Summary of experimental designs and results

Tissue	*Fixation*	*Post-fixation*	*Embedding*	*Sections (μm)*	*Stain*	*MMC*	*Comments*
Operative and biopsy specimens	Carnoy's	—	Paraffin	6·0	AB/S	+++	GTA fixed and paraffin embedded intestinal mucosa is unsatisfactory for light microscopic identification of MMC
	GTA 5%	—	Paraffin	6·0	AB/S	–	
Biopsy specimens	GTA 5%	—	Araldite	1·5	AB/S	–	Routine processing for semithin plastic sections and AB/S gave poor technical and staining results
	GTA 5%	OsO_4	Araldite	1·5	AB/S	+	
Operative and biopsy specimens	GTA 5%	—	Araldite*	1·5	AB/S	–	Post-fixation with OsO_4 and staining after Araldite removal resulted in identifiable MMC, very weak staining pattern
	GTA 5%	OsO_4	Araldite*	1·5	AB/S	+	
Operative and biopsy specimens	Carnoy's	—	Araldite*	1·5	AB/S	+	Staining results after Carnoy's fixation for semithins were poor, marked improvement after OsO_4 post-fixation
	Carnoy's	OsO_4	Araldite*	1·5	AB/S	++	
Biopsy specimens	GTA 5%	—	Araldite	1·5	AII/MB	–	Routine processing for plastic sections and AII/MB give excellent and distinct staining pattern of MMC and other cellular components, MMC with blue granules
	GTA 5%	OsO_4	Araldite	1·5	AII/MB	+++	
Biopsy specimens	Carnoy's	—	Araldite	1·5	AII/MB	(+)	Identification of MMC feasible; non-specific background staining, interfering with MMC identification
	Carnoy's	OsO_4	Araldite	1·5	AII/MB	(+)	

*Araldite was removed before staining.

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Manual screening for immune antitetanus antibodies by means of latex coated with tetanus toxoid

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Techniques for the selection of donor plasma containing suitably high concentrations of antibody for the production of anti-tetanus immunoglobulin include immunoelectrophoresis (IEOP),[1] haemagglutination,[2] automated haemagglutination[3] and tetanus toxoid coated latex particles[4] used in both automated[5] and manual methods. At the Trent Regional Transfusion Centre, Sheffield, IEOP and automated coated latex methods have been used to select blood donors whose serum contains a sufficiently high concentration of tetanus antitoxin for the preparation of antitetanus immunoglobulin. At present this concentration is set at 4 IU/ml.

The manual latex technique has been developed to replace these methods; it is rapid, easy to perform and requires the minimum equipment. Consequently, large numbers of samples can be screened by relatively inexperienced staff, with no outlay for complex automated equipment. The method described can also be used to estimate the response to tetanus toxoid by an individual.

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Material and methods

Ten ml of 2% latex (Intex 191 International Synthetic Rubber Co Ltd, Hythe, Southampton) in 0·1 *M* glycine-saline buffer pH 8·2 and 5 ml of the glycine-saline buffer were put into a glass container. Ten ml of the tetanus toxoid (Blood Products Laboratory, Elstree) containing 110 Lf/ml were added without mixing followed immediately afterwards by 4 ml of 20% bovine serum albumin. The contents of the container were then mixed and placed in a water bath at 60°C for 20 min. The product of this process is granular and is therefore either gently treated with a sonic disintegrater, or passed through a fine hypodermic (25 gauge) needle until particles which give no visible reaction with negative sera but react with positive sera are obtained. The sensitised latex was diluted 1/3 with 0·1 *M* glycine-saline buffer.

In order to use the sensitised latex to select plasma suitable for immunglobulin preparation, the latex suspension must be standardised. Antibody standard prepared by the Blood Products Laboratory was diluted in human serum which was known to have no antitetanus activity. Dilutions were prepared equivalent to 6, 4 and 3 IU/ml. One drop of sensitised latex was added to one drop of each dilution on a glass microscope slide, mixed to an area of 2 cm diameter, and rotated on a Luckham's rotary plattern mixer at 110–120 rpm. The test was timed, and the point at which the 6 IU/ml standard gave good flocculation, the 4 IU/ml standard gave a slight reaction, and the 3 IU/ml standard had not reacted was noted. This time was then used as the standard screening time (normally 3 to 5 min). As the breakdown of the granular, sensitised latex particles is difficult to standardise, the time varies for each batch of sensitised latex. To screen blood donations for levels of antitetanus antibodies ≥4 IU/ml, one drop of sensitised latex was mixed with one drop of donor serum, rotated at 110–120 rpm for the standard screening time, and examined for flocculation using the naked eye. Those sera which produced flocculation were regarded as containing ≥4 IU/ml and were, therefore, regarded as suitable for immunoglobulin preparation.

The latex reagent may also be used in a quantitative test to determine the immune status of an individual in respect of antitetanus toxoid. Dilutions of antibody standard provided by the Blood Products Laboratory were prepared in human serum known to have no antitetanus activity. The dilutions prepared were 16, 8, 4, 2, 1 and 0·5 IU/ml of tetanus antitoxin. The dilutions were tested as in the screening method, and the time to flocculation for each level of antibody dilution was determined. The patient's serum was then tested under the same conditions, and the time to flocculation noted. By comparing the time obtained for the patient's serum to flocculate the sensitised latex with the times taken for the controls to flocculate the latex, an estimation of patient's antibody level could be made. In practice, the patient's sera and standard dilutions could be tested together. Whenever possible, serum samples taken before and after tetanus immunisation were tested simultaneously.

Results

The results obtained when screening blood donor samples are shown in the Table.

Comparison of immunoelectrophoresis (IEOP) and manual latex methods

Activity	*No of donor samples*	*%*
<4 IU/ml on both tests	2417	94·5
≥4 IU/ml by IEOP	141	5·5
≥4 IU/ml by latex	139	5·4
≥4 IU/ml IEOP; <4 IU/ml latex	2	0·1
≥4 IU/ml latex; <4 IU/ml IEOP	Nil	
Total	2558	

Titrations of positive samples gave comparable results by both IEOP and manual latex methods. These results indicate that the test is suitable for selecting blood donations for the preparation of antitetanus immunoglobulin.

The quantitative method was used in parallel with IEOP on a series of eight cases either suspected of having, or diagnosed as, clinical tetanus.[6] The two methods gave comparable results between 1 IU/ml and 32 IU/ml. The manual latex method did, however, detect activities down to 0·5 IU/ml. The manual latex and IEOP methods were also used to study individual response to tetanus toxoid immunisation. The response to immunisation was detected earlier when using the latex method as this method is more sensitive.

The results show that the manual latex method is useful in ascertaining the immune status of an individual in respect of antitetanus toxoid.

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Letters to the Editor

Investigation into paediatric bilirubin analyses in Australia and New Zealand

I read with interest the paper by Watkinson, *et al* in your issue of January 1982.[1] This paper provides valuable information regarding standardisation of total bilirubin assays, however I cannot totally agree with comments by the authors on estimation of conjugated bilirubin. The authors maintain that conjugated bilirubin cannot be reported accurately and advocate ranking results as (μmol/l):

<25
25–50
50–100
100–150
150–200

stating "This approach should be adequate for patient care and not lead to over-interpretation of results."

The authors themselves show with three histograms in Fig. 2, that this is in fact not so. These histograms show clearly that it is not possible for laboratories to perform conjugated bilirubin assays accurately enough to place correctly a value in one of these ranks. In the two neonatal plasma samples assayed (samples D and E), number of labs = 75) the results fall into the first two ranks (D) and first three ranks (E). In the specimen assayed with the high conjugated value, results for the laboratories range from 0 to 150 μmol/l—that is, the first four ranks.

With such large between-laboratory variation I feel it is meaningless to offer conjugated bilirubin results assayed by diazo techniques.

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Reference

[1] Watkinson LR, St John A, Penberthy LA. Investigation into paediatric bilirubin analyses in Australia and New Zealand. *J Clin Pathol* 1982;**35**:52–8.

Dr Watkinson and colleagues reply as follows:

Robert McKenzie's letter has given us the opportunity to further discuss the problem that exists in the measurement of conjugated bilirubin in neonates as shown in our paper (reference above).

Our survey clearly showed that there was a very wide dispersion of results for the measurement of conjugated bilirubin in plasma. From the limited information gained on conjugated bilirubin analyses we intended (a) to demonstrate clearly this dispersion and (b) to generate discussion on the need for conjugated bilirubin measurement.

It is our proposal that the need for this analysis requires review by the biochemistry laboratory and the clinical staff. Such discussion may as Mr McKenzie suggests result in the laboratory no longer offering a conjugated bilirubin assay but we feel that from our data and discussion with some clinicians that this may be too narrow an outlook at present. Our suggestion was an intermediate stance which while giving clinicans the benefit of the assay, would also identify the state of the art and hopefully prevent overinterpretation of the results.

We felt that our recommendation on ranking was valid by the fact that a significant percentage of laboratories as shown below did rank the results correctly.

There is no doubt that the analysis should be improved. If laboratories react to our investigation and seek improvement in their performance then the ranking we suggested is probably acceptable for this seemingly necessary analyte.

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	Laboratories ranking		
μmol/l	*Sample D (%)*	*Sample E (%)*	*High conjugated sample (%)*
< 25	87	86	5
25–50	13	13	8
50–100	—	1	49
100–150	—	—	38

n = 75
Sample D mean = 13 μmol/l
Sample E mean = 14 μmol/l
High conjugated sample mean = 87 μmol/l

Silicone lymphadenopathy

Silicone lymphadenopathy is a rare complication of silicone joint prostheses which may give rise to clinical suspicion of malignancy and be biopsied. It is important therefore that the surgical pathologist is familiar with this condition and the following case-report should be of interest.

A 60-year-old-man complaining of chest pain was found to have enlarged axillary lymph nodes and an opacity on chest radiography. Lung cancer with lymph node metastases was diagnosed and an enlarged axillary lymph node was biopsied. This showed numerous epithelioid and giant cell granulomata with refractile non-birefringent particles in many of the giant cells (Figure). No tumour was seen in the node but a transthoracic needle biopsy confirmed the diagnosis of pulmonary carcinoma. Enquiries about previous injections and operations on the arm revealed that the patient had had prosthetic finger