receiving full dose heparin (Δ kaolin clotting time; 56, 29, and 19 seconds) and four had been considered to have equivocal lupus inhibitor tests. On testing of subsequent samples from three of the equivocal patients, one with a Δ kaolin clotting time of 29 seconds was found to have a definite lupus inhibitor while the two others (19 and 14 seconds) were negative. The Δ kaolin clotting time in the two positive patients with normal PTTs was 14 and 34 seconds and in the positive patients with normal APTTs the Δ kaolin clotting time range was 14–150 seconds with a mean (SD) of 24.6 (21.6) seconds.

Discussion

This study was prompted by the increasing number of requests received by our laboratory for the lupus inhibitor test and the consequent drain on laboratory staff resources. Our policy had been to perform a full lupus inhibitor test on all requests, provided the patient was not receiving therapeutic anticoagulation. In our one year study this led to a lupus positivity rate of 10.4%. As already mentioned, if less sensitive assays such as the PTTK or APTT were used as screening tests a small but significant number (5 and 20%, respectively) of patients positive for lupus inhibitor would be missed.

In our attempts to develop a simplified yet sensitive screening test for the lupus inhibitor the Δ kaolin clotting time between the 8:2 and 10:0 points was evaluated. This is the most sensitive area of the curve for lupus inhibitor detection and least affected by heparin. If this assay alone had been used we would have detected all true lupus inhibitor positive samples (that is, no false negatives). In addition, however, there would have been seven "false" positive results in the 386 samples. Based on the results of this study we

intend to introduce the Δ kaolin clotting time as an initial screen. If the result is less than 14 seconds the sample is considered to be negative and if greater than or equal to 14 seconds a full test is performed. The full test would help exclude false positive results due to heparin and distinguish equivocal results which should be repeated. In addition, the use of the Δ kaolin clotting time will provide a rough quantitation for sequential studies and considerably reduce laboratory work load.

The secretarial assistance of Mrs C Young is appreciated.

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Immunoturbidimetric assay for rheumatoid factor using an enzyme immunoassay microplate reader

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Several methods for detection of rheumatoid factors in serum are currently commercially available.^{1–3} The earliest assays developed, and those still most widely used, rely on the agglutination of latex particles coated with immunoglobulin G when exposed to sera con-

taining rheumatoid factors.¹ The assay described in this paper is turbidimetric, originally developed for use of chemical analysers (such as Cobas Bio, Kone CD),⁴ and modified for use with a microplate enzyme immunoassay reader.

Material and methods

Samples were received from patients being investigated for various arthritides. Fifty samples were tested and were shown by the Cobas assay to have rheumatoid factor activity between 50 and 500 IU/ml.

TURBIDIMETRIC ASSAY

The rheumatoid factor kit (Orion Diagnostica, Espoo, Finland) was supplied with all the reagents required to carry out the assay, including reference serum standar-

Technical methods

dised against the World Health Organisation standard for rheumatoid factors. The enzyme immunoassay (EIA) hardware was a microplate reader Biotek EL-310 (Biotek Instruments, Burlington, USA). Microwell strips (Nunc Catalogue No 469949, Kamstrup, Denmark) were used without pretreatment.

The procedure was as follows. Sera and a 200 μ l aliquot of the supplied reference serum were heat inactivated at 56°C for 30 minutes. While the samples were incubating, the rheumatoid factor reagent (aggregated human IgG) was reconstituted with 2 ml of sterile 0.9% sodium chloride, gently mixed and allowed to stand at room temperature for at least 30 minutes. Ten microlitre volumes of patient samples were added to two consecutive wells of a microwell strip. Fifty microlitre, 20 μ l, and 10 μ l volumes of the reference serum were added to three consecutive sets of paired wells of a microwell strip; 10 μ l of 1:2 dilution of the reference serum, in reference serum diluent, were added to a fourth pair of microwells—these corresponded with 500, 200, 100 and 50 IU/ml calibration standards, respectively. Two hundred microlitres of diluent buffer were added to all wells. Ten microlitres of the rheumatoid factor reagent were added to the first microwell (test well) of each pair of microwells (the second well acts as a sample blank), mixed, and allowed to stand at room temperature for 15 minutes. The microwells in a microwell carrier tray were then placed on the EIA microplate reader and the absorbance of each microwell determined at 405 nm. The difference of absorbance of each pair of microwells was calculated by subtracting the absorbance of the blank microwell from the test microwell. A calibration curve was constructed with the difference in absorbance being plotted on the y axis and reference calibrator values of the x axis.

COBAS BIO TURBIDIMETRIC ASSAY

The Cobas Bio turbidimetric assay was carried out according to the instructions detailed in the Orion rheumatoid factor reagent instruction sheet.

Results

A calibration curve was constructed on 25 separate occasions in the manner described. The table shows the mean absorbance and coefficient of variation (CV) of the absorbance for each of the calibrators used.

The interbatch reproducibility was determined using the values obtained for the calibrators on 25 consecutive batches of tests. The coefficient of variation (CV) was calculated to be between 10% and 13% (table) over the range of the calibrators—that is, 50 to 500 IU/ml. Intrabatch reproducibility was determined 20 times in one batch of tests—the coefficients of variation were 6% and 8%, respectively.

The results obtained for the turbidimetric assay

Table Mean and CV absorbance for calibrators

	Calibrators value (IU/ml)			
	50	100	200	500
Mean absorbance	43	62	86	106
CV%	10	13	10	12

Figure Correlation between Cobas Bio and microadaptation assays.

were compared with those obtained using the Cobas Bio turbidimetric assay for 50 patient samples (fig 1). The coefficient of correlation was 0.92.

Discussion

The Orion Diagnostics turbidimetric assay for rheumatoid factor correlated well with the latex agglutination tests.⁴ The modified method described was developed because of limited access to one of the recommended chemical analysers. It was shown to correlate well with the Cobas Bio turbidimetric assay. It was also shown to have an acceptable coefficient of variation. Because of the widespread use of enzyme immunoassays in microwell format, and proliferation of associated hardware, this modified assay could allow many laboratories to perform more easily a turbidimetric assay for rheumatoid factor. Interfacing of a microcomputer with the microplate reader would allow automatic data reduction and printing of the results. An average batch of 40 patient samples and calibrators requires about 20 to 30 minutes of direct technician time. The microadaptation reduces the cost of reagents for the test by 70% to 80% of the cost for the methods described in the manufacturer's information sheet, as well as the cost for latex agglutination tests, without loss of reproducibility.

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Simple latex agglutination method for typing pneumococci

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Serotyping of *Streptococcus pneumoniae* was important before the introduction of antibiotics when intravenous administration of type specific antisera was the only treatment available for invasive pneumococcal infections. Now that pneumococcal vaccines are available, the typing of pneumococci has reassumed importance, especially as these vaccines contain only a limited number of polysaccharides.

Since its description by Neufeld in 1902,¹ the capsule "swelling" (Quellung) reaction has been regarded as the standard method of typing pneumococci. It is, however, somewhat labour intensive as a routine test. We describe a simple slide agglutination method for typing pneumococci by means of specific antibody adsorbed to latex particles.

Material and methods

Five hundred consecutive strains of pneumococci isolated from clinical specimens were collected. Each isolate was confirmed as *S pneumoniae* on the basis of colonial morphology and sensitivity to optochin.

A pure culture of each isolate was obtained on a blood agar plate from which a broth culture in Todd and Hewitt broth was made (overnight incubation at 37°C). The organism on the plate was then serotyped by staphylococcal coagglutination according to the method described by Kronvall,² and the broth culture was used for serotyping by a latex agglutination method. The method of Severin³ was used with several modifications. The antiserum (Statens Seruminstitut, Copenhagen) was diluted 1/20 with sodium glycine buffer (pH 8.2). Equal volumes of the diluted antiserum and latex suspension (polystyrene latex particles—0.81 µm in diameter, Difco) were mixed and incubated in a water bath for two hours at 37°C. An equal volume of 0.1% albumin solution in sodium glycine buffer was then added as a preservative. The latex agents were stored at 4°C. The reagents for

both typing methods have to be brought to room temperature before performance of the tests.

In the latex test one drop of sensitised latex particles was added to a drop of the broth culture of the organism on a black tile. The drops were mixed for two minutes and examined for agglutination. In positive cases a distinct agglutination formed. When the suspension remained milky the test was negative. Each strain was tested against a range of pneumococcal antisera to determine the monotype (nine types) or serogroup (13 groups). The types or groups were chosen to be the same as the antigen types included in the 14 and 23 valent pneumococcal vaccines. One hundred strains were referred to another laboratory for serotyping by counterimmunoelectrophoresis (CIE).

Results

Of the five hundred isolates collected, 488 were tested. Most of the strains (390 strains, 80%) were isolated from sputum culture. Thirty two strains (6%) were isolated from blood or cerebrospinal fluid and the remainder from eye, ear, and peritoneal swabs.

The table shows the results of the serotyping. The

Table Serotype distribution of 488 strains of pneumococci relative to coverage of appropriate vaccines

Serogroup or serotype vaccine related		
14-valent vaccine	23-valent vaccine	No (%)
1	1	1 (0.2)
2	2	0 (0.0)
3	3	48 (9.8)
4	4	5 (1.0)
	5	2 (0.4)
6 (6A)	6 (6B)	47 (9.6)
7 (7F)	7 (7F)	5 (1.0)
8	8	22 (4.5)
9 (9N)	9 (9N, 9V)	57 (11.7)
	10 (10A)	6 (1.2)
	11 (11A)	23 (4.7)
12 (12F)	12 (12F)	0 (0.0)
14	14	26 (5.3)
	15 (15B)	14 (2.9)
	17 (17F)	26 (5.3)
18 (18C)	18 (18C)	12 (2.5)
19 (19F)	19 (19A, 19F)	46 (9.4)
	20	8 (1.6)
	22 (22F)	17 (3.5)
23 (23F)	23 (23F)	62 (12.7)
25		0 (0.0)
	33 (33F)	11 (2.3)
Other types		50 (10.2)
Total		488