

they remained unstained in all the tests. It should be noted that the sections tended to come off the slides at 60°C on all occasions.

Discussion

The properties necessary for the acid alcohol fastness of tubercle bacilli are an intact cell or the lipoidal portion of the cell mycolic acid or both.⁵

Nyka^{6,7} has previously shown that periodic acid oxidation of tubercle bacilli during fixation and on paraffin sections caused them to stain strongly by the methenamine silver and Ziehl-Neelsen techniques and that without oxidation the stain was poor or negative. Whether the stain is intensified by the oxidation of the ethylene groups of the organisms' unsaturated lipids⁸ or by the oxidation of the polysaccharide constituents of the bacillus to form aldehydes stainable by basic fuchsin is not known.

In this experiment, which was performed with epoxy resin sections, the same results occurred. It was also found that bromination for longer than 60 s produced a negative stain which could not be reversed by oxidation. Bromination for 30–60 s and subsequent oxidation produced a positive result. It follows that it is essential the tissue remains in the bromine vapour for the shortest possible effective time.

It seems probable that mycolic acid is extracted by the presently used resin solvent ethanolic sodium hydroxide or blocked in the case of bromine.⁹ It

seems probable that the positive result after oxidation is dependent on the structure of the intact cell rather than the mycolic acid.

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Micromodified cytomegalovirus antibody screening test

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Recent increased interest in post-transfusion cytomegalovirus (CMV) infections in young infants¹ and immunosuppressed patients² has resulted in clinical requests for blood screened for CMV antibodies.³ Detection of these antibodies is possible by a variety of tests, including complement fixation,⁴ fluorescent antibody techniques,⁵ enzyme linked immunoabsorbent assays,⁶ indirect haemagglutination tests,⁷ or radioimmunoassay.⁸ One procedure which is particularly attractive to blood transfusion centres is the indirect haemagglutination assay,

which utilises well known serological techniques and is adaptable to rapid screening procedures.

Although a commercial indirect haemagglutination assay kit is available,⁹ its cost precludes its use for routine blood donor screening. We report our experience with a micromodification of the test which makes the method economical while maintaining sensitivity and specificity.

Material and methods

CMV indirect haemagglutination assay kits (Cetus Corporation, California, USA) were used according to the manufacturer's directions. In brief, human group O cells are supplied sensitised with CMV antigen. These cells are incubated with test serum in V-bottomed microtitre plates, and the agglutination patterns are observed. Results may be read after 90 min and settling patterns remain constant for up to 18 h.

The procedure was modified to enable the test to be performed in microtissue culture trays (Nunclon

1-63118). CMV sensitised cells supplied with the kit were reconstituted with 1.5 ml of the Cetus kit dilution buffer rather than with distilled water. The cells were then left for 30 min before use. Reconstituted cells were stable for up to 14 days when stored at 4°C–6°C.

Serum and control samples to be tested were diluted 1/6 in phosphate buffered saline containing Tween (PBST). The buffer contained 8.0 g/l sodium chloride, 0.2 g/l potassium dihydrogen phosphate, 2.17 g/l disodium hydrogen phosphate · 7 H₂O, 0.2 g/l potassium chloride, 0.2 g/l sodium azide, and 0.5 ml/l Tween at pH 7.4 ± 0.1. It could be stored at 2°C–8°C for up to 30 days.

Serum samples (2 µl) were added to sensitised cells (2 µl) already dispensed in a microtray. After a brief vortex mixing, the tray was left at room temperature for 30 min and then placed on a light box with the viewing surface at an angle of 45°. At 30 min the reaction could be read: negative results showed streaming of cells to the lower end of the well, while with positive results the cells remained in the pattern in which they had settled. Reaction patterns remained stable for up to 24 h, and positive and negative reactions were clear cut.

CMV enzyme immunoassays were performed using a commercial kit (MA Bioproducts CMV EIA, cat No 30-327 U). The CMV immunofluorescent assay used was the method of Stagno.¹⁰

The serum samples used for testing were from neonatal cord blood sera and from routine blood donors. Samples were stored frozen at –25°C until tested.

Results

One hundred and thirteen samples were tested by both the indirect haemagglutination assay kit method (macro) and the modified (micro) technique. Concordance was 100%.

Twenty one sera were titrated in CMV antibody negative sera from 1/6 to 1/1500 and retested at each dilution by both the micro- and macrotechniques. There was no significant difference in sensitivity between the two different methods.

One hundred and ninety six neonatal and 173 donor sera specimens were tested by both the enzyme immunoassay and micro indirect haemagglutination assay techniques (Table). There was a 97.3% concordance ($p < 0.001$).

Enzyme immunoassay and micro indirect haemagglutination assay discrepant results were then tested by the immunofluorescence assay method. All of the six micro-indirect haemagglutination assay positive, enzyme immunoassay negative specimens were positive by immunofluorescence

Comparison of results obtained using micromodified indirect haemagglutination assay and enzyme immunoassay methods

	Micro-modified indirect haemagglutination assay		
	Positive	Negative	Total
Enzyme immunoassay			
Positive	192	4	196
Negative	6	167	173
Total	198	171	369

assay. Of four enzyme immunoassay positive, micro-indirect haemagglutination assay negative sera, three were negative by immunofluorescence assay and one was positive. Micro-indirect haemagglutination assay and immunofluorescence assay thus give very similar results with respect to enzyme immunoassay discrepancies.

The micromodification, using smaller amounts of reagents, resulted in considerable cost savings. From the standard 100 test kit, about 1500 tests could be performed.

Discussion

Booth, using the method of Yeager,⁸ found the indirect haemagglutination assay method to be the least satisfactory in comparison with enzyme immunoassay and radioimmunoassay. Other workers¹¹ have found the indirect haemagglutination assay more sensitive than the enzyme immunoassay, while Castellano¹² found both tests in close agreement. In our hands the micro-indirect haemagglutination assay compared favourably with results obtained by enzyme immunoassay and immunofluorescence assay techniques, methods well recognised for their sensitivity.

Micromodification of commercially available test kits can often reduce the cost of each test by a substantial factor.^{13,14} The micromodification described allows testing for CMV antibodies without loss of sensitivity or specificity. This should now permit CMV antibody screening of donor blood economically and efficiently.

Addendum

Owing to recent manufacturer's modifications to the Cetus assay kit we now find the kit cells need to be reconstituted in Cetus buffer, left at room temperature for 2 h, centrifuged, the supernatant discharged, and the original volume reconstituted with fresh Cetus buffer. Reactions should be read at 10 min and an agglutination free pattern indicates a negative result. Equally satisfactory results to those described in this paper can be obtained with this modification.

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Letters to the Editor

Erythrocyte ferritin concentration in patients with myelodysplastic syndromes

We have investigated the erythrocyte ferritin concentrations of 45 patients with myelodysplastic syndromes. The mean concentration of heart type ferritin was five times normal and spleen type ferritin was 15 times normal, with no significant difference between the various diagnostic subgroups.

The diagnostic criteria used were those of the FAB group,¹ slightly modified by threshold values for abnormal blood counts and with the addition of a further group of idiopathic macrocytosis.² Of the 45 patients studied, nine had refractory anaemia, 15 had idiopathic sideroblastic anaemia, 11 had refractory anaemia with excess blasts, two had refractory anaemia with excess blasts in transformation, four had chronic myelomonocytic leukaemia, and four had idiopathic macrocytosis. Patients were aged 26-82 years (mean 62.6 years). Twenty four patients had received no blood transfusions at the time of investigation. Erythrocyte ferritin concentration was measured in leucocyte free samples by a two site immunoradiometric assay with antibodies to heart and spleen ferritin,³ and serum ferritin assay was used as a measure of iron stores. Results are shown in the Table.

Patients with myelodysplastic syndromes may have extremely high concentrations of

Erythrocyte ferritin concentration in normal subjects* and patients with myelodysplastic syndromes (ag/cell)

	No of subjects	Mean	Range
Normal subjects*	37		
Heart type		123.1	65-357
Spleen type		12.4	5-34
All patients with myelodysplastic syndromes	45		
Heart type		314.2	43-7556
Spleen type		48.9	4-4243
Refractory anaemia	9		
Heart type		187.9	43-7556
Spleen type		24.8	4-4243
Sideroblastic anaemia	15		
Heart type		392.7	80-1313
Spleen type		66.2	11-467
RAEB plus RAEBT	12		
Heart type		354.2	158-2397
Spleen type		61.3	17-1713

*From Peters *et al*³

RAEB = refractory anaemia with excess blasts.

RAEBT = refractory anaemia with excess blasts in transformation.

erythrocyte ferritin and the mean concentrations of both heart type and spleen type ferritin are greater than normal. Twenty seven of the 45 patients had either heart type or spleen type ferritin concentrations above the normal range, and in 19 patients the concentrations of both were increased. There was no significant difference between patients with refractory anaemia, idiopathic sideroblastic anaemia, or those with marrow blasts in excess of 5%. Those patients who had received one or more transfusions had a mean serum ferritin of 1337 µg/l with a mean erythrocyte ferritin of 410 ag/cell (heart) and 73 ag/cell

(spleen). Those who had not been transfused had a mean serum ferritin of 160 µg/l, with a mean erythrocyte ferritin of 239 ag/cell (heart) and 33 ag/cell (spleen). These values represent a significantly higher serum ferritin concentration in the transfused group (p < 0.001), but there is no significant difference for either type of red cell ferritin. When only the 24 patients who had no transfusions are considered, 17 had serum ferritin concentrations in the normal range (below 300 µg/l), but of these seven had abnormally high concentrations of both heart and spleen type erythrocyte ferritin and two had abnormally