nmol/l. The mean red cell folate level for these 30 samples was 266 nmol/l, which is slightly lower than that of the whole group of 51. The remaining 21 samples with normal serum levels ranged from 5.4-17.9 nmol/l.

These data would seem to indicate that if serum samples alone were used to assess the folate status of hospital patients, a folate deficiency may only be correctly noted in approximately ⅔ of patients tested, with the further complication arising from the observation that hospital patients may well have a sub-normal folate level not related to a folate deficiency.

The data from our assays would also refute the idea of establishing a clinically significant serum range because of the normal serum levels encountered which are related to a low red cell level.

Whilst the findings given here are not new, they need to be re-emphasised in the light of so few red-cell folates being requested. More readily available results due to more laboratories able to assay with the introduction of commercial radioisotope kits, and inappropriate testing may well greatly devalue the folate assay in terms of assessing a patient for folate status.

Yours sincerely

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