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EDITORIAL

Quality Assurance of Immunophenotyping

In all countries the cost of delivery of health care has become a major concern as the cost continues to rise. Increasingly there is a request that there be audit of, and accountability for, this expenditure. This is particularly so in the case of diagnostic procedures and investigations. In many countries governments are now demanding some form of external quality assurance in this area to demonstrate that there is good value for the money that is spent by them, by health insurance agencies or by the patient.

Another concern of most governments is the escalating medical cost of human immunodeficiency virus (HIV) infection and AIDS. Because of these two areas of public and government interest the 1992 International Congress of Immunology in Budapest held meetings of both national and regional organisers of cellular phenotyping Quality Assurance Programs (QAP) to compare experiences and results, and to pool information. While quality assurance programs in other diagnostic immunology areas have been in existence for more than 15 years it is significant that this area of cell phenotyping (or cell markers) has been chosen for immediate international effort. A further meeting is planned for early next year. This is undoubtedly due to widespread concern about HIV virus infection and the acquired immunodeficiency disease (AIDS).

The problems encountered in acquired immunodeficiency disease are mainly due to the decrease in CD4+ T lymphocytes.[1-3] A reduction in the numbers of CD4+ T lymphocytes is associated with an increased risk of clinical complications. Therefore the accurate reproducible measurement of these cells has become of great clinical significance because it has been used to monitor disease progress, to decide when to commence antiviral therapy[4] and prophylaxis for *Pneumocystis carinii* pneumonia;[5] and to assess prognosis.[6]

It is now recommended that all HIV infected persons have their level of CD4 T+ lymphocytes determined every 3-6 months.[7]

With the continuing rise in number of HIV infected persons throughout the world the need for CD4 counting facilities has increased and will continue to increase. Recently it has been suggested that CD4+ T lymphocyte levels are included as a criterion for classifying HIV-infected people by the Centers for Disease Control (CDC) and the proposed AIDS surveillance case definition for adults and adolescents.[7]

Measurement of whole blood absolute numbers of CD4+ T lymphocytes is determined from the total white blood cell count, the lymphocyte count and the percentage of lymphocytes that are CD4+ T lymphocytes. There is analytical and biological variability in these three determinations. For a total white blood cell count the analytical variability (coefficent of variation, cv) is quoted as 2.2-7.7% for automated counts and 9.3-17.6% for manual counts,[7] and for the differential count it is 1.9%-5.3% for automated and 12.5-27% for manual methods.

At the meeting in Budapest it was evident that QAP's in immunophenotyping in different regions vary in their administration, demands and establishment. At present some involve assessing flow cytometer performance by microbeads only while others consist of the distribution of cells for phenotyping and classification of results according to variance from the mean; some cir-

culate samples two or three times per year while others send out samples at intervals of a month or less.

A QAP in immunophenotyping was first introduced in Australia in 1988 and it is now a requirement that any laboratory, large or small; university, hospital or private, that receives funds for these tests from the Government or from Health Funds participate in a recognised QAP. Therefore diagnostic laboratories of all these types now take part in the program and there were 62 who participated in 1992 including some laboratories in New Zealand, Hong Kong, PR China, Malaysia, Saudi Arabia, and USA. Transportation has been less of a problem than first envisaged and samples sent to Shanghai and Los Angeles have given results close to the consensus mean. A regional QAP is therefore possible.

The next question is whether or not participation in a QAP has any role in improving or educating the participating laboratories. The answer seems to be that it does. It has been the Australasian experience that determination of lymphocyte subsets has shown the greatest improvenment of all the assays that are examined in its QAP. Between 1988 and 1991 the coefficient of variation (cv) for CD3 fell from 9.6 to 6.9 and for CD4 from 22.8 to 8.9. In the past four years there have been changes in methodology which have contributed to this. One is the increasing use of flow cytometry (97%-1991; 74%-1988) instead of manual methods. Another is use of whole blood lysis instead of density separated mononuclear cells. In 1988 most, if not all, participating laboratories used discontinuous density gradients which are created by layering blood over a medium of higher density (usually ficoll/hypaque). Because there is an variable overlap of cell size and density there is the potential for inadequate separation, reduced yield and change in the composition due to a biased cell loss. Other techniques to produce a cell preparation, eg erythrocyte agglutination or monocyte depletion, commonly result in a variable and selective loss of up to 45% of the cells and are not recommended for lymphocyte preparation. Whole blood lysis avoids these problems and has now been adopted by than 95% of laboratories. From the cv values obtained for all the assays performed in 1988 and 1991 in the Australasian QAP [10] it is apparent that there is greater improvement in the assay of CD4+ than CD8+ T cells. Why is there a greater dispersion with CD8? One reason is that CD8 is also expressed on NK cells. These cells may be larger than CD8+ T cells and may or may not be 'gated' out. Some operators using computer determined gating may include some NK cells whereas operators who set more restricted forward scatter limits will exclude most. Subtraction of NK cells from CD8 is likely to over-compensate since not all NK cells bear CD8. If a laboratory does not have double labelling facilities it can exclude most of these larger cross reacting cells by setting 'tight', restricted gates. In determining numbers of CD4+ or CD8+ T cells the most reliable method is by double labelling with CD3 and the appropriate subset marker.

With ongoing studies and rigorous quality assurance and training the cv of a select group of laboratories for CD4 assays has been observed to fall from 6% to 4.1% for the percentage and 29.4% to 8.4% for absolute counts. [7] An analysis of data from a CAP study [7] showed that the SD of the percentage of CD4+ T cells was 3.5% to 5% regardless of the percentage of CD4 T cells in the sample.

A workshop was organised by the Centers for Disease Control in November 1991 and it published its recommendations in 1992 to improve the accuracy and reliability of CD4+ T lymphocyte results. It also provided recommendations concerning laboratory safety, specimen collection, storage and transport, flow cytometer control, analysis, reporting and quality assurance. [7] The National Committee for Clinical Laboratory Standards has also published tentative guidelines on quality assurance and immunophenotyping of peripheral blood lymphocytes. [9]

Briefly the recommendations of these reports are:

Flow cytometer quality control.

Although flow cytometers differ in their requirements in general align the optics daily to make certain that the brightest and tightest peaks are produced in all parameters. For this a stable calibration material (eg microbeads) is used which has known forward and side scatter, phycoerythrin (PE) and FITC peaks.

Calibrate the machine daily for optimal performance, maintain records and monitor machine performance.

Determine the sensitivity daily to make sure that low level fluorescence can be distinguished from auto fluorescence and to determine if the distance between peaks exceeds the minimum required.

Specimen processing

One of the areas of concern is the error in determining the WBC. This is particularly so when the count is low as frequently happens in HIV infected people. On automated counts it is recommended that 10,000 to 30,000 cells be counted. If a specimen is rejected or signalled by the instrument a manual differential of at least 400 cells should be performed.

Monoclonal antibodies recommended:

FITC Isotype	PE Isotype	Comments
		Isotype specific negative control to set cursor and define positivity

CD45	CD14	sets gates; lymphocytes are CD45+ and CD14-
CD3	CD4	only cells that are CD3+ and CD4+ are T cells
CD3	CD8	only cells that are CD3+ and CD4+ are T cells
CD3	CD19	measures B cells; accounts for all lymphocytes
CD3	CD16/56	measures NK cells.

To reduce the variability of this important laboratory measurement it has been recommended that (i)the test protocol be standardised, (ii) the laboratory use CDC or other published guidelines for flow cytometry, and (iii) the laboratory participate in a recognised external proficiency testing/performance program. Laboratory accreditation, licensing or certification is helpful in giving emphasis to these recommendations.

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