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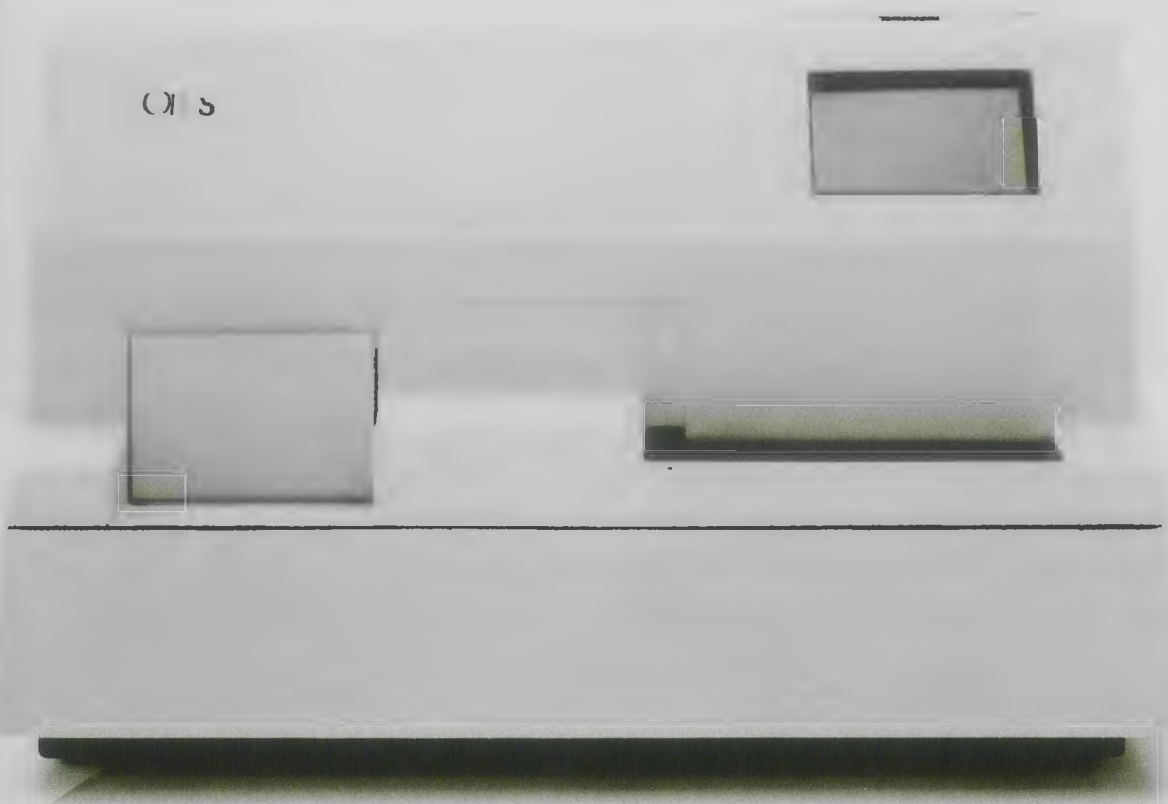

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NEW ZEALAND JOURNAL OF
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Subscriptions to the Journal for non-members requiring delivery in New Zealand is $NZ33.00 for 1 year surface mail paid. Single issues are $NZ12.00 surface mail paid.

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DIRECTIONS FOR CONTRIBUTORS

From Vol. 36 No. 1 all papers published will be in the form known as "Vancouver Style" or Uniform Requirements for Manuscripts submitted to Biomedical Journals. Full details may be found in the New Zealand Journal of Medical Laboratory Science, Vol. 45, No. 4, page 108 to 111 or from the Editor.

Intending contributors should submit their material to the Editor, M. Gillies, Microbiology Laboratory, Auckland Hospital, Auckland, New Zealand. 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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DATES OF PUBLICATION

The months of publication for 1992 are March, May, August and November.

Evaluation of two commercial assays used in screening sera from patients with suspected Leptospirosis

Annette Chereshsky MSc(Hons), Diana R Martin PhD,
Leptospira Reference Laboratory, Clinical Microbiology II Unit, New Zealand Communicable Disease Centre, P O Box 50 348, Porirua.

Address for correspondence: Annette Chereshsky

Abstract

Two commercial assays, for the detection of antibodies to *Leptospira interrogans* serovars, were evaluated for their sensitivity, specificity, convenience of use, and cost effectiveness for use as screening tests.The assay manufactured by Diagnostic Pasteur, France, was found to be more sensitive, more specific, simpler to use, and less expensive than the reagents produced by Lee Laboratories, USA.

Key Words

Leptospira antibody tests, leptospira serovars, slide agglutination

Introduction

Leptospirosis is a widespread acute zoonotic disease which has a high prevalence in New Zealand. The causative agent, *Leptospira interrogans*, is subdivided into 202 serovars grouped on the basis of serological relatedness into 23 serogroups(1). Serovars known to cause leptospirosis in New Zealand are *L. hardjo, L. pomona, L. copenhageni, L. ballum, L. canicola, L. bratislava,* and *L. tarassovi* (2).

Usually the diagnosis of leptospirosis is confirmed serologically. Most hospital and private laboratories in New Zealand use commercial reagents for screening patients' sera for antibody to leptospira. In many instances laboratories receive a single serum to be tested, since acute and convalescent sera are not always available. The serum is usually tested in a single dilution or undiluted and the results of such screening tests provide information only on whether or not a particular serum contains leptospiral antibodies. Sera found to be reactive are referred to one of the major laboratories where they are retested using the WHO recommended microscopic agglutination test (MAT)(3). Live Leptospira antigens of the seven serovars prevalent in New Zealand are used in the MAT.

The screening assay provides an important laboratory result to which a clinician will respond pending the outcome of the confirmatory testing. Therefore, the ability of an assay to detect leptospiral antibody is very important.

We evaluated the performance of two reagents currently available in New Zealand: (1) Lee-Leptospira Antigens, Lee Laboratories, USA (LLA), and (2) HS Leptospira Antigen Kit, Diagnostic Pasteur, France (DP). Both assays utilise slide agglutination procedure. Methodologies used were as stated by the manufacturers.

Materials and Methods

The DP kit consists of one vial (1 ml) of *Leptospira* antigen and one vial (0.5 ml) of positive control serum. According to the manufacturer the leptospiral antigen is a *Leptospira interrogans* species-specific preparation which can be used for screening sera regardless of infecting serogroup.

Lee Laboratories produce six pools of antigens (5 ml/vial), individual serovar-specific antigens (5 ml/vial), and a positive control serum for each antigen (3 ml/vial). Each pool is supposed to detect antibodies to the Leptospira serovars included in that pool. To give coverage for most of the Leptospira serovars present in New Zealand we selected three of the pools as follows: Pool No I. *(L. ballum, L. canicola, L. icterohaemorrhagieae);* Pool No 3*(L. automnalis , L. pomona, L. wolfii);* Pool No 4 (L. *australis ,L. hyos, L. georgia)*; and one individual antigen, *L. hardjo. (L. bratislava* and *L. copenhageni* serovars present in New Zealand, are not included in any of the Lee reagents, but because of cross-reactivity antibody to *L. bratislava* should be detected by pool 4 and *L. copenhageni* by pool I.)

For the evaluation a total of 100 sera were used. These sera were selected and categorised on the basis of their previously known MAT titre as follows: "no detectable antibody", "serologically positive" (detectable antibody $\leq$ 200), and "serologically diagnostic" (> 200).

Sera with no detectable antibodies were, in the main, specimens referred to our laboratory for Legionella and Lyme disease serology. Sera in the "serologically positive" and "serologically diagnostic" groups were all obtained from patients with either suspected or confirmed leptospirosis.

Sera were tested by both assays, using the single serum dilution recommended for each assay. Results were recorded as either negative or positive and no titrations were performed. The study was conducted blind, in that the category to which the serum belonged was unknown to the person performing the assay evaluation.

The assays were evaluated for their sensitivity and specificity in detecting antibodies to the leptospira serovars found to be

Table 1: *Results of each assay per serum category*

		Serum category				
Assay	**Reaction**	**"No antibody"**	**"positive"†**	**"diagnostic"‡**	**Combined total "positive" and "diagnostic"**	**Total positive or negative by assay**
LLA	Positive	7	20	24	44	51
	Negative	30	16	3	19	49
DP	Positive	0	28	25	53	53
	Negative	37	8	2	10	47
Total sera tested		37	36	27	63	100

† "postive" (titre $\leq$ 200 by MAT)
‡ "diagnostic" (titre > 200 by MAT)

present in New Zealand. Positive and negative predictive values were determined. Additionally, the assays were assessed for convenience of use and cost effectiveness.

Results

The assay results are given in Table I.

Sensitivity:

Forty-four of the 63 sera with leptospira antibody by MAT were detected by LLA (sensitivity 70%) and 53 were detected by DP (sensitivity 84%). When sera categorised as "serologically positive" only were considered, the sensitivity of LLA was 56% (20/36) and DP was 78% (28/36). Maximum sensitivity occurred when sera with "diagnostic" levels of antibody (>200 MAT) were considered: LLA 89%; DP 93 %.

Specificity:

Whereas the DP kit showed 100% specificity, accurately identifying all 37 sera with no antibody to Leptospira the LLA reagents were only 81% specific (30/37).

Predictive values:

Positive predictive values (true positive/(true positive + false positive)) for each assay were: LLA 86%; DP 100%. Negative predictive values (true negative/(true negative + false negative)) for each were: LLA 61%; DP 79%.

Overall the accuracy of the DP kit (90%) was better than that of LLA (74%). (Accuracy = true positives + true negatives / total tested x 100.)

Serovar-specific antibody detection:

To examine the ability of the assays to detect serovar-specific leptospiral antibodies, 56 of the sera which contained antibody to a single serovar, were tested. Results showed that only four sera (7%) were not detected by DP whereas 18 (32%) sera with serovar-specific antibodies were not detected by the LLA reagents used (Table 2).

Of the four positive sera not detected by DP, one serum contained antibody to *L. bratislava* , another antibody to *L. hardjo* and the other two antibody to *L. tarassovi.*

The only serovars which were consistently detected by the LLA reagents were *L. ballum* and *L. canicola.*

When we examined the reactions given by the 38 sera positive with LLA (Table 2) we found that 26 (68%) had agglutinated with all three pools of antigens and the *L. hardjo*-specific antigen, regardless of the serovar-specific antibody present in the serum (Table 3). In fact there appeared to be no correlation between the serovar-specific antibody present in a serum and the agglutination shown by the serum with the pools of antigens; and only one serum agglutinated alone the pool containing the appropriate serovar.

The convenience and cost effectiveness of using each of the assays are summarised in Table 4.

Discussion

By screening sera for the presence of leptospiral antibodies, laboratories provide a very valuable service in establishing the presumptive diagnosis of leptospirosis. For this reason there is a need to evaluate the testing procedures used by diagnostic laboratories in New Zealand to ensure that the results they provide are meaningful. As stated, most diagnostic laboratories undertake screening assays for leptospiral antibodies, the confirmatory testing being undertaken by only a few of the larger laboratories.

To be useful a screening assay must be both sensitive and specific. False-positive results can lead to misdiagnosis and wrongful treatment, whereas false-negative results are misleading and appropriate treatment may not be given. A useful screening assay, also, should be simple and inexpensive to perform.

In this study we compared the performance of two assays (LLA and DP) which are currently available on the New Zealand market. A greater difference in sensitivity between DP and LLA was observed when "positive" but not "diagnostic" sera were considered (DP 78%; LLA 56%). The sensitivity of both assays significantly increased when sera with

Table 2: *Detection of antibodies to a specific Leptospira serovar*

		Reaction Obtained			
		LLA		DP	
Specific antibodies present in serum	Number tested	Positive	Negative	Positive	Negative
L. ballum	4	4	0	4	0
L. bratislava	7	4	3	6	1
L. canciola	4	4	0	4	0
L. copenhageni	5	3	2	5	0
L. hardjo	15	9	6	14	1
L. pomona	16	11	5	16	0
L. tarassovi (hyos)	5	3	2	3	2
Total	56	38	18	52	4

Table 3: *Specificity of positive reactions in 38 sera tested with LLA assay*

Specific antibody present in serum (corresponding pool).	No of sera positive with corresponding pool only.	No of sera positive with one alternative pool.	No of sera positive with either 2 or 3 pools.	No of sera positive with all 4 reagents	Total no of positive sera regardless of the antigens in the pool.
Ballum (pool No. 1)	-	1	1	2	4
Bratislava (pool No. 4)	-	1	1	2	4
Canciola (pool No. 1)	-	-	-	4	4
Copenhageni (pool No. 1)	-	-	1 (excluding corresponding pool)	2	3
Hardjo (L. hardjo antigen)	-	-	-	9	9
Pomona (pool No. 3)	1	-	5	5	11
Tarassovi (pool No. 4)	-	1	-	2	3
TOTAL	1	3	8	26	38

Table 4: *Practical aspects of performing the tests*

Category	Assay LLA	DP
Ease of reading the slide agglutination	Strongly visible	Fine agglutination less visible
Approximate time taken for testing one serum†	≈ 15 min‡	≈4 min§
Approximate cost of reagents used to test 100 sera	$174.80*	$59.60*
Delivery time of each kit after ordering	2 months	I week

† Sera tested in batches of ten
‡ Time includes testing one serum with four reagents as well as time needed to test all positive control sera with these four reagents
§ Time includes testing one serum and one positive control serum with one reagent
* Based on prices paid at the time of purchasing and includes only the cost of reagents used, not the full cost of reagents purchased

"diagnostic" levels of antibody were tested (DP 93%; LLA 89%). Overall, in our hands the sensitivity of DP was found to be higher than that of LLA although a greater sensitivity for both assays would seem desirable.

When specificity of the assays was considered, DP was 100% specific whereas false-positive results were recorded for 19% of the antibody-negative sera with the LLA reagents. When evaluating a method used for screening-out negative patients, the most important criterion for judgment of the quality of that test is the negative predictive value. This value expresses as a percentage true-negative results compared with the total number of negative results detected by the method under evaluation. A high negative predictive value indicates a high number of true-negatives and a low number of false-negatives. With our testing DP gave a negative predictive value of 79% whereas the LLA reagents gave a value of only 61%.

Although the DP assay is not intended to detect serovar-specific antibody, we were concerned to determine if each assay (DP and LLA) adequately detected antibody to the various leptospirae prevalent in New Zealand. In this regard the LLA reagents failed to detect antibody in 32% of serovar-specific sera tested compared with only 7% for DP. Failure to detect antibody by either of the assays was not related to the titre of antibody as shown by MAT. Most concerning, with our testing, was the failure of the LLA *L. hardjo* antigen to detect antibody in 40% of sera with that serovar-specific antibody. *L. hardjo* is the most common serovar causing leptospirosis in New Zealand.

In reviewing the practical aspects of performing the tests we found that the agglutination given by the reagents of LLA was more clearly visible to the reader. However, the DP assay has an obvious advantage as a screening test, being a single species-specific preparation which can detect antibodies to all leptospira serovars. In contrast, LLA reagents comprise many serovar specific antigens which have to be used to test each serum making the assay more time-consuming, labour-intensive, and costly.

Acknowledgements

We are very grateful to Mrs Sue Walker for assistance in the review of the manuscript and for preparing sera for the evaluation. This paper is published with the authority of the Director-General of Health.

References

1. Ferguson IR. Leptospirosis update. *BMJ 1991;* **302:** 128-9.
2. Public Health, Department of Health. Zoonosis. *NZ Med J;* 1985; **98:** 503-4.
3. Faine S (Editor). Guidelines for the control of leptospirosis. World Health Organisation, Geneva, 1982.

BOOK REVIEW

"MULTIPOINT METHODS IN THE CLINICAL LABORATORY — A HANDBOOK" (1991) ISBN 0-901144-28-2

Authors: Mary Faiers, Robert George, Julian Jolly, Philip Wheat

Public Health Laboratory Service
61 Colindale Avenue,
LONDON NW9 5DF

Reviewed by Graham Cameron
Microbiology Dept, Auckland Hospital

Many laboratories use multipoint inoculating devices for a number of procedures including antimicrobial susceptibility testing and identification procedures. The technique is economical in materials, and media preparation etc need not be a burden in any laboratory with sufficient workload to support its use. In this handbook, the authors have produced a useful document for laboratories which are either interested in starting to use the method, or which would like to expand the method further.

The text is divided into seven chapters, each dealing with a different application or method.

Chapter One is an interesting but brief history of the development of the method. Chapter Two is concerned with antimicrobial susceptibility testing and is by far the most comprehensive, occupying about one half of the volume. Topics covered are the choice of both agents and media, the storage of media, inoculum preparation, Q.A. and Q.C., and interpretation of results. No specific breakpoint levels are recommended, but very comprehensive charts of the various lists published by acknowledged authorities are included.The choice of which levels to use has been left to the user laboratory.

My only criticism is that no specific information is given regarding the solvents suitable for preparation of the various antimicrobials, nor about their long term storage. This segment is of a rather more general nature than I would have thought appropriate.

The second half of the book is concerned with the following topics.

Chapter Three: the identification of Enterobacteriaceae, Chapter Four; the identification of gram positive organisms, Chapter Five; techniques for urine specimens, Chapter Six; a number of specialised uses such as MIC/MBC and Chapter Seven discusses the application of computers and reading devices.

Overall this 95 page handbook would be of considerable value to any current or prospective user of this simple readily adaptable method. Of necessity, such a relatively slim volume cannot contain every detail of every topic presented, but it is well referenced and should provide an excellent resource for further reading. This handbook should be a good investment for any laboratory seriously considering using this method.

N.Z.I.M.L.S. 47th ANNUAL SCIENTIFIC MEETING WELLINGTON AUGUST 26—28th 1992

OUTLINE PROGRAMME

Wednesday 26 August

WORKSHOPS
1. Anti Nuclear Antibodies : Half Day, morning
2. DNA Probes in Microbiology : 10.00 — 16.00
   Run by Med Bio Enterprises

Thursday 27 August

ANNUAL SCIENTIFIC MEETING OPENING CEREMONY

GENERAL FORUM

Speakers: Dr C. Feek "Steroids in Sport"
Dr C. Burgess "Fenoterol, The Inside Story"
M. Lynch "Pacific Paramedical Training Centre"

FORUM "NEAR PATIENT TESTING"
Speakers to include: Mr Jim Le Grice of Med Lab South, General Practitioners, ICU Staff and Industry Representatives. There will be time for discussion and audience participation.

CONCURRENT FORUMS — Immunology. Other disciplines if required

47th ANNUAL GENERAL MEETING N.Z.I.M.L.S.

SPECIAL GENERAL MEETING N.Z.I.M.L.S

Friday 28 August

CONCURRENT FORUMS: Biochemistry
Transfusion Science
Cytology
Histology
Haematology
Microbiology
Immunology

WORKSHOP — DNA Probes in Histology, full day. Run by Med Bio Enterprises.

CLOSING CEREMONY

GUEST SPEAKERS FOR ABOVE FORUMS INCLUDE:

Dr E. Dax, Director National HIV Reference Lab, Fairfield Hospital, Australia, who will speak on "Rapid HIV Tests" and "Evaluation of HIV Kit Tests" on Friday morning.
Dr Mark Jones and Dr Michael Humble of Wellington Hospital who will speak in a seminar on "Respiratory Tract Infections" on Friday morning.
Drs Diamond and Nguyan from Hospital Henri Mondor, Paris, who will speak on "Computer Assisted Interpretive Reporting of Coulter VCS and Elite/Profile Data". They hope to demonstrate a Bone Marrow decision support system, and a case study system under Windows 3.0.

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Do not state, for example, "The results will be discussed". Tables and figures may be included, but within the space allocated.

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NZIMLS CONTINUING EDUCATION
SPECIALIST INTEREST GROUP UPDATE

FROM THE IMMUNOLOGY SPECIAL INTEREST GROUP (ISIG)

Convenor: Gillian McLeay
Contact address: Laboratory Training Centre,
Building 18,
Auckland Hospital,
Private Bag 92024,
Auckland

. . . Of Seminars and Scientific Meetings

Probably, by the time you read this edition of the Journal, the Waikato/BOP seminar (9 May) will be over. History will decide whether it was a success or not, but hopefully the intention to make it relevant to the people at the bench, by having an informal programme with plenty of time for discussion, will have been achieved. And, not least in importance, it will have been fun.

All seminars have problems with finding speakers, (not to mention the audience), with dates which either clash with work or leisure time, and distances to be travelled. It all costs — time and money, and both seem in short supply. Relying on goodwill alone poses real problems for the organisers, and application of strong arm tactics to gain support is the norm.

Gerry Campbell, Wellington ISIG, has got the ISIG ANA Workshop organised for 26 August, but still needs papers of an immunodiagnostic nature for the Immunology Forum at the NZIMLS Scientific Meeting on 27 August. He is seeking short, informal presentations or group discussions, with the abstracts for the programme being equally short and informal. As published in the last Journal, the Friday forum on HIV is finalised.

. . . Of Finances and other Money-Related Things

Those members who opened their March Journal will be aware of the exponential growth in contributions describing the activities of the different Special Interest Groups. We are really on a roll, reminding me of the heady days when the Branches came into existence and brought the Institute within reach of all its members. Sadly, the Branches seem to have had their day, but perhaps the SIGs will be more than worthy replacements and escape the crippling apathy which led to the demise, for example, of the Auckland Branch.

Over recent months I have trod on a few toes by questioning the right of the NZIMLS to dictate to the SIGs on how they organise their activities (i.e. timing of seminars and allocation of funding received from the Institute). I know the money available is not limitless, and will have to be divided even further as new SIGs are set up — there are two in the pipeline at the moment.

In the past, disgruntled members, as well as non-members of the Institute, have questioned the amount of the annual subscription. Adequate financial support of the SIGs, with freedom to operate in the way that suits their needs, will show value for money to NZIMLS members, and may provide a real incentive for non-members to join the Institute. After all, the SIGs are merely extensions of the NZIMLS at a regional level.

. . And Closer Educational and Other Relationships

Links with Massey University and ISIG are now well established. John Clarke (Associate Professor — Microbiology) has sent some information on his proposed Immunology course for comment and suggestions. This is an informal arrangement, and his material will be passed to those ISIG members who have offered to act in an advisory capacity for Immunology.

Professor Clarke writes . . . "The BMLS class of 31 students (second year) has been separated off into a distinct practical stream in most subjects and are already developing into a cohesive group. Tonight they have organised a dinner and they are organising a spokesperson. It may be that they would like to meet some SIG representatives so if you, or anyone else, are passing this way, please let me know and we may be able to arrange a meeting with the class. I have no doubt that the Institute will wish to convey to the class the idea that the Institute regards them as important to the future of this profession."

"Learning by Post" is the name Angela Wheeler (Diagnostic Laboratory, Auckland) is calling the correspondence courses she is running under the auspices of ISIG. She has six QTA students, four MLTB Certificate students and two enthusiastic souls for Continuing Education. There will be a small fee to cover the cost of photocopying and postage. Contact Angela (PO Box 5728) if you wish to participate.

The ISIG Network News continues to link up members all over the country and keep them informed, although most of the material still comes from Auckland, despite pleas for contributions from other areas.

There has been criticism over having a separate newsletter, rather than utilising the NZIMLS Journal and Newsletter. However, contrary to public opinion, as you can see, we publish in the Journal also, ,but find that the Network News gives a certain amount of flexibility and informality, and reaches a slightly different audience.

The mailing list continues to grow slowly and I should like to take this opportunity to welcome Jane Clark and Jane Humble (Wellington), Jackie Wright (Whakatane) and Paul Bolton (New Plymouth) to the ISIG Network.

FROM THE TRANSFUSION SCIENCE SPECIAL INTEREST GROUP

Having read our newsletter in the March Journal you will have been thinking for a couple of months "Oh yes, I should send something to Sheryl for publication in the TSSIG newsletter". Don't delay any longer. Write it! Send it! Articles, questions, comments, even an idea for a topic that you would like referred to someone to write a review about. Send it to: Sheryl Khull, Secretary TSSIG, Red Cell Serology Laboratory, Wellingtion Regional Blood Service, Wellington Hospital, Private Bag, Wellington 6002

Remember, it is YOUR input that puts the 'I' in the TSSIG.

CONFERENCE

Planning is under way for the Transfusion Science content of the NZIMLS Annual Scientific Meeting in Wellington this August. There will be a wet workshop, the subject of which is as yet undecided, as well as opportunities for you to present formal scientific papers and to take part in an informal panel discussion.

Why not present your: case study
research
assessment of kits or equipment
new techniques

Papers should be of 15 minutes duration. Full details were published in the March Journal. If you have something you wish to share, or a topic you would like discussed, please get in touch with the co-ordinator of the Transfusion Science conference programme:

Stewart Dixon, Wellington Regional Blood Service, Wellington Hospital, Private Bag, Wellington 6002

N.I.C.E. V.I.S.I.T.

(National Immunohaematology Continuing Education — Various Interesting Speakers on Immunohaematology Topics)

You will already have received some information about the NICE VISIT. TSSIG is proposing a travelling road show during November with one-day blood banking seminars being organised in provincial centres throughout the country. It is intended to have a travelling speaker dealing with a specialised topic and a number of papers that will be presented by different speakers at each location. Local contributions will also be encouraged.

We want this VISIT to be the kind of event that you want it to be. We are planning to use provincial centres that should be convenient for you to attend. Also we're asking for your input into the topics which you would like to hear about. The VISIT will have a general interest for those working in all sizes of laboratories. It won't be above your head, neither will it be superficial.

A questionnaire was included with the initial results of the February NIPSurvey, to be returned to: Mr Les Milligan, Otago Regional Blood Centre, Dunedin Hospital, Private Bag, Dunedin

If you have lost yours, or come up with some more ideas, please contact Les, or any of the Members of TSSIG.

NICE WEEKEND

The NICE (National Immunohaematology Continuing Education) Weekend was held at the THC Wairakei Resort Hotel on 25 - 26 April. 43 Delegates from as far afield as Invercargill, Whangarei and Sydney registered and attended the meeting.

Socially the weekend was a success with very few of the delegates failing to take advantage of the soothing waters of the large outdoor thermal pool until the wee small hours on Saturday and Sunday mornings.

There were two full days of oral and poster presentations and once again this year everyone who attended was very enthusiastic about the meeting.

The papers presented covered a wide range of topics as the abstracts illustrate. The following are the abstracts of the papers and posters presented.

Oral Presentations

1. RHESUS IS A MONKEY

David Wilson, Manawatu Regional Blood Centre, Palmerston North Hospital, Palmerston North

We show great enthusiasm for correctness when we employ our serological techniques.

Why do we not show the same enthusiasm for the correct use of the nomenclature which describes our findings?

Some of the more obvious errors (and a few more) are discussed.

2. GAMMA-CLONE^{R} ANTI-HUMAN GLOBULIN REAGENTS

Ian Steed, Biotek, Auckland

We welcome this opportunity to share with you an in-depth profile of Gamma's new line of Coombs reagents, which are the first wholly monoclonal Anti-IgG and Polyspecific Anti-Human Globulin reagents produced by a US manufacturer.

3. RELIEVING PA PA PA — PAIN

Yvonne Geeraedts, Immunohaematology, Taranaki Base Hospital, New Plymouth

Testing commercial papain and an in-house product in parallel to compare their ability to detect weak Rh antibodies.

4. AN ALTERNATIVE ENZYME METHOD

Marina Wigmore, Medlab Thames, Thames Hospital, Thames

Over the past four years the Thames Hospital blood bank staff have experienced sensitivity problems with various enzyme techniques, with problems more clearly defined by external QC surveys primarily the NIPS Survey. Using these as indicators of success/failure of sensitivity the problems seem to have improved with the use of the Alternative Method.

The enzyme method was sourced from the Waikato Blood Centre who are also currently using the same method and are our reference laboratory. For routine Crossmatch, Antenatal and Group/Hold Screens, the method is simple to use and read. Even used in an urgent situation with minimum incubation times, sensitivity is not compromised.

5. ANTISERA — THE BEST BUYS FOR 1992

Linda Pinder, Auckland Regional Blood Centre, Auckland Hospital, Auckland

The Auckland Regional Blood Centre has recently completed an evaluation of ABO and D typing reagents and monospecific Anti-IgG Coombs reagents prior to bulk purchase for 1992. Performance and prices of the reagents tested will be discussed and a special mention made of the new Monoclonal Anti-IgG now available.

6. BEP-II CONDEMNED TO THE GAS CHAMBER

Joy Tippett, Immunohaematology, Taranaki Base Hospital, New Plymouth

A brief account of the problems encountered with HIV testing when contaminated distilled water is used in a Behring ELISA Processor and the methods used to overcome these.

7. BONE MARROW HARVESTING

Eileen Chappell, Manawatu Regional Blood Centre, Palmerston North Hospital, Palmerston North

An overview of the separation of bone marrow for autologous grafting using the Haemonetics 30 blood component separator will be presented.

8. LETTING GRAVITY DO THE WORK

Ray Scott, Auckland Regional Blood Centre, Auckland Hospital, Auckland

The availability of a new plasma components collector offers a means of producing Red Cell Concentrates and fresh cell free Plasma simultaneously without the requirement for centrifugation. A review of the performance of this device and the potential uses will be presented.

9. SOME ASPECTS OF WORKING IN A VERY SMALL LAB

Aileen Hindess, Laboratory, Taupo Hospital, Taupo

Some of the difficulties of maintaining expertise in a very small outlying laboratory are discussed.

10. ARBC LOCAL AREA NETWORK

Paul Clark, Auckland Regional Blood Centre, Auckland Hospital, Auckland

In March 1992 the Auckland Regional Blood Centre installed its long awaited 25 station Local Area Network. Novell Netware V3.II is the network operating system. The major software packages on the system are Microsoft Windows 3, Microsoft Word for Windows 2, Microsoft Excel 3.0 and Borland Paradox 3.5. I will share some of the frustrations and some of the joys of the first month with the new system.

11. EFFICIENCY GAINS THROUGH COMPUTING

Sue Lindsay, PAXUS Services Ltd, Wellington

In the last decade the computer has moved from the enthusiasts workbench to being the main tool in all kinds of businesses, homes and educational environments.

If there is one activity that is crucial to any size organisation or department, it is the communication of information to the people who need it. Improved communication will also improve productivity and reduce both costs and errors.

In addition to the benefit of improved information flow, equipment is easily shared between departments, making the best use of each dollar invested in computers.

There are other areas I would like to cover and you're bound to have questions.

12. THE NEXT GENERATION OF AUTOMATION

David Akeroyd, Abbott Diagnostic Division, Auckland

Laboratory Automation involving both Virology Testing and Data Flow has been a rapidly developing field.

Abbott Diagnostics Division have developed a variety of systems that meet automation requirements. A presentation will be made discussing current and future strategies for the Blood Bank Laboratory.

13. WELCOME TO DELPHIC — A COMPUTER BASED BLOOD BANK PROGRAM

Geraldine Heta, Blood Bank, Auckland Hospital, Auckland

The 23 September 1991 will always be remembered as the day that we went "live" on computer at the Blood Bank, Auckland Hospital. And our lives have never been the same since. Whilst the gestation period was long, it was relatively trouble-free. Unfortunately the same cannot be said for the post-natal period. This complex system at times seemed to have a mind of its own and frequently took offence when programmes were improved or new formats were introduced.

To justify computerisation a staff member was "replaced" on paper. I have yet to meet a staff member who has been abused verbally to the extent that our dear friend Delphic has.

However, all is not lost. On the positive side the system has allowed us to keep accurate records on previous transfusions and blood group antibodies. We are also able to register units of blood and have an individual record of the management of each unit.

14. BLOOD DONOR RECRUITMENT SOLVED

Judith Palea'ae, Immunohaematology, Wanganui Base Hospital, Wanganui

With increasing theatre workloads and especially the national demand for more plasma we were experiencing difficulty in contacting enough donors to fulfil this need.

A lot of time was spent on the phone or sending letters for a relatively small response.

3½ years ago we decided to remedy this by recruiting donors from a different angle. From averaging about 2500 donors a year we are now bleeding 3500 with the ability to bleed another 1000 without too much pain or effort if we needed to. The only pain would be for the ever demanding accountants and budget experts!!

14. CATCH THEM YOUNG

Faye Martin, Immunohaematology, Memorial Hospital, Hastings

A summary of nine years experience of high school mobile collections is presented.

16. DONOR RECRUITMENT AND RETENTION IN THE 90's

Gerard Verkaaik, Immunohaematology, Wairau Hospital, Blenheim

Mae West, famous designer of WW1 survival gear, and mamographers nightmare, is reputed to have coined the phrase ... "There's no such thing as BAD publicity, only PUBLICITY."

Very recent developments in donor politicking can be turned to advantage. They also present a new challenge to Blood Banks faced with aging and declining rolls.

Ideas will need to be pooled and a new look taken at recruitment and retention in the 90's.

17. SAFETY AWARENESS

Lorraine Rimmer, Auckland Regional Blood Centre, Auckland Hospital, Auckland

In July 1991 the Auckland Area Health Board's Safety Committees ran a Safety Awareness Week. The objectives of the week were

- to network with AAHB staff regarding H & S issues
- to extend functions of H & S
- to increase management support
- to raise staff morale
- to raise the image of the AAHB to the public

The ARBC's theme for the week was "The Safety of Ourselves and our Fellow Workers". A very successful week was organised by our very active H&S Committee and many positive H&S activities have now been implemented.

18. COURSE IN TRANSFUSION MEDICINE — "SELF AND NON-SELF"

Max Love, Immunohaematology, Hutt Hospital, Lower Hutt

A report on this course held in Melbourne in April 1992 under the sponsorship of the Royal College of Pathologists of Australia, Australasian Society of Blood Transfusion, Red Cross Blood Banks and Victorian Immunohaematology Discussion Group is presented.

19. USER PART CHARGES

Ian Bardsley, Laboratory, Hawera Hospital, Hawera

The introduction of User Part charges has caused some donors to reconsider their position as donors.

The effect of the introduction of User Part Charges on donor attitudes is discussed.

20. BLOOD TRANSFUSION AND THE TRANSMISSION of CMV

Bronwyn Kendrick, Manawatu Regional Blood Centre, Palmerston North Hospital, Palmerston North

An important but not as often thought about virus in blood transfusion as are HIV, HBsAg, HCV and T pallidum.

The history, biology, diagnosis and prevention of the infection through transfusion are presented.

21. HTLV-1 TESTING IN NEW ZEALAND

Helen Brady, NZCDC, Porirua

Over the last two years the New Zealand Communicable Disease Centre has offered a screening service for Human T Lymphocyte virus (HTLV-1) for the Blood Transfusion Service. This retrovirus is endemic in some areas of the world and has been isolated from aborigines in the Northern Territory of Australia. With New Zealanders' fondness for overseas travel and our diverse ethnic population, is this another retrovirus which will be routinely screened for in our blood supply? A brief summary of the testing results so far, what tests are used and what is the current state of confirmatory assays will be presented.

22. REGIONAL HCV TESTING — OTAGO AND SOUTHLAND

Les Milligan, Otago Regional, Blood Centre, Dunedin Hospital, Dunedin, Lindsey Browning, Immunohaematology, Southland Hospital, Invercargill

Following an introduction, a resumè of results to date is presented.

23. ANTIBODY PRODUCTION — A CASE STUDY

Diane Murton, Wellington Regional Blood Centre, Wellington Hospital, Wellington

On 19.09.91, Leo, a 57 year old male cancer patient, first appeared with a positive antibody screen. Over the next two months until his death in early December we obtained compatible and incompatible crossmatches, negative and positive antibody screens, followed by doubtful and eventually identifiable antibody investigation results. Leo produced anti-Fyb and anti-Jka which made 95% of donor blood incompatible.

24. COLD AGGLUTININ DISEASE — AN UNUSUAL CASE

Linda Giddy, Immunohaematology, Taranaki Base Hospital, New Plymouth

Cold Agglutinin Disease is a condition most associated with the winter months — or is it? Follow the investigation that lead to the diagnosis of Cold Agglutinin Disease in the middle of summer.

25. Rh(D) ANOMALIES DETECTED IN ANTENATAL WORK AND A SUGGESTED MONITORING SCHEDULE

Diane Whitehead, Christchurch

A Rh(D) positive patient presented with a positive antibody screen identified as anti-D at her pregnancy test at six months. Tests confirmed that the patient's blood group was Rh(D) mosaic Category VI. A Scheme was devised to monitor the antibody status of the patient during the remainder of her pregnancy. Limited family studies were used to trace the inheritance of the D mosaic group.

After a home birth, a second patient presented with concern about whether she required treatment with Rh(D) immunoglobulin. Tests showed that the patient also had a Rh D anomaly. Brief comment will be made concerning the usefulness of the patient's history in investigating a blood group anomaly.

Implications for cost effectiveness and monitoring of problems of this type in a small to medium sized laboratory will be discussed.

26. SEVERE HAEMOLYTIC DISEASE OF THE NEWBORN CASED BY ANTI-E

Marie Willson, Laboratory, Gisborne Hospital, Gisborne

A case history of severe HDN caused by anti-E is outlined and the difficulties encountered because of the isolation of the laboratory.

27. THE BMLSc DEGREE AT MASSEY UNIVERSITY

Chris Kendrick, Manawatu Regional Blood Centre, Palmerston North Hospital, Palmerston North

1992 sees the beginning of University Based degree Training for Medical Laboratory Science in New Zealand. A report of the first intake of students and the involvement of the profession in the course content at Massey is presented.

Poster Abstracts

1. SPLENECTOMY FOR PYRUVATE KINASE DEFICIENCY

Geoff Herd, Immunohaematology, Northland Base Hospital, Whangarei

A fully studied erythrocyte pyruvate kinase deficient family is presented.

The proband and her sister required regular red cell transfusions for 10 and 8 years respectively until splenectomy was performed.

Eight months post splenectomy no further transfusions have been necessary.

2. OPTIONS — A COMPUTER BASED LEARNING TOOL

Alison Dent, Auckland Regional Blood Centre, Auckland Hospital, Auckland

A 'practical' look at computerised education.

3. ABBOTT HIV I/II 3RD GENERATION ASSAY

Christine Martin, Abbott Diagnostic Division, Auckland

Abbott Diagnostic Division has released a new direct Enzyme Immuno Assay (EIA) for the detection of specific IgE and IgM antibodies to human Immuno-deficiency Virus type 1 and 2.

A presentation will be made showing the performance and clinical utility of this assay.

4. PROBABLE FALSE POSITIVE AWAITING CONFIRMATION

Grant Storey, Waikato Regional Blood Centre, Waikato Hospital, Hamilton

The indeterminant HIV Serology findings on a donor are presented and an attempted conclusion is drawn.

5. THE SORIN COMMITMENT TO VIRAL HEPATITIS

Brian Fowler, Pacific Diagnostics, Auckland

Abstract not available at time of print.

6. DOPED UP ON BLOOD

Stephen Silk, Immunohaematology, Hutt Hospital, Lower Hutt

The 1988 Olympics were nicknamed the 'Anabolic Olympics'. Let us hope the Barcelona Olympics do not become the 'Haematocrit Olympics'.

7. Rh IMMUNOGLOBULIN — HOW MUCH AND AT WHAT COST?

Sheryl Khull, Wellington Regional Blood Centre, Wellington Hospital, Wellington

The dose of Rh immunoglobulin indicated postnatally varies with the size of the foetomaternal haemorrhage (FMH). Until 1990 we screened for the presence of passive anti-D in the maternal serum to determine whether one dose of Rh immunoglobulin was sufficient. This practice leads to overtreatment. Suitable ways to determine the size of the FMH include acid elution quantitation, rosette screening and clinical evaluation. Considering the minimal clinical impact of Rh prophylaxis failure, extravagant efforts to detect large FMH are probably not a high priority use of transfusion laboratory resources.

8. BENEFITS OF LEUCOCYTE DEPLETION OF RED CELLS AND PLATELETS

Stephen Jones, OBEX Medical, Auckland

Clinical problems associated with Leucocytes in red cells and Platelets include:

- Alloimmunisation and refractoriness
- Graft versus host disease
- Non-haemolytic febrile transfusion reactions
- Immunomodulation
- Occlusion of pulmonary vasculature
- Histamine release
- Fibronectin depletion
- Cytomegalovirus infection (CMV)
- Thrombocytopenia (transfusion related platelet decrease)

The answer to these problems — "Leucocyte Free" Red Cells and Platelets through the use of high efficiency bedside filtration.

9. NEURAMINIDASE — AN UNWANTED ENZYME

Will Perry, Biological Laboratories Division, Salmond Smith Biolab, Auckland

Bovine Serum Albumin (B.S.A) is widely used in a number of applications in immunohaematology.

The performance of the B.S.A. is affected by its purity.

This poster will address the problems associated with the use of B.S.A. contaminated with a Neuraminidase-like impurity.

10. SEMEN BANKING

Roger Austin, Immunohaematology, Taranaki Base Hospital, New Plymouth

Roger has fathered 48 children throughout the North Island in the last 9 years. How did he do it? What does his wife think? These and other questions answered in explicit detail.

11. "FAST FOOD" TECHNOLOGY

Janette Dixon, Scianz Corp, Auckland

This poster takes a brief look at convenience "ready to go" products for use in the immunohaematology laboratory — with a focus on blood grouping by microtitre methods and solid phase antibody screening.

Descriptions of the principles involved are given as well as a summary of the instructions for use.

12. A RAPID SELF-CONTAINED IMMUNOASSAY FOR HEPATITIS B SURFACE ANTIGEN

Katya Dmitrieff, Hoechst NZ Ltd, Auckland

A rapid monoclonal capture polyclonal indicator sandwich ELISA to detect Hepatitis B surface antigen has been developed. The glass fibre matrix and unique capillary wash format are combined with a fluorescent substrate to give a sensitive and rapid test. The use of the OPUS random access analyser gives the test "walk away" automation. The test is a convenient and reliable method for screening for HBsAg.

13. BCC-1 GRAVITY FILTRATION SYSTEM

Pat Bayer, Tuta Laboratories, Sydney

An innovative method for separating plasma and red cells will be shown.

The BCC-1 gravity filtration system which has been developed by Tuta Laboratories will be displayed. Principles of filtration, method of use and merits of the system will be described.

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If you would like to receive further information on any of the papers or posters presented, do not hesitate to contact the authors directly.

EVEN IF YOU DON'T UNDERSTAND THE ABOVE HEADLINE, NOW YOUR IMx WILL.

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Articles of Interest

Antibody Identification — A Case Study

by Sheryl Khull, Red Cell Serology Laboratory,
Wellington Regional Blood Service, Wellington Hospital.

Mrs S was admitted for an elective caesarian section at 39 weeks gestation, after a normal pregnancy — her third. A blood sample was sent to the laboratory for Group and Screen. Immunohaematology records showed that we had identified anti-HI in Mrs S's serum during two of her earlier pregnancies, in 1986 and 1989.

Routine test results confirmed that Mrs S was group A Rh Positive, but her antibody screen was positive with both of the screening cells by both indirect antiglobulin and two-stage papain techniques. The sample was referred for investigation. Delivery suite was notified and a further specimen requested.

Since the caesarian section was immiment, an antibody identification panel was set up using low ionic strength antiglobulin technique as soon as possible on receipt of the specimen. Grade '8' reactions were obtained with all ten group O panel cells, but the auto control was negative. At this point there were three possible explanations:

1. Her anti-HI had increased its thermal amplitude to a degree where it was able to activate complement under the conditions of the test.
2. Mrs S had developed several antibodies, which together reacted with all panel and screening cells.
3. Mrs S had developed an antibody to a high frequency antigen present on all cells so far used for testing.

Each of the possibilities would require quite different laboratory investigations:

1 a) Maintaining reactants at 37*x*C throughout testing might avoid Interference by an antibody that was essentially cold-reacting.
   b) Use of anti-IgG in the antiglobulin test would only detect IgG bound to the red cells at 37*x*C, not activated complement.
   c) Selection of A1 red cells could be expected to yield a compatible crossmatch.

2 a) Mixtures of antibodies often reveal themselves by reacting at different strengths with different red cell samples.
   b) Different techniques may enhance one antibody while inhibiting another.
   c) Using more red cell samples increases the changes of finding one which happens to be negative for all antigens concerned.
   d) Phenotyping the patient's red cells will indicate which antibodies she could possibly produce.

3 a) An antibody to a high frequency antigen is one of the most difficult antibodies to identify without some clues to its possible identity. Large special panels of cells lacking high frequency antigens (Bombay, Rh null, Ko, Lu(a-b-), Vel negative, Fy(a-b-), MkMk, etc, as well as the more than a hundred high frequency antigens which have not yet been assigned to *a* blood group system) are not readily available.

By definition, cells which lack a high frequency antigen are rare, but not all are equally rare in all populations. For example, (Fy(a-b-) is in fact the most common phenotype (68%) in American Blacks. In New Zealand's Polynesian population, the Jk(a-b-) phenotype, which lacks the high frequency antigen Jk3, occurs in a significant, though still low, percentage (0.89%). This makes anti-Jk3 a good first guess in a New Zealand patient with an antibody to a high frequency antigen. (Mrs Smith had A Polynesian-sounding middle name).

To make life easier for New Zealand blood bankers, in 1982 Heaton and McLoughlin described the Urea Lysis test, in which Jk:3 cells are rapidly lysed by 2M urea, while Jk:-3 cells are not. This test is so quick, easy and cheap, that it is used as a screening test on New Zealand donors in order to detect Jk:-3 blood donors, most of whom have no atypical antibodies. In just one more minute we knew that Mrs S's cells failed to lyse in 2M urea, and were therefore reasonably certain that her serum contained anti-Jk3. The transfusion director contacted her obstretrician to determine the need for obtaining Jk(a-b-) Blood. Fortunately her caesarian section was uneventful and Mrs S did not require transfusion.

Baby S's cord blood had a positive direct antiglobulin test (grade '8') and we were able to identify anti-Jk3 in an eluate from the cord red cells. Anti-JK3 can cause clinical haemolytic disease of the newborn, but baby S was healthy and did not require any treatment.

This case turned out well, with no requirement for transfusion. The urgent need to identify an antibody reacting with all panel cells can be a very difficult situation. Although we frequently think of urea lysis testing only in relation to screening blood donors, this case reminds us of its usefulness in solving serological problems in patients.

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Question and Answer

Question:
A question from a small rural hospital has caused heated debate among our medical staff. A unit of red cells had been issued to the ward for transfusion. The technologist who issued the blood noticed the unit sitting on the counter at the nurses'; station an hour and a half later. She took the temperature of the unit (23*x*C) and advised against transfusing. The patient's attending physician insisted the blood was fine since he had four hours in which to administer the unit, so he would just subtract one and a half from four hours. What should future procedure be? Should this unit have been transfused?

Answer:
Blood and blood components should not be obtained from the Transfusion Service until transfusion is imminent. The blood or component should be infused as soon as possible after delivery to the patient care area.

Red-blood-cell-containing components may not be returned to the blood bank for storage or reissue if greater than 30 minutes have elapsed from the time of issue or if there is any evidence of system entry or inappropriate storage conditions.

It is true that the maximum recommended length of time for infusion is four hours. This time is generally counted from the time the bag is spiked and hung at the bedside. The limit is applied to lessen the chance of bacterial contamination (which can occur any time the system is opened or can be pre-existing from the time of collection) from becoming a clinically relevant problem. Another issue surrounding the practice of allowing blood to be held for extended periods on the ward is that of identification. Any interruption of standard procedure increases the possibility that an error can occur. What would happen if a second unit of blood had been transported to the ward for a different patient and they both sat at the nurses' station for a period of time? Clearly this type of occurrence would increase the likelihood of a transfusion error and should be avoided. Good practice dictates that blood and blood components should be handed directly to the staff person responsible for the transfusion and that the infusion should begin as soon as possible thereafter.

Should the unit in question have been transfused? There is no right answer to this question. It was certainly appropriate for the technologist to bring the situation to the attention of responsible staff. The recommendation not to transfuse was also appropriate. Routinely allowing transfusion under these circumstances would not be in keeping with the spirit of the

standards of the AABB. Such practice might also be found to be in conflict with the requirements of other regulatory agencies. The final decision to transfuse must, however, remain with the attending physician, who often makes an informed choice in consultation with the medical director of the blood bank. There should be documentation in the patient's chart any time a therapeutic decision is made outside of the context of standard procedure. The hospital's transfusion commitee may want to address this issue, especially if the practice of delayed infusion is common or handled lightly.

Source: AABB News Briefs — June 1991.
Answer provided by Ann McMican, MS, MT(ASCP) SBB

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HEPATITIS IN CHINA

One of our colleagues, Narelle Cosier, is presently living in Chang Chun in northern China. She sends this cutting from the local newspaper.

"A Major Step to Check Hepatitis"

"China is to inoculate all new-born babies against Hepatitis B, from January 1. This will be a major step to improve the general health level of the nation.

"China has a high incidence of virulent hepatitis. It is estimated that about 120 million Chinese carry the hepatitis B virus, and that babies of about 40% of the pregnant women carrying the virus are likely to be infected. Infected babies are most likely to become chronic hepatitis B virus carriers. Some of them will even develop cirrhosis or liver cancer when they grow up.

"So the decision to inoculate new-born babies is a significant one, and could help check the spread of hepatitis B among future generations of Chinese.

"The new vaccine, the result of much painstaking effort by scientists during the Sixth Five-Year Plan Period (1981-85) and the Seventh Five-Year Plan Period (1986-90), is an effective weapon against the disease. Now that it is possible to produce enough vaccine to inoculate all new-born babies it is time to meet the demand of the people.

"The Chinese Government has shown great concern for the prevention and treatment of virulent hepatitis B. That should stimulate the widespread use of the vaccine on babies. Departments in charge of public health at all levels must ensure the smooth implementation of the inoculation project by finding enough people and allocating enough funds and materials for it."

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PARENTAGE TESTING

Extracts from Information and Protocols as used in the Paternity Testing Laboratory Auckland Regional Blood Centre.

PRINCIPLES OF GENETIC MARKER TESTING IN CASES OF DISPUTED PARENTAGE

1. One half of the genetic information in a child is maternal in origin and one half is paternal.
2. Only one man can be the biological father of a child.
3. Genetic markers used follow Mendelian Laws of Inheritance.
   - a child cannot have a genetic marker that is absent in both parents (direct exclusion).
   - a child must inherit one of a pair of markers from each parent.
   - a child cannot have a pair of identical genetic markers unless both parents have the marker (indirect exclusion).
   - a child must have a genetic marker if it is present as an identical pair in a parent.
4. The test method used must be reliable and reproducible and measure a characteristic with a known inheritance pattern.
5. The gene system must be polymorphic (distributed in the population) and the gene frequencies established by testing an appropriate sample of random individuals.

USEFUL GENETIC MARKERS IN PARENTAGE TESTING

Red cell antigens

These are genetic markers found on the surface of the red cell, and are often known as blood groups. ABO, Rh, MN, Kell, Duffy, Kidd. Other markers are used in special cases. The tests for red cell antigens depend on observing agglutination of red cells when they are mixed with an antibody which reacts with the marker expressed on the cell membrane. These markers are stable for several weeks if properly stored.

Red cell enzymes

These are genetic markers found in the red cell. The three usually tested for are EAP (erythrocyte acid phosphatase), PGM (Phosphoglucomutase) and GPT (glutamic pyruvate transaminase).

Serum proteins

These are genetic markers found in the liquid portion of the blood. The two we mainly test for are Gc (group specific component) and Hp (haptoglobin). In selected investigations Gm (gamma marker) and Km (kappa marker) are used. Most enzymes and proteins are tested by placing the sample in a support material and separating the markers in an electrical field.

The basic principle of these tests is that the inherited protein molecules vary in electrical charge depending on their genetically determined amino acid composition. By selecting the proper test conditions one can separate molecules with minimal differences in charge. These tests are very reliable and the markers are not generally affected by storage or shipping.

Human leucocyte antigens (HLA)

Genetic markers found on white cells. A locus and B locus. In special cases C and D locus markers are investigated. The tests for HLA antigens depend on observing the killing of lymphocytes by antibodies directed to the genetic markers on their surface. Samples for HLA must reach the testing facility within a specified period of time so that living lymphocytes from the blood sample can be collected.

Desoxyribonucleic acid (DNA)

Recent advances in the area of genetic research have demonstrated the existence of a vast amount of genetic variation which has previously gone unrecognised. The human chromosome has a large number of individual segments or regions which are recognised by cloned human DNA probles. Genetic variation within an individual's

chromosomes at or near the probe specific site can often be recognised after digesting (restricting) the chromosomal DNA with different bacterial enzymes and separating the segments by electrophoresis. The genetic variation is observed by looking at the length of DNA fragments. The genetic variation is called restriction fragment length polymorphism (RFLP). The three single locus DNA probes used in our procedure are very informative and used in conjunction with the conventional blood tests will usually result in a calculated probability of paternity that exceeds 99.5%.

ESTIMATING THE POSSIBILITY OF PATERNITY

Using statistical calculations based on sound mathematical principles it is now possible to estimate a probability of paternity. The calculation uses the genetic information obtained by testing of the individuals concerned and compares this with tables of genetic data gathered on testing a large number of routine New Zealanders, either European or Polynesians.

In this calculation, each genetic marker in the child is assessed as to its probability of being inherited from the mother or putative father. From genetic frequency data, it is then calculated the chance of that marker being inherited from the putative father or a random population of other possible fathers. This procedure is then repeated for each genetic marker and the values so obtained can be multiplied which results in a figure known as the paternity index.

Paternity Index

This is a figure representing the genetic odds in favour of paternity given the various observations of blood types available. In general a paternity index ABOVE 19 is regarded as being statistically significant i.e. below this figure little can be stated regarding the probability of paternity.

Probability of Paternity

This genetic expression combines the data obtained from the paternity index and the non-genetic information, that is any information that would indicate that the accused man had the opportunity to father the child. This non-genetic data is known as the prior probability. Paternity laboratories usually, unless specifically requested, take the stance that they are neutral in this respect and adopt a prior probability of 50% or 0.5. Thus the probability of paternity is not weighted one way or the other, and is based solely on the genetic data. The probability of paternity then compares the chance of the tested man being the father of the child in question with other men in the random population who are assumed to have an equal chance of having fathered the child. The probability of paternity is expressed as a percentage and many laboratories now follow the predicts as follows:

Probability of Paternity (%)	Prediction of Paternity
99.8-99.9+	Practically proven
99.0 - 99.7	Extremely likely
95.0 - 98.9	Very likely
90.0 - 94.9	Likely
80.0 - 89.9	Hint
80	Not useful

Comments

Very often, using conventional typing tests e.g. red cell markers and HLA, it is possible to obtain a probability of paternity above 99% and on occasions much higher. The more tests that are done, the higher the probability becomes in non-exclusion cases. Where the probability of paternity is below 99% it is sometimes worthwhile performing additional tests such as protein or red cell enzyme markers. Furthermore DNA testing can also be performed which is a powerful tool increasing the probability of paternity to even higher levels, and in most cases above 99.7%. In paternity cases, where the probability of paternity is above 99.7% (practically proven) no further tests are necessary as it would be exceedingly rare for such a finding to be overturned by performing additional tests. Indeed some experts would state that above 99% no further testing is required. This would be our own experience, although there may be exceptions that could occur.

★ ★ ★ ★ ★ ★

SPACED-OUT BLOOD

The effectiveness of white blood cells and blood volume decreases for those who venture into space, according to researchers involved in the Spacelab Life Sciences-1 (SLS-1) mission flown aboard the space shuttle 'Columbia'.

The SLS-1 crew conducted studies last summer on blood,cell, kidney, lung, and cardiovascular functions while in space. Consistent with findings in similar experiments, the SLS-1 crew found that white blood cells do not respond as well in space as they do on earth.

Source: The Component Therapy Digest
Fenwal Division of Baxter Healthcare Corporation

★ ★ ★ ★ ★ ★

Cold Autoimmune Haemolytic Anaemia — A Review

by Sheryl Khull, Red Cell Serology Laboratory,
Wellington Regional Blood Service, Wellington Hospital

The current theory of immunity holds that there are clones of B cells in every human which would make antibodies to all possible antigens, including the antigens of 'self'. The 'forbidden' clones that could make antibodies to self are normally suppressed by specific T suppressor cells. In autoimmune disease, T cell surveillance is impaired, allowing forbidden clones of immunocytes to proliferate and produce autoantibodies.

With increasing age the immune system is more prone to malfunction, making autoimmune disease more common in older patients. Genetic predisposition may also be a factor, since autoimmune diseases tend to occur in families.

In idiopathic cold autoimmune haemolytic anaemia only one clone is involved and the autoantibody produced is monoclonal. In warm AIHA several clones must be involved, since the autoantibody is polyclonal. The loss of T cell regulation appears to be non-random, and the specificity of the autoantibodies which are produced show a pattern which is typical of the type of AIHA.

Immunohaematologists classify AIHA into two broad categories, depending on whether the autoantibody reacts optimally at cold or warm temperatures. We call these, rather obviously, cold-type and warm-type AIHA. Cold-type AIHA is also known as cold haemagglutinin disease. It is important that the types be distinguished, since they have different treatments and prognoses, and require different types of laboratory investigations. Although some cases may present difficulties, the distinction is usually quite clear.

Cold AIHA is less common than warm AIHA. The middle-aged to elderly are most often affected. Young patients are usually secondary cases. The patient is typically pale and slightly jaundiced with symptoms of chronic anaemia and sometimes haemoglobinuria or painful extremities associated with cold. Low grade anaemia may result from more frequent haemolytic episodes during winter, although

the patient may feel well during summer.

The haemoglobin is usually moderately low. Red cell morphology is less abnormal than found in warm AIHA, although spherocytes are often noted. In vitro autoagglutination of blood is characteristic, and often quite noticeable on the slide prepared for examination of red cell morphology. It may even be obvious as clumping of the anticoagulated blood sample, especially if it is refrigerated. The DAT is usually positive, with the red cells coated only with C3d.

The autoantibody is typically an IgM, complement-binding antibody. It is monoclonal in idiopathic CHD, but polyclonal in secondary disease. Its optimal temperature is 0-4°C, but it may have a wide thermal range (up to or even above 30°C). The antibody specificity is often in the I system. It has a high titre (greater than 64, and often in the thousands). It is found in warm-separated serum, since it is in reversible, thermal-dependent equilibrium with red cells, and therefore not found on the red cells at 37°C.

In cold AIHA red cell destruction is predominantly via intravascular complement-mediated haemolysis. At low body temperatures, within the thermal range of the antibody, antigen-antibody complexes form. As temperature rises, these dissociate. Complement activation occurs optimally at 37°C and less with lower temperatures. In the area of temperature overlap, haemolytic episodes occur.

The amount of red cell destruction, and hence severity of disease, is related to the thermal aplitude of the autoantibdy, and the degree of exposure to the cold, and to a lesser extent; complement activation thermal range, the rate of production of complement components, and the level of C3b INA. Early inactiviation of C3b reduces haemolysis since coating of cells with C3d blocks the binding of newly activated C3.

SEROLOGICAL INVESTIGATION OF COLD AIHA

The investigation of suspected AIHA requires appropriate specimens. Red cells for DAT should be collected into anticoagulant, since the anticoagulant prevents in vitro complement activation. DAT's on clotted specimens that have been refrigerated may be positive, due to complement components which have been bound to the cells in vitro by naturally-occurring cold autoantibodies of no clinical significance.

Serum is required for investigation of unbound autoantibody, and for determination of any alloantibodies present. The blood sample should be maintained at 37°C until the serum is separated from the red cells, to avoid loss of the autoantibody by autoabsorption.

The one test most indicative of the immune nature of an acquired haemolytic anaemia is the Direct Antiglobulin Test. However, a positive DAT may occur coincidentally in a patient. A significant number (one study 8%) of hospital patients have a positive DAT, usually due only to complement, without evidence of haematological disease. A positive DAT will demonstrate any antibody or complement on the red cells, but it cannot distinguish between auto and allo-antibodies. A positive DAT may indicate HDN, a transfusion reaction or AIHA. In patients with strong cold-reacting autoantibodies, the red cells may have to be washed in several volumes of warm saline before being suitable for direct antiglobulin testing.

The initial DAT is performed using polyspecific antiglobulin reagent, which contains anti-IgG and anti-C3. Once a positive DAT is found, classification of the type of protein coating the red cells can be determined using monospecific antiglobulin reagents. Since the five main classes of immunoglobulins have different heavy chains (IgG, M, A, E & D have gamma, mu, alpha, epsilon and delta chains respectively) antibody specific to the heavy chain can determine the class of immunoglobulin coating on the red cells. Because the IgM autoantibody is seldom active at 37°C, it is often not detectable on the red cells. However, the binding of complement components is not a reversible process and these are more readily detectable, most typically C3d.

The detection and characterisation of antibodies in the patient's serum is required in the serological evaluation of AIHA. The serum may contain only autoantibodies; autoantibodies and alloantibodies; or only alloantibodies (the autoantibodies having been absorbed onto the patient's red cells).

The specificity of cold autoagglutinins can usually be determined by parallel titrations of serum against pooled adult and cord cells, since the specificity is usually in the I system (usually anti-I). Tests for specificity often require a range of reaction temperatures since a strong antibody may react to a similar titre and avidity with adult and cord cells at 4°C, and only reveal its preference at less optimal temperatures. It must be remembered that adult cells, and more specially cord cells, vary in the strength of their I antigens.

Although anti-I is the most common specificity of cold autoantibodies, especially idiopathic cases or those secondary to Mycoplasma pneumoniae infection, other specificities have been reported. Anti-I is more common in cases secondary to infectious mononucleosis.

Specificities outside the I system are rare, but cases due to anti-Pr have occurred. Anti-Pr reacts equally well with adult and cord cells, and is different to I system antibodies in that it is destroyed by enzymes. Isolated cases with other specificities have been reported.

In patients with Paroxysmal Cold Haemoglobinuria, the specificity of the autoantibody is almost always anti-P, i.e. it reacts with P1 and P2 cells but not Pk or p cells, (as does the naturally occurring allo-anti-P produced by Pk individuals.)

Cold agglutinins should be titrated. A titre of less than 64 at 4°C is considered normal, but in severe cases of CHD the titre is often in the thousands. Although it is a useful guide, the titre of an autoagglutinin at 4°C is not a direct measure of its clinical significance.

The thermal range of cold antibodies should be determined by testing at a range of temperatures. Autoantibodies reactive only below 30°C are not likely to be a cause of in vivo red cell destruction.

Titrations and thermal amplitude studies may be performed using red cells suspended in 30% bovine albumin rather than saline. Occasionally, tests in saline may give equivocal results but tests in albumin dramatically reveal the pathological nature of the autoantibody.

CROSSMATCHING AND TRANSFUSION OF PATIENTS WITH AIHA

Although the anaemia resulting from cold AIHA is usually mild, these patients occasionally need blood transfusion. Transfusion of patients with AIHA carries several added risks beyond the risks present in every blood transfusion.

The autoantibody may cause premature destruction of any transfused red cells, just as it does with the patient's own cells. However, acute symptomatic transfusion reactions are rare, and the transfused cells usually survive about as well as the patient's own cells. In CHD with depletion of complement components, transfused plasma may increase haemolysis by introducing more complement. In these cases washed red cells may be indicated.

The major difficulties in finding compatible blood for a patient with CHD are likely to be technical ones. Problems often arise as soon as you try to find out the patient's blood group, since the patient's red cells are already agglutinated, and the patient's serum agglutinates all red cell samples tested. To minimise autoagglutination, the cells can be washed in warm saline.

To determine the true serum group, the test may be performed strictly at 37°C, at which temperature ABO agglutinins usually still react but cold autoantibodies do not. Serum grouping may be possible using cord cells of known ABO group if the autoantibody has anti-I specificity. The results may be confirmed at usual temperatures using autoabsorbed serum.

If other red cell antigen testing is required, especially if the only antiserum available requires an antiglobulin technique,

special procedures and precautions must be employed to avoid false positives. The use of anti-IgG in typing tests may yield reliable results if the cells are not coated with IgG.

Although transfused red cells are likely to be as compatible as the patient's own cells with the autoantibody, any incompatibility with alloantibodies is equally likely to result in a haemolytic transfusion reaction as it is with any other patient. Before blood transfusion can be considered, some method of determining the presence of alloantibodies in the patient's serum is necessary. Routine antibody screening and crossmatching procedures may be confused by the presence of autoantibody.

In cold AIHA where the autoantibody is not reactive at 37°C, tests performed strictly at 37°C will be free of autoantibody interference while revealing any clinically significant alloantibodies. This method is simple, quick, suitable even for recently transfused patients, and works in most cases of cold AIHA.

Using anti-IgG instead of polyspecific antiglobulin reagent will avoid interference from complement activation by cold autoantibodies. Those alloantibodies detectable in vitro only by the complement activation they produce would also be missed. These are not common, but clinically significant examples such as anti-Jka do occur. Laboratories which use PEG antiglobulin technique already use anti-IgG reagents in a technique which is senstitive enough to detect most clinically significant antibodies.

Cold autoabsorption is useful for removing interfering cold autoantibodies with a high thermal amplitude. This method is reliable if the patient has not been transfused recently, but any transfused cells in the circulation may absorb cold-reacting alloantibodies. Serum clotted and harvested at 4°C and warm-washed anticoagulated cells are required. The cells are enzyme treated to enhance their antibody uptake. Multiple absorptions are sometimes required before the autoantibody is no longer detectable at 37°C.

Since cold autoantibodies are IgM, they can be inactivated with dithiothreitol. Any IgM alloantibodies present will of course also be inactivated, but immune IgM alloantibodies seldom occur without an IgG component, so the risk is minimal.

Formaldehyde-fixed rabbit erythrocytes or stroma (RESt) absorb anti-I, anti—H and anti-HI, allowing further testing for alloantibodies. A commercial kit is available if you don't happen to have rabbit blood in your bank. However, some important alloantibodies, such as anti-D, may also be depleted in samples treated with RESt.

Once the procedures for revealing alloantibodies have been used, minor modifications of routine crossmatching methods are generally suitable for selecting compatible blood. Autoabsorbed serum may be used. Crossmatching strictly at 37°C will avoid interference from most cold antibodies. Tests in saline, rather than albumin or LISS, may be preferable.

In cold AIHA, the use of prewarmed blood for transfusion is controversial. Certainly large volumes of cold blood should not be infused rapidly. Warming blood for transfusion is probably of most significance for severely ill patients. In all cases it is beneficial to keep the patient warm.

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THE RELATION BETWEEN RESPONSE TO HYPERTONIC STRESS RECOVERY AND THE SWIRLING OF STORED PLATELETS

Les Milligan, Suzanne Williams, Kaye Stewart, Linda Barrett

The nature of platelet response to osmotic shock and its relationship to platelet swirling were studied in an attempt to establish whether the observable swirling action of stored platelets is a practical and reliable screen for the assessment of platelet viability.

Blood was collected from random male and female donors and platelet concentrates were prepared using the standard Dunedin method.

Hypertonic Stress: the addition of hypotonic solution to platelet rich plasma causes two successive changes in the platelets which can be measured in a spectrophotometer. There is a sudden drop of light absorbancy followed by a rise towards the original level. This reversal reaction may be used as an indicator of platelet viability after storage (the hypertonic stress was expressed as a percentage recovery).

We were able to establish that:

i) if the HST showed a recovery of >50% swirling was evident (hypotonic stress response depends on the presence of intact platelets and a stabilised pH).
ii) while it is possible to maintain platelet viability during extended periods of storage (5 - 7 days), there is considerable variation in the efficiency with which platelet viability is maintained.

We were able to establish that observable swirling of platelets in suspension is a practical and reliable in vitro indicator of platelet viability.

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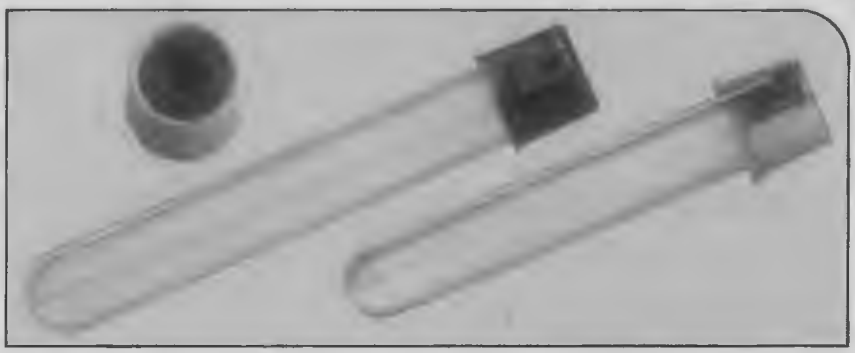

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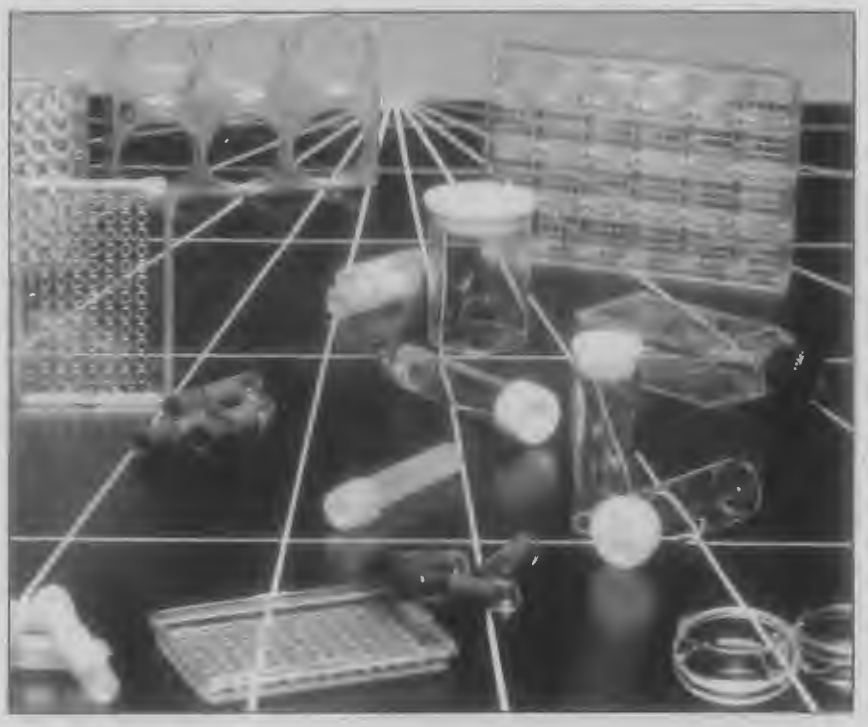

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Editor
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Members wishing to receive their publications by airmail should contact the Editor to make the necessary arrangement.

Membership Sub-Committee Report — March 1992

Since the November meeting there have been the following changes:

	12.3.92	26.11.91	26.8.91	23.5.91
Membership	1188	1188	1297	1202
less resignations	3	6	37	33
less G.N.A	9	3	1	7
less deletions	-	-	81	-
less deceased	2	1	-	2
less duplications	-	1	1	-
	1174	1178	1177	1160
plus applications	14	9	10	22
plus reinstatements	-	-	-	116
	1188	1188	1187	1298
Composition				
Life Member (Fellow)	12	12	12	12
Life Member (Member)	5	5	5	5
Fellow	21	21	21	21
Member	670	670	666	711
Associate	393	392	395	462
Non-practising	61	60	60	57
Honorary	26	28	29	29
Total	1188	1188	1188	1297

Applications for Membership
A. HAYWARD, Tauranga; D. MILNER, Taranaki; J. MIDDELKOOP, Waikato; J. KEITH, Hamilton Med Lab; C. RAWSON, Hamilton Med lab; J. DUNLOP, Christchurch; A. ABDI; K. HARRIS, Greenlane; J. SEXTON, Tauranga; K. WILLS, Palmerston North; A. WEBB, Wanganui; T. DAWSON, Greenlane, W. GOBLE, Hamilton Med Lab; R. MATTHEWS, Lower Hutt.

Gone No Address
J. WARREN, Blenheim; A. MOTYL, Princess Mary; D. RUDD, Blenheim; K. PATCHETT, Blenheim; P. WASTNEY, Medlab Sth; F. DOIDGE, Medlab Sth; T. CLARK, Med Lab Sth; J. DAVIES; J. MYRING, Princess Mary.

Resignations
D. ALLEN; M. DIXON, National Womens; C. SIMS, Waikato.

Deceased
C. ELLIS, Middlemore; M. HILBOURNE, Auckland.

The Pacific Way

Pacific Paramedical Training Centre News — Abstract from the Annual Report.

Regional Health Laboratory Quality Assessment Programme.

This activity commenced in May 1990, with the sending to 17 Pacific Island Laboratories, and one South East Asian Laboratory samples for analysis. Samples are despatched monthly to the participant laboratory. Six weeks after despatch of the sample an immediate report is posted to all laboratories in the survey showing the results that were obtained by the referee laboratory. Three weeks later a final report is sent to all laboratories responding to the survey. The report reviews the results of each participating laboratory and contains comments aimed at assisting the laboratory to improve its performance.

Each Biochemistry survey to date has consisted of three lyopholysed human serum samples and corresponding diluents. Target Values and Acceptable Ranges were established at the Clinical Chemistry Department of Wellington Hospital. Although 22 different chemical analyses were offered for each sample, the participants were asked to perform, in duplicate, only those tests they routinely performed. The laboratory response rates were 62% and 56% and the most commonly performed were sodium, potassium, urea, creatinine, glucose and total protein. The median of all replies was 44% "out of range". It was alarming to find that 60% of all sodium and creatinine tests were outside the acceptable range.

The first Microbiological survey consisted of smears and cultures of micro-organisms for identification and antibiotic sensitivity testing. Generally the staining of smears and identification of Staphylococci provided no difficulties. The use of inappropriate antibiotics for sensitivity testing caused some concern, particularly as comments on this problem have been sent to participating laboratories during past years. Also highlighted were incorrect methods and lack of internal quality control. It seems that not much work has been done to improve the standard of antibiotic testing over the past few years.

The Haematology survey consisted of a set of blood films for staining and comment upon the cellular picture. Generally laboratories did well in this exercise. Some were not prepared to suggest a possible diagnosis after examining the film, and some had difficulties with identifying immature white blood cells. Comments on the morphology of erythrocytes caused some concern as anaemia is a common occurrence in the Pacific Islands.

The Immunohaematology survey consisted of a blood grouping and compatibility exercise which was generally well done. Many laboratories are now using the methodology demonstrated at the Annual Bloodbanking course held at our Training Centre. Two points of concern that emerged from this exercise was the use by some participants of outdated reagents and the lack of internal quality control.

Some minor difficulties were experienced with the running of the "Regional Quality Assessment Programme" but these will be rectified by the Centre as more experience is gained.

Participation by Pacific laboratories is generally good and those laboratories that have a less than 50% record will be approached to see if the programme is relevant to their needs. As records and data accumulate with the programme, there will be a need in the near future to obtain a computer for storage and analysis of results.

Finally, the Pacific Regional Quality Assessment Survey of Health Laboratories undertaken by the Centre is the first comprehensive programme of its type in the region. Response from the participating laboratories has been generally good and the Centre hopes to further improve on this in 1992 by having more frequent contact with those laboratories who to date have been tardy with their replies. Feedback from laboratories is that they wish to continue with the programme even if they do not reply to all samples. In August of this year, Clare Murphy, Quality Control Biochemist, undertook an assessment on behalf of the P.P.T.C. of some of the Pacific Island Laboratories to assist those who require help in improving performance.

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Clinical Chemistry Quality Assurance in the Pacific

Solomon Islands, Vanuatu, Fiji, Western Samoa.
Brief Report by Clare Murphy for the P.P.T.C.

During August of 1991 I was fortunate enough to be able to visit five biochemistry laboratories in four countries of the South Pacific, and to observe and participate in their clinical chemistry quality control programmes. This was made possible through the support of the Pacific Paramedical Training Centre, which encouraged and funded the tour.

First I visited Honiara, the capital of the Solomon Islands. The laboratories at the Central Hospital are, like the hospital itself, in need of replacement, but they are well staffed and equipped. The major biochemical analyser is the Hitachi 704. Two specifically local problems in operating the 704 are maintaining a pure water supply (Honiara's water is high in calcium) and machine servicing. Lack of finance and distance contribute to a low level of technical back-up.

Port Vila, the capital of Vanuatu, is strikingly French in appearance but multilingual, fortunately for this monoglot English speaker. The Central Hospital used to be the British hospital in Condominium days. The laboratories are spacious, a quality emphasised in Biochemistry by a marked lack of equipment. As in Honiara there are problems with water purity, glassware and plastic pipette tips necessarily recycled through lack of finance are consequently less clean than is desirable. There was a dearth of technical books. The only copy of Monica Cheesbrough's "Medical Laboratory Practice in Tropical Countries" belonged to the Canadian volunteer.

The Colonial War Memorial Hospital in Suva, Fiji, was the largest visited on this trip. It is a solid early 20th century laboratory building teeming with staff and students. The Biochemistry laboratory has recently been re-equipped with two semi-automated general chemistry analysers, which have gone a long way towards improving analytical performance. However, specimen separation, identification, and laboratory documentation, could be better organised.

Lautoka Hospital's Biochemistry laboratory has a similar organisation and structure to Suva's, but there is more manual equipment.

While in Suva I was able to attend the 8th Convention of the Fiji Medical Laboratory Technologists Association. The theme of the conference was "Learning Together". It was a very enjoyable and professionally run occasion, and an opportunity to learn and to discuss ideas with people which I may not otherwise have enjoyed.

SPECIALIST LEVEL EXAMINATIONS 1991
EXAMINERS' REPORTS

CLINICAL BIOCHEMISTRY

SUMMARY:
Candidates should read the question and structure the answer as directed in the question.

Paper One was long, but required only brief note form type answers as indicated on the paper. Candidates who did not attempt all parts of the question put themselves at a serious disadvantage.

The examiners recommend that calculations be a compulsory part of the examination. Candidates should at least be competent in the basics of calculations, as covered in NZJ Med.Lab.Tech. Volume 38 (1984) in a revision series of calculations by T.A. Walmsley.

PAPER ONE:

Question 1
No passes from 5 candidates.
This relatively simple calculation was answered very poorly and I am dismayed for the future of our profession if students are unable to adopt new technology and to critically investigate methods.

Question 2
3 passes from 6 candidates.
One answer was excellent. Applying pKa data to amino acids was poorly answered while interpreting the immunoelectrophoresis strip was well answered.

Question 3
3 passes from 7 candidates.
Cholesterol was answered extremely well, in contrast the bilirubin was poorly answered. Candidates failed to identify EDTA contamination as the reason for the negative iron result and that glucose oxidase only reacts with beta D glucose. Freshly prepared glucose solutions should be left for at least 2 hours for mutarotation to take place (glucose at equilibrium = 36% alpha and 64% beta).

Question 4
2 passes from 9 candidates.
All 9 candidates did this short answer question which unfortunately attracted poor answers. In particular, the problems of haemolysed sera with the CK method and the effect of thiol groups were not well understood. Enzyme levels in liver disease and alkaline phosphatase isoenzymes were also poorly answered.

Question 5
5 passes from 9 candidates.
The calibration of a spectrophotometer was very poorly answered. In contrast to this flame photometry and atomic absorption were answered well.

Question 6
7 passes from 8 candidates.
An average mark of 64% for this question indicated a generally good standard of answer.

Question 7
No passes from 4 candidates, highest mark 10%.
Not a single candidate made an acceptable attempt at this question which only asked for details of one application of HPLC in clinical chemistry.

Question 8
4 passes from 9 candidates.
The majority of candidates gave a good answer for blood pigments and the emergency treatment of a needle stick injury. The clinical answers on hyperparathyroidism and Padget's disease were poorly answered.

Question 9
2 passes from 6 candidates.
In part (a) most candidates knew what galactoaemia was and one person named the enzyme deficiency. Galactorrhoea is NOT excessive faecal galactose.

In part (b) most candidates wrote adequately on neuroblastoma and phaeochromocytoma but the questions on a catecholamine method were poorly answered. Two candidates copied the normetanephrine method from Question 1, and another gave the method for VMA, no-one knew the reference range. These are examples of the problems encountered.

Question 10
6 passes from 8 candidates.
One answer was excellent. Most understood ELISA and EMIT techniques while the question on spectral interferences was answered less well.

PAPER TWO

Question 1
7 passes from 8 candidates.
Most of the candidates who answered this question had a very good understanding of Near Patient Testing and its implications.

Question 2
1 pass from 4 candidates.
Candidates failed to structure their answer in line with the question. Candidates were unfamiliar with reaction rate kinetics and assumed that all reactions were linear with time.

Question 3
3 passes from 6 candidates.
All candidates were familiar with the metabolic pathway and clinical details of porphyria. Very few actually discussed the laboratory investigation of porphyria (as was asked in the question).

Question 4
3 passes out of 4 candidates.
Mediocre answers for such a common analyte.

Question 5
3 passes from 7 candidates
Most candidates reviewed the QC to date, but failed to identify the different analytical processes carried out by the random access analyser.

Question 6
5 passes from 7 candidates.
The place of glycosylated proteins in diabetic management was well understood but interpreting the GHb data seemed to prove very difficult.

HAEMATOLOGY

NUMBER OF CANDIDATES
Six candidates sat the examination and four gained a pass mark.

COMMENTS ON PAPERS
The examination consisted of two theory papers.

PAPER ONE:
The mean mark for this paper was 54.25%. The range was 37.75% — 68.75%.

SECTION A:
This section was worth 43 marks. The mean mark was 22. The range was 15.75 — 28.25.

The section consisted of eleven questions, ten of which required the candidates to examine and comment on blood or bone marrow photomicrographs with accompanying clinical and laboratory data.

The photomicrographs in this section were of the following conditions:

1. Gaucher's disease
2. CDA type 1
3. ALL with myeloid markers
4. CLL with associated paraprotein
5. Hairy cell leukaemia
6. Large granular lymphocyte syndrome
7. Haemophagocytic syndrome
8. Non-secretory myeloma
9. McLeod phenotype
10. Leishmaniasis

The quality of the answers was variable with half the candidates failing to gain half marks. In particular, three candidates failed to positively identify an obvious Gaucher cell in question one. In question two only one candidate offered a diagnosis of CDA. The haemophagocytic histiocytes in question seven proved a difficulty for most candidates with a number considering these to be Reed Sternberg cells. In question nine all candidates commented on the presence of acanthocytes in the blood film, but none recognised the significance of these cells in conjunction with the abnormal NBT test. Most candidates gave a diagnosis of chronic granulocytic disease. While four of the six candidates correctly identified the intracytoplasmic parasites in question ten, no candidates elucidated hypersplenism as the cause of the pancytopenia, in spite of the fact that splenomegaly was indicated in the clinical features.

Question eleven required candidates to comment on the carrier status of three members of a family with haemophilia B. Although most candidates were able to correctly identify the carrier status, the associated comments indicated a poor understanding of the genetic basis for the transmission of X linked disorders.

SECTION B:
This section was worth 57 marks. The mean mark was 32.25, the range 19.5 to 40.5

Question 1
Cytogenetic abnormalities. Very well answered indicating that most candidates are conversant with the FAB classification of the acute leukaemias and the associated morphological and cytogenetic features. Only one candidate failed to gain half marks.

Question 2
Polymerase chain reaction. This was poorly answered. Three candidates failed to gain any marks and none of the candidates exhibited adequate knowledge of this topic. This is of great concern as the PCR reaction is a rapidly developing technology, which has much prominence in the literature and is destined to be a widely used technique in many diagnostic haematology laboratories in the future.

Question 3
Evaluation of APTT reagent. This was generally well answered, although some candidates were unfamiliar with the role of low molecular weight heparin and its laboratory control.

Question 4
Reasonably easy marks were available for writing brief notes on four current topics. Only one candidate was unable to gain half marks in this question.

Question 5
Classification of aplastic anaemia. Only two candidates were able to provide an adequate aetiological classification of the aplastic anaemias. While most candidates recognised drugs and infection as causative agents in aplastic anaemia, specific examples such as Chloramphenicol were not generally alluded to.

Question 6
Laboratory findings in von Willebrand's disease. Well answered, with all candidates providing the required information in tabular form.

Question 7
Haemoglobin electrophoresis. Generally well answered with most candidates well familiar with the relative electrophoretic mobilities of haemoglobin variants at alkaline and acid pH's.

Question 8
Workload units. Poorly answered. Most candidates could name a system for the measurement of laboratory workload units and had an understanding of how the workload unit was derived. However, the application of a workload recording system as a tool in laboratory management was poorly understood.

Question 9
Bull analysis. Only two candidates gained half marks. Generally there was a poor understanding of the underlying principle for the use of patient results in the control of haematology analysers. This is disturbing given the widespread use of the Bull analysis system on modern multi parameter haematology analysers.

PAPER TWO:
The mean mark for this paper was 54.25%. The range was 34% to 65.5%.

Question 1
This question required the candidates to discuss the use of computers in the haematology laboratory. It was attempted by only one candidate who failed to gain half marks. It surprised the examiners that given the recent widespread implementation of clinical laboratory computer systems, more candidates didn't opt for this question. It would appear that while many laboratory workers have become skilled keyboard operators, they appear to have little appreciation of the use of computers in process control and their potential for information handling.

Question 2
This involved the differential diagnosis and an investigation of a patient suffering from a macrocytic anaemia. All six candidates attempted this question. The mean mark was 11.5 out of 25. The range being 7 to 13.5. Three candidates failed to gain half marks.

Candidates were generally able to give a good account of megaloblastic anaemia. However, there was a very poor appreciation of the other possible causes of a macrocytic anaemia, including MDS, alcohol, CHAD, drugs and CDA. In addition, the laboratory investigation of a macrocytic anaemia was not well handled by some candidates.

Question 3
This required the candidates to discuss the chronic B cell lymphoproliferative disorders. Three candidates attempted the question with only one gaining greater than half marks. The mean mark was 9.5 out of 25, the range 5.5 to 13.5

While all candidates who attempted this question discussed B CLL, only one candidate recognised the full spectrum

of chronic B cell disorders. No candidates alluded to the FAB proposals for the classification of these lymphoproliferative conditions. The candidates who failed to gain half marks did so largely through a failure to discuss the role of the laboratory in the diagnosis and management. In particular, there was little appreciation of the role of lymphocyte surface marker analysis and cytogenetic studies. The less common disorders of SLVL, Hairy cell variant, and leukaemic phase of NHL were generally not recognised.

Question 4
All candidates attempted this question which required them to discuss PNH. The mean mark was 14.25 out of 25. The range 11.5 to 21.5.

This question was generally well answered with only two candidates failing to gain half marks. While the laboratory investigation of PNH was on the whole well handled some candidates experienced problems in relation to the clinical details. There was also little appreciation of the recent developments in the detection of GPI determinants (CD55, CD59) on leucocytes and erythrocytes for the diagnosis of PNH.

Question 5
Five candidates attempted this question on haemopoiesis and stem cells. The mean mark was 15 out of 25 with a range of 6-19.

Generally well answered with only one candidate failing to gain half marks. Those candidates who passed this question showed a good appreciation of the current concepts and terminology for the differentiation pathways of haemopoiesis. Some deficiencies, however, were noted in respect of growth factors, in particular their cloning, recombinant technology and pharmacological applications.

Question 6
This required candidates to write examples of laboratory safety protocols. The question was attempted by three candidates with one failing to achieve half marks. The mean mark was 13 out of 25, the range 12-14.5.

Although this question generally elicited answers of an acceptable standard, most candidates exhibited obvious deficiencies in their knowledge of basic laboratory practice and safety standards. While safety manuals should be available in all laboratories it would appear that candidates are often not aware of the standards and protocols laid out in these manuals. It behoves charge technologists to ensure that laboratory workers receive ongoing education in acceptable laboratory safety practice.

GENERAL COMMENTS:

We were disappointed that the overall standard of results was so low. Of the four candidates who passed the examination only two achieved a mark of greater than 60% and no candidate exceeded 65%.

It was obvious that a number of candidates lacked the depth of knowledge required at this level. In addition the examination technique was often poor and candidates obviously need coaching in this aspect of examinations. Too often candidates failed to write down all the facts. Examiners cannot make an assumption that candidates know more than they have submitted on paper, even though as an examiner you feel that the candidate probably has a greater knowledge of the subject.

Candidates must also be discouraged from simply reiterating the information supplied in the examination paper. What is required is interpretation and reasoned argument not a regurgitation of the question.

In conclusion, some candidates presenting themselves are inadequately equipped to sit this examination.

This may be a result of senior laboratory staff being unable to commit time to coaching, as staffing numbers fall in most laboratories. The fact that the examination no longer forms part of the path to attainment of the Diploma in Medical Laboratory Technology may also have a bearing.

MICROBIOLOGY

Seven candidates sat the examination — Five passed:
Overall marks ranged from 45-63%.

The standard of answers to questions in Paper I was low with marks ranging from 33-61%.

The standard for Paper II was better with marks ranging from 41-68% and only one candidate getting <50%.

The following comments are to highlight those areas that were not so well answered. For more details refer to the marking schedule.

When discussing the identity of micro-organisms candidates frequently forget to describe colonial morphology. The size and shape of parasite eggs and cysts are often not described.

ex *Cardiobacterium hominis* — some did not describe colonial or microscopic morphology.

Acanthaemoba, Cryptosporidium, Clonorchis, D. fragilis — lack of detail on size and shape of parasites.

PAPER ONE:

SECTION A

Question 1
(a) *Fusarium oxysporum*: No one mentioned direct examination of material (hyphae).

(e) *Ps.cepacia:* Poorly answered. Many candidates did not know much about identifying characteristics. Only a few mentioned the use of selective media ex. PCA.

(f) *Blastocystis hominis:* Not all candidates know that B. hominis is a commensal of the human gut. The clinical significance of this organism is controversial which is what we wanted brought out in the answer.

Question 2
(a) A few candidates outlined a method. This was not required.

(b) Well done - all candidates except 1 gained equal to or greater than half marks.

(c) 2 candidates answered this question well and 1 omitted an answer.

SECTION B

1. Quite well answered except for those that drew tables and not flow charts.

2. This question was adequately answered by most candidates.

3. This question was poorly answered by all candidates. More technical articles are appearing in Microbiology journals using these parameters. Candidates should be able to define them.

SECTION C

Question 1
(a) Although this question was generally well answered one point that was not specifically stated is that the calibrated disc test must be followed exactly as it is calibrated under specific conditions whereas in the comparative method the test and control organisms are tested on the same plate so there are less constraints on this method.

Although candidates said that in the comparative method, the zones of inhibition of control and test organism are compared, they did not say what the control organism is, ie., known susceptible strain.

(b) Little mention was made of how to test for chromosomally mediated penicillin resistance in *N. gonorrhoeae.*

(c) The use of oxacillin discs to test the sensitivity of staphylococci to methicillin and reasons why, were well known. Not so the use of oxacillin discs for testing sensitivity of *S.pneumoniae.* Hardly anyone stated the concentration of the discs (1.0ug) or knew the zone size of a senstitive strain.

(d) Although some candidates mentioned a 99-99.9% kill in the serum bactericidal test, they would not have known this, as they did not do a colony count on their original inoculum.

(d) Most candidates answering this question did not give details of testing for high level resistance to aminoglycosides ie., concentrations of antibiotic in discs or agar.

Question 2
1 candidate attempted 2a and gave an adequate answer. 2 attempted 2b with adequate answers. 4 attempted 2c and had no knowlege of the more specific tests available for the diagnosis of Epstein Barr Infection.

SECTION D

Question 1
The answers to this question were in the main very disappointing. It is a worry that some candidates at this level don't know much about mycobacteria species that might well be considered certificate level material.

Most candidates did not mention the microscopic morphology of the bacilli.

Growth on media with glycerol is not an identifying characteristic of *M. tuberculosis* as other mycobacteria grow on this media too. However a description of the colonies on this media is an identifying characteristic.

Question 2
The recent advances that were expected in this answer included the use of radiometric culture methods, and nucleic acid probes. Mention of PCR and ELISA was also counted. In their discussion of these methods, candidates often failed to say what the advantages (and disadvantages) of these new techniques are.

PAPER 2:

The questions on Listeria and Yersinia were well answered by most candidates, particularly the laboratory diagnosis of these bacteria. Candidates were weaker on the epidemiological and clinical aspects of Listeria and Yersinia infections.

The question on Hepatitis C was attempted by three candidates and the answers reflected a lack of knowledge of this topical subject.

The answers to questions 1 and 2 again reflected the Candidates' lack of knowledge of the clinical aspects of infectious diseases. In question 1, no Candidate mentioned that the case could be a partially-treated bacterial meningitis due to the previous Augmentin therapy and that the CSF should be tested for pneumococcal or meningococcal antigens. Most Candidates, however, mentioned amoebic meningitis as a possible cause. Question 2 was answered by three Candidates and none mentioned acute toxoplasmosis, which would be one of the commonor causes of acute pharyngitis and cervical lymphadenopathy in a young adult.

Question 6
3 candidates answered this question.
Range of marks 11-14.

One of the main objectives of an infection control programme would be to lower the risk of an infection during hospitalisation. No one said this.

The most poorly answered section was "evaluation of the programme"

ie; have your objectives been achieved
have nosocomial infections decreased
have interventions been effective and efficient
(what is the cost benefit)
is the surveillance accurate
is education in infection control methods occurring
are policies reviewed and modified and communicated

NEW PRODUCTS AND SERVICES

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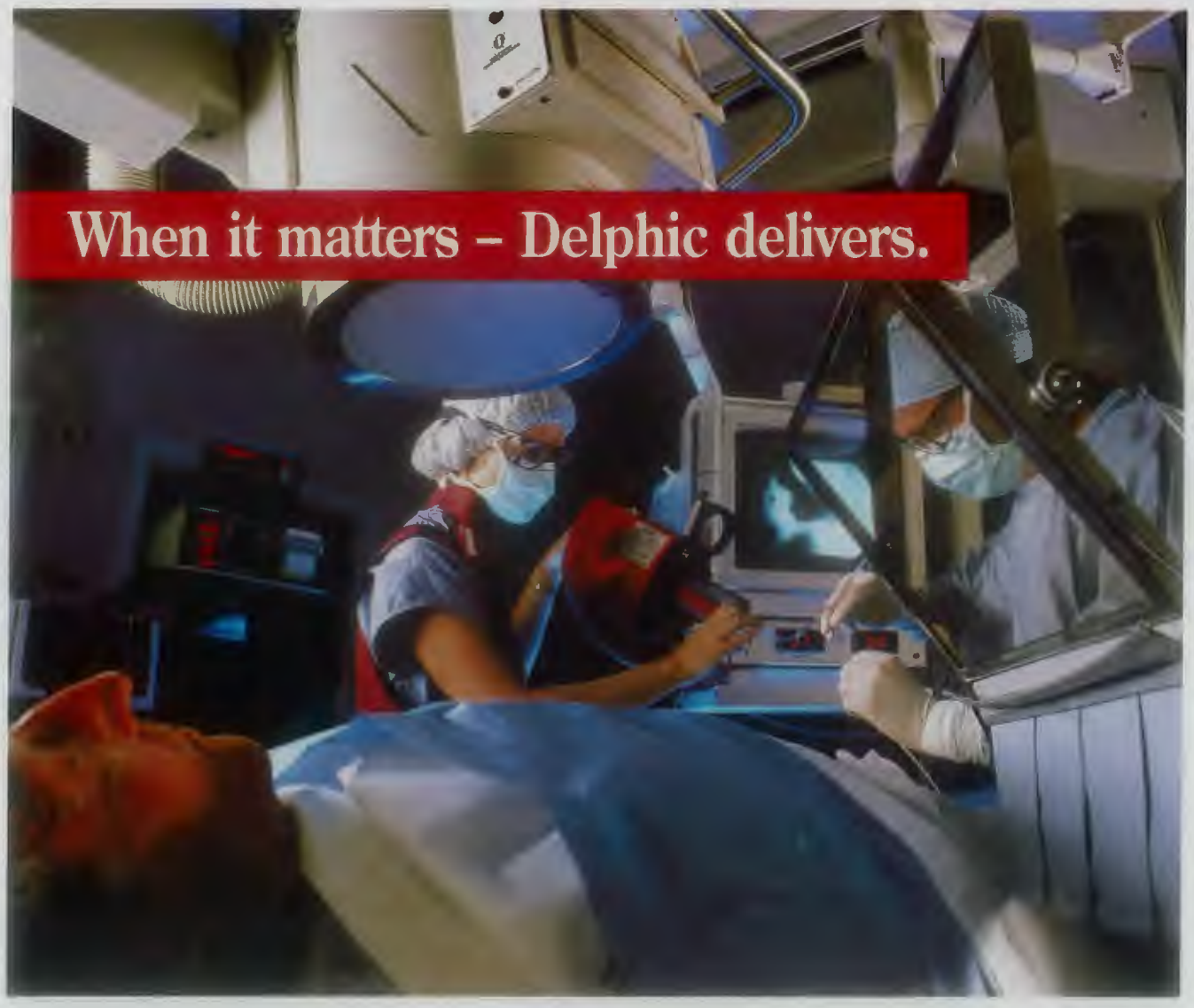

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