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Quality Assurance of Immunodiagnostic Tests in Australasia : II Five Year Review

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SUMMARY **A Royal College of Pathologists of Australasia (RCPA) sponsored quality assurance program in clinical immunopathology has, over a 5 year period, demonstrated:**

- **enrolment by the majority of immunodiagnostic laboratories in Australia and New Zealand;**
- **improved compliance with the program over time eg. increasing numbers returning their replies by the due date;**
- **different commercial techniques give different mean values for the same analyte. This appears to be due to the use of different reference materials in each technique;**
- **greater utilization of nephelometric techniques in quantitating immunoglobulins, C3, C4, CRP and rheumatoid factor resulting in better accuracy and precision;**
- **improvement in the frequency of detecting anticentromere antibody as most laboratories use proliferating cell lines as substrate for anti-nuclear antibody (ANA) detection;**
- **improved interlaboratory concordance of ANA titers by the provision of reference standards;**
- **improved detection of antibodies to extractable nuclear antigens (counter-immunoelectrophoresis being more sensitive than immunodiffusion);**
- **the Farr and radioimmunoassay technique for the demonstration of antibodies to native DNA have greater sensitivity than the Crithidia assay;**
- **improvement in accuracy and precision of cell phenotype analysis with the use of whole blood and cell flow cytometric techniques;**
- **development of techniques to rank each laboratories performance on a rating scale based on the average number of tests outliers (from the consensus mean) per mailing.**

However deficiencies in performance are still being observed. These relate to both technical factors causing systematic errors and in the provision of interpretive comments on the laboratory result. Continuing education and participation in quality assurance programs are emphasized to monitor and improve performance over time.

An assessment of the performance of any diagnostic laboratory service plays a vital function in ensuring accuracy and consistency of results within and between laboratories, detecting substandard or deficient results and providing a structure for optimizing methodology and critically appraising the relevance and cost of diagnostic tests.[1] With this in mind the Royal College of Pathologists of Australasia initiated a program of Quality Assurance in clinical immunodiagnostic tests in 1981.

In 1988 this Quality Assurance Program (QAP) was transferred to our care at Flinders Medical Centre. Over the last 5 year period each participating laboratory in Australasia has been sent, at regular intervals, a number of test samples, including replicate samples, to assess accuracy and interbatch precision. The results were then compared both within and between laboratories and results were also analysed according to methodology. In selected tests, laboratory performance was also ranked. At the end of 1988 we reported the results from the first year's operations.[2] In this communication we summarize some conclusions from the program over the last 5 year period. We were particularly interested to note whether regular participation in the program led to improved performance over time as such a finding

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would validate the usefulness of the program and encourage non participating laboratories to enrol.

MATERIALS AND METHODS

Four independent and specially designed QAP modules in Clinical Immunology were offered each year. The first module involved general imunochemical tests and serum samples were distributed at approximately 6 weekly intervals over the 12 month period. Each laboratory was required to supply specific details of their methods together with the result of the various requested analytes: immunoglobulins (IgG, IgA, IgM), IgG subclasses, C3, C4, C reactive protein (CRP), rheumatoid factor (RF), B_2-microglobulin, transferrin, α_1 antitrypsin, C1 esterase inhibitor, serum electrophoresis, total serum protein and characterization of paraprotein.

The second (autoantibody) module involved the assessment of specific autoantibodies in selected sera distributed on regular occasions. The antibodies assessed were RF, ANA, ENA and anti-DNA, anti-cardiolipin, ANCA, anti-gastric parietal cell and anti-thyroid.

The third (allergy) module involved the estimation of total IgE and specific IgE.

The fourth (cell phenotype) module involved the enumeration of lymphocyte subpopulations in preparations of blood mononuclear cells obtained from healthy subjects and leukemic patients.

Blood samples were obtained from patients or healthy volunteers (volumes up to 250 ml were required),- the serum separated and stored at -20°C prior to dispatch. For some test samples, plasma was obtained from patients with Guillain-Barre syndrome by plasmapheresis, converted to serum with Ca^{++} and then spiked with serum containing high levels of autoantibodies, CRP or IgE. Some samples consisted of pools of sera from many donors.

Test samples were preserved with 0.1% sodium azide and posted to participating laboratories at monthly or greater intervals, with the requirement that the results were to be returned to the QAP office within 3 weeks. For overseas laboratories samples were dispatched by air courier.

On occasions an interpretive clinical comment of a test result was also requested. Full details of the methodology of the program is published elsewhere.[2] For the purposes of this communication the consensus mean is defined as the arithmetic mean of the results for that analyte after the elimination of any markedly disparate outlying results.

RESULTS

Enrolment and compliance

The number of laboratories enrolled in the various modules over the last 5 years and the respective compliance (ie % number of laboratories returning all results within the 3 week allowed period) are shown in Table 1. The majority of laboratories were situated in Australia and New Zealand, however, 5% were from overseas (Malaysia, China, Hong Kong, Singapore, Saudi Arabia and USA). Compliance and accuracy of results were not related to geographical location.

Different commercial techniques

Over the five year period it has been consistently observed that the mean consensus value obtained for an analyte may vary according to the method used for quantitation. For example, the mean Ig, rheumatoid factor (RF) or α1-antitrypsin level varied by 40% according to which of the 3 commercial nephelometric diagnostic assays were used (Beckman technique giving lower levels than Behring, Fig. 1-3). However, for CRP quantitation good

Table 1. Enrolment and complicance of laboratories

	Immunochemical module		Autoantibody module	
	No labs enrolled	% compliance	No labs enrolled	% compliance
1988	72	30	84	30
1989	120	NA	91	NA
1990	130	48	108	62
1991	128	56	110	61
1992	132	73	116	77

NA = not assessed

	Allergy module		Cell phenotype module	
	No labs enrolled	% compliance	No labs enrolled	% compliance
1988	NA	NA	22	NA
1989	NA	NA	35	NA
1990	NA	NA	42	81
1991	77	NA	52	67
1992	76	75	62	75

NA = not applicable or not assessed

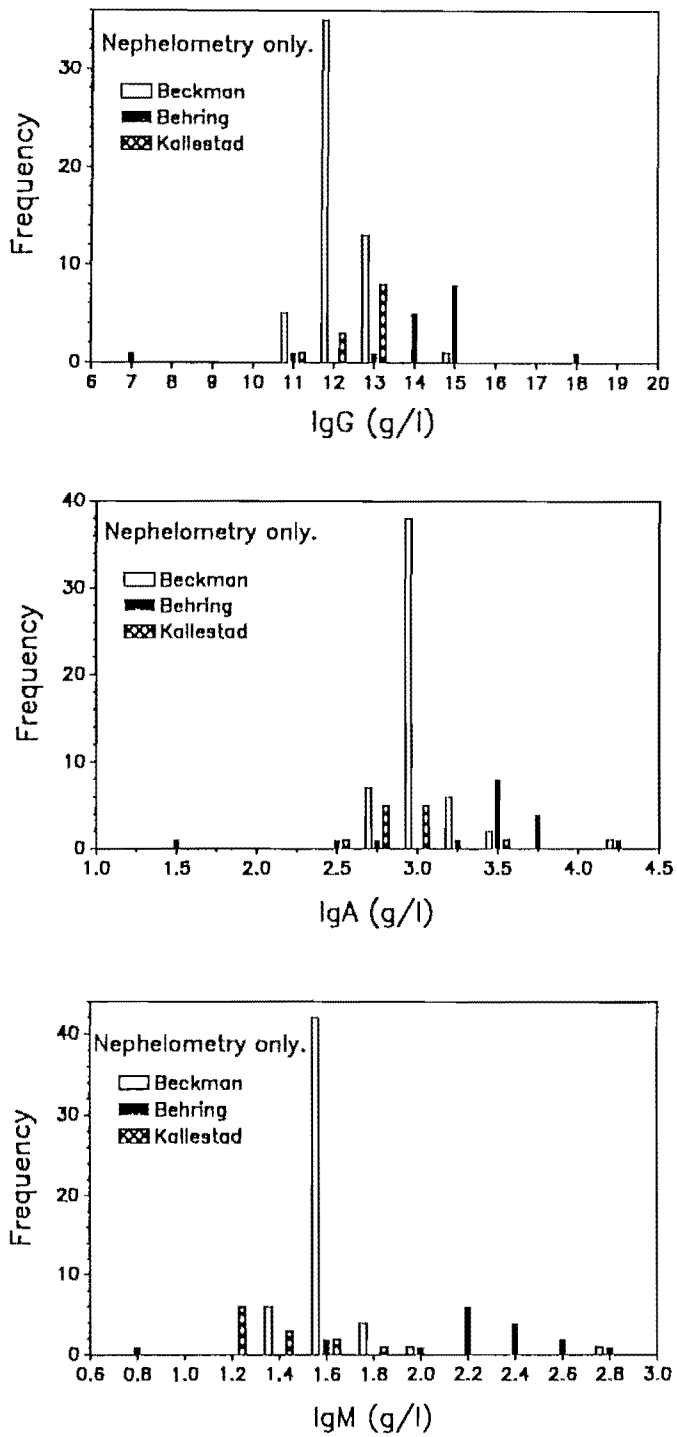

Fig. 1 Frequency distribution of immunoglobulins in a serum sample according to the commercial source of the nephelometric technique (n = 82 laboratories).

concordance was observed for all 3 nephelometric assays. Similarly the Pharmacia kit for specific IgE for cat dander and rye grass gives a mean lower score than the Kallestad kit (Fig. 4, 5).

Over the period of study nephelometric techniques for quantitating serum proteins have become more popular replacing the more traditional methods (Table 2). This has resulted in a smaller scatter of results for the nephelometric techniques as compared with other techniques and improved precision (Table 3). In most laboratories the intralaboratory coefficient of variance (CV) for replicate samples for the nephelometric techniques is between 3-5%.

Antinuclear antibodies and DNA antibodies

Most laboratories are accurate in detecting moderate or strongly positive ANA and in describing the pattern. In 1988 only 58% of participants could detect the anti-centromere pattern whilst 96% of laboratories could do so in 1992. This improvement closely parallels the frequency of the use of the HEp-2 cell line as ANA substrate (only two laboratories continue to use the rodent substrate in 1992). In 1988 68% of participating laboratories could correctly identify a SSA positive sera whilst in 1992 the frequency was 90%. However, only one third of laboratories could correctly identify a sample containing Scl-70 antibodies. Counterimmunoelectrophoresis (CIE) was more sensitive in detecting antibodies to extractable nuclear antigens than immunodiffusion (ID), (Table 4). Throughout the program the Crithidia assay for the detection of antibodies to DNA has been shown consistently to be less sensitive than the Farr or radioimmunoassay (RIA) technique, eg. for a specimen containing 20 IU/ml of DNA antibodies by RIA, only 50% of laboratories gave a positive result using the Crithidia assay.

Table 2 Change in immunoglobulin assay metholds

	1988	1991
Total	102	115
Nephelometry	46	81
Turbidimetry	20	19
RID	24	10
Rocket	2	0
FL polarisation	0	2
Unknown	10	2

Table 3 Precision of methods for C3 and C4

Method	No of labs	C3 mean precision error	C4 mean precision error
Rate nephelometry	34	0.03	0.004
Nephelometry	12	0.02	0.004
Turbidimetry	12	0.02	0.010
RID	19	0.07	0.018

Table 4 Sensitivity of methods detecting ENA

ID	:	8/19 Detected SS-B only 1/19 Negative 1/19 Detected Sm 9/19 Detected SS-A and SS-B
CIE	:	20/20 Detected SS-A and SS-B

ID = Immunodiffusion
CIE = Counter Immunoelectrophoresis

Table 5 Improvement in coefficient of variance for detecting T cell subsets

YEAR	CD3	CD4	CD8
1988	9.6	22.8	23.2
1991	6.9	8.9	17.6
1992	5.6	11.9	16.0

% Coefficient of variance

Table 6 Cell phenotyping methods

	Enrolments	Microscopy	Flow cytometry
1988	23	6	17
1992	62	3	59

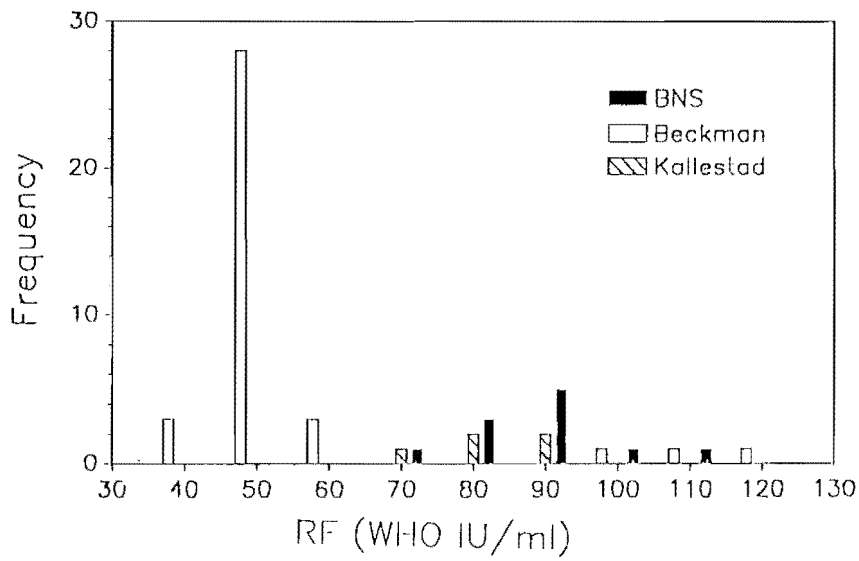

Fig. 2 Frequency distribution of RF in a serum sample according to the commercial source of the nephelometric technique (n = 53 laboratories).

The provision of a reference preparation, standardized against a calibrated WHO reference reagent (in IU/ml), allowed laboratories to standardize their ANA positive test sera in IU/ml. This led to improved interlaboratory concordance with smaller dispersion of the results around the mean, as compared with the results expressed in dilution titers, (Fig. 6). However, this improvement was dependent on the test serum having a homogenous ANA pattern. Furthermore, some participating laboratories disputed the value of the WHO reference preparation supplied by us, as it did not agree with comparative studies using their own "WHO reference material".

Cell phenotyping

There are currently 62 laboratories enrolled in the cell phenotyping module and significant improvement in accuracy of cell subset quantitation has been observed since 1988 (Table 5). This improvement coincides with the greater frequency of the use of flow cytometric techniques (Table 6) and with the use of whole blood techniques which do not require density gradient separation of mononuclear cells (56 of 59 laboratories currently use the whole blood method). The use of dual labelling methods has also increased, which ensure that the cells included in the CD8 population are also CD3 positive.

Sequential observations on individual laboratory performance

By noting the number of outliers of results (>3SD from the consensus mean) for the same technique per year a conclusion could be made concerning laboratory performance over time. Many laboratories have shown improvements in single or all modules, however, a smaller number have deteriorated. Figure 7 demonstrates the frequency distribution for the performance rating of the 133 laboratories enrolled in the immunochemical module for 1992. By noting the degree of dispersion (scatters of the data over time) it will be possible to identify improvement or deterioration in the overall performance of

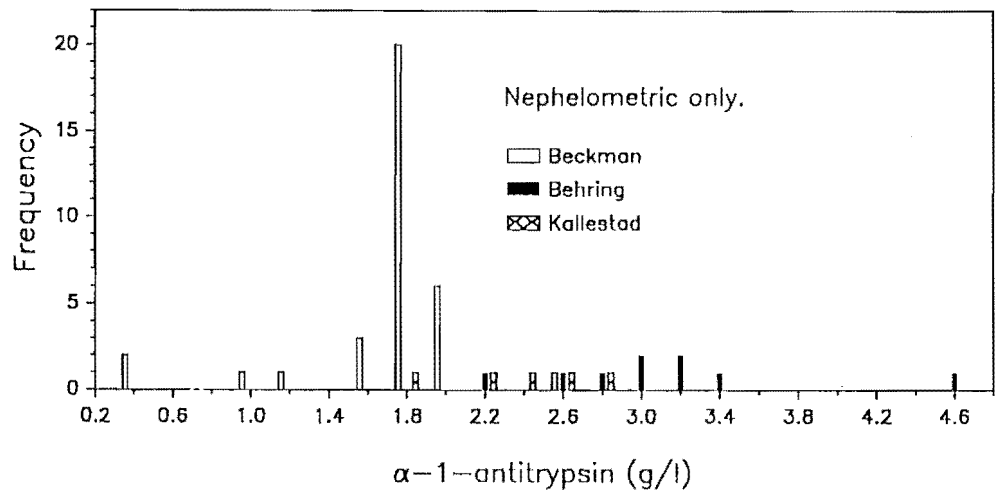

Fig. 3 Frequency distribution of α_1-antitrypsin in a serum sample according to the commercial source of the nephelometric technique (n = 48 laboratories).

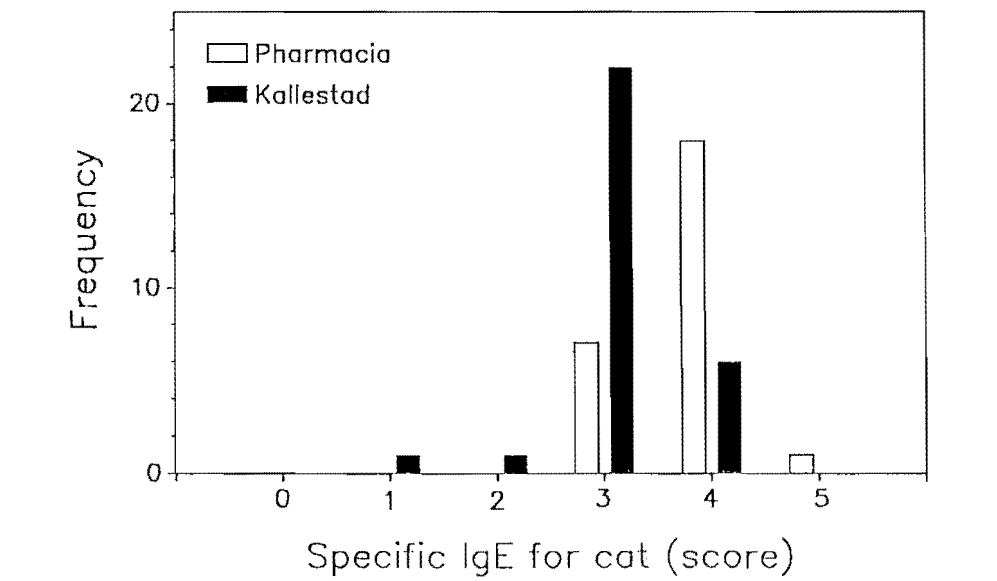

Fig. 4 Frequency distribution of specific IgE for cat allergen according to the commercial source of the immunoassay kit (n = 48 laboratories).

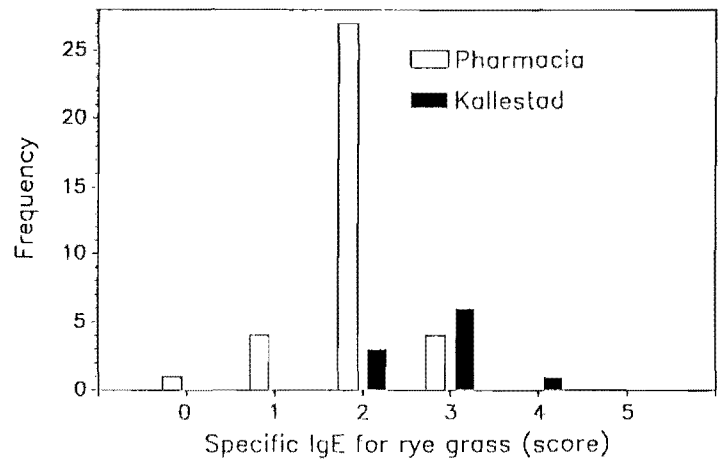

Fig. 5 Frequency distribution of specific IgE for rye grass allergen according to the commercial source of the immunoassay kit (n = 46 laboratories).

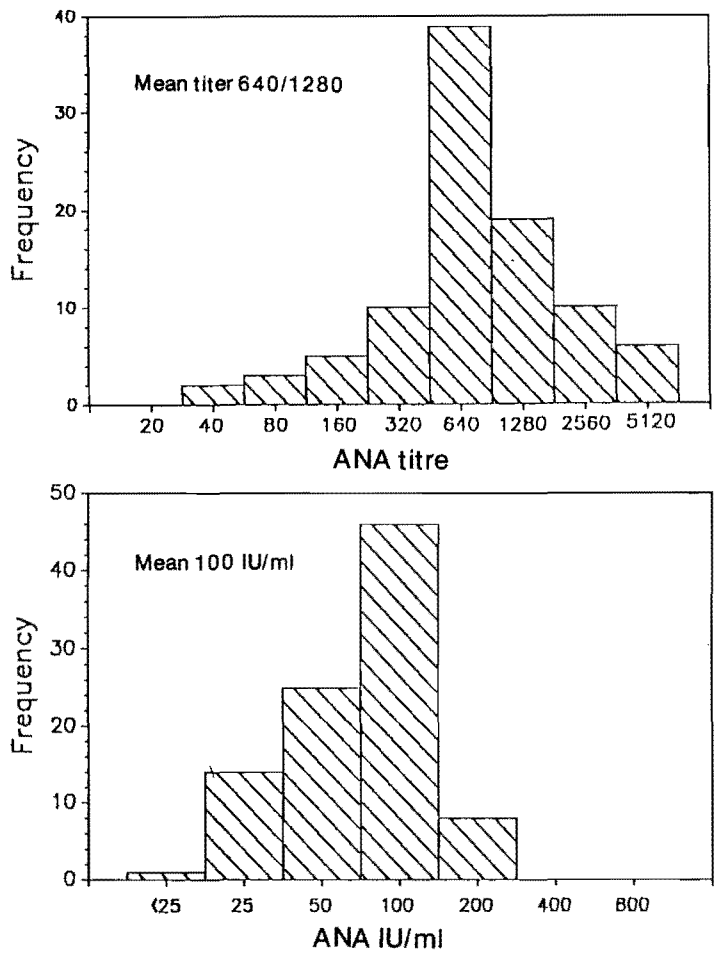

Fig. 6 Frequency distribution of ANA expressed in dilution titre (upper panel) and after conversion to iu/ml by comparison with reference standard (lower panel). Note the tighter distribution after standardization.

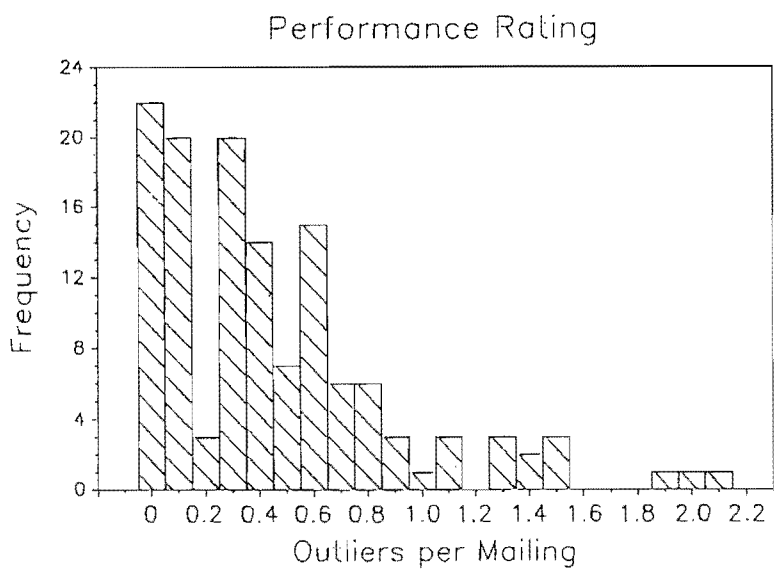

Fig. 7 Frequency distribution of performance rating (mean number of outliers (>3SD from consensus mean)per mailing) of 133 laboratories enrolled in the immunochemical module for 1992. Twenty two laboratories performed in highly consistent fashion with no outliers at any time.

the program over time whilst individual laboratories can also plot their sequential performance. Performance ratings have been assessed for all 4 modules of the QAP for 1991 and 1992.

Interpretive comments on laboratory results

The immunology results for 3 selected patients with immune disorders (recurrent infections, Sjogren's syndrome and angio edema) were distributed to participating laboratories. Each laboratory was asked to assess the case study and add interpretive comments. In general the replies were disappointing. Several laboratories made no attempt to add interpretive comments stating that they had no attending immunopathologist. Furthermore, an examination of the replies suggested that many were completed by staff with only a superficial knowledge of immupathology. Only a minority of laboratories were successful in giving the correct interpretation or suggesting further appropriate tests to arrive at a clinical diagnosis and most of these were from teaching/university/large private laboratories. These laboratories answered correctly in a consistent fashion on each occasion.

DISCUSSION

Over the years 1988-1992 an ongoing assessment has been made of the majority of the laboratories in Australia and New Zealand who perform immunodiagnostic tests useful in the diagnosis and management of immune disorders. During this period an increasing number of laboratories have joined the program including laboratories in South East Asia, China and elsewhere. Geographical location, with possible prolonged transit time of analyte, does not appear to lead to any major difficulties with regard to receipt of samples (including whole blood specimens) or return of replies within the due date or accuracy of results, a finding also noted in a previous study.[2] These conclusions are encouraging and suggest that a QAP can be successfully applied to laboratories situated over wide geographical regions. Laboratories have increasingly shown their support for the program by improving their compliance in returning their replies by the due date and by communicating with the QAP office for advice concerning poor performance and for information regarding technical developments. Each year the QAP committee issues a detailed, technical report summarizing the years activities and the overall performance in each of the 4 modules assessed and also presents similar information at the annual scientific meeting of the RCPA. The conclusions resulting from the programs results obtained in 1988 have been previously published and mainly related to observations concerning the serum immunoglobulins, paraproteins and autoantibodies.[2] In the current report we

have extended these findings and have made some additional observations regarding improvements in performance over the last five years. In general, accurate and consistent performance has been observed in laboratories with tests utilizing modern techniques with standardized internal reference preparation. Pleasingly, general improvements in selected areas of performance have also been observed over this period (eg improved accuracy and precision of cellular phenotyping, improved accuracy of autoantibodies to extractable nuclear antigens and centromere, greater utilization of more sensitive and precise techniques etc). This improvement seen with regular participation in a quality assurance program has also been documented with other QAP's in laboratory medicine (eg External Quality Assurance Scheme of the UK). However there are several observations which are a cause for continuing concern. The first relates to the different commercial techniques. Each commercial manufacturer appears to be using internal reference preparations which differs between methods giving rise to a different test sample value. Provided each laboratory generates their own normal ranges for each technique this would not have a great clinical effect but it does lead to some difficulties in comparing values between laboratories. However, there are still many laboratories that use published normal ranges. Attempts should be made by each company producing diagnostic methods to standardize their internal reference materials.

The second concern relates to the provision of interpretive comments on diagnostic laboratory reports. An assessment of this area suggested that only a minority of laboratories perform this function in a reliable and consistent fashion giving useful clinical information to the requesting physician. The performance of many laboratories was consistently poor (despite them having a good record in getting accurate numerical results). An examination of these interpretive comments suggested that inexperienced or junior staff were completing the returns and some laboratories acknowledged that qualified immunopathologists were not vetting all results issuing from their units. We believe that all results should be viewed, prior to dispatch, by immunopathologists who are trained to correlate the clinical information with the numerical data and can then add, if required, an informed comment.

Over the last 2 years we have developed a performance rating (average number of test outliers from the consensus mean, per mailing) for each laboratory which will allow us to closely monitor the performance in each of the 4 diagnostic modules. This will give useful information regarding the overall performance of the program with time (ie. whether no change, improvement or deterioration is occuring) and will also allow individual laboratories to monitor their own performance. However, it will be important to identify any possible bias of a "consensus mean" due to the increasing use of a particular commerical technique (with its own mean value which might differ from another technique) as this could skew the consensus mean value towards that of the most commonly used method. Despite this potential difficulty, however, we believe the information obtained from performance ratings will allow us to make conclusions as to whether a QAP program is a useful and valid exercise in assessing laboratory performance and in alerting poor performer of their deficiencies. We also believe that participation in QAP provides an important forum for exchanging ideas and information on technical matters and in providing continuing education in laboratory medicine.

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