Figure 3 Negative control (PBS substituted for antibody to CMV) in the same case as fig 1.

reagent alone or of peroxidase linked avidin (Dako; catalogue no. P364), diluted 1 in 500, or streptavidin-biotinylated alkaline phosphatase complex (Dako; catalogue no. K 391) produced the same result. Incubation in

the enzyme development solutions alone or substitution of a PAP complex for the streptavidin or avidin containing reagent did not produce nuclear staining, although control CMV containing sections showed typical positive staining with the PAP method.

Discussion

We believe that the nuclei of endometrial epithelial cells sometimes contain biotin during and shortly after gestation. Biotin may take the form of intranuclear inclusions, which increases the resemblance to viral infection. This topic has already been discussed in the literature¹ but as it seems to be little known, we would like to emphasise the importance of including negative immunostaining controls. We advise that methods employing avidin be avoided when immunostaining gestational endometrium for CMV.

- 1 Yokoyama S, Kashima K, Inoue S, Daa T, Nakayama I, Moriuchi A. Biotin-containing intranuclear inclusions in endometrial glands during gestation and puerperium. *Am J Clin Pathol* 1993;99:13-17.

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Comparison of two automated quantitative immunoassays for the determination of C reactive protein concentrations

E A Barclay, J E Coia, P C D Kale, R G Masterton

Abstract

Two quantitative, automated methods for the determination of C reactive protein (CRP) were compared: turbidimetry (Cobas Fara II, Roche, Welwyn Garden City, UK) and fluorescence polarisation TD_x, Abbott, Wokingham, UK). One hundred and twenty routine serum samples submitted for measurement of CRP were tested using both procedures. The results were compared using regression line analysis and showed a high degree of correlation ($r^2 = 0.99$, X coefficient = 1.01, constant = 0.11). C reactive protein can be accurately measured using the automated turbidimetric method which can be recommended as an alternative to fluorescence polarisation.

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Serum C reactive protein (CRP) concentrations rise and fall rapidly as part of the "acute phase" response.¹ Despite the non-specific nature of this response, CRP measurement provides a sensitive indicator of inflammation in a variety of conditions and is an objective index of the clinical response.² Early studies of the clinical use of CRP measurement were dogged by a lack of standardisation and of quantitative assays which could be performed quickly and conveniently. Recently, an international reference standard for proteins in human serum has been published.³ This, coupled with the availability of rapid, automated and quantitative assay systems, has facilitated the development of CRP measurement as a routine clinical laboratory investigation.

Fluorescence polarisation⁴ is currently in widespread use, while the alternative method,

Central
Microbiological
Laboratories, Western
General Hospital,
Crewe Road,
Edinburgh EH4 2XU
E A Barclay
J E Coia
P C D Kale
R G Masterton

Correspondence to:
Dr J E Coia

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Measuring CRP:
correlation between Abbott
TD_x and Roche Cobas
Fara methods.

immunospectrometry,⁵ also lends itself to automation. The greatly increased demand for CRP measurements prompted us to compare these methods.

Methods and results

One hundred and twenty sera submitted to the laboratory for the measurement of CRP were examined. Samples giving a value of 0.5 mg/dl or higher were chosen to give an even spread of results across all ranges. All specimens were initially tested using fluorescence polarisation and subsequently within four hours by the turbidimetric method, and within 24 hours of receipt by the laboratory. Specimens were stored at 4°C before processing, and equilibrated with room temperature before testing.

The reference fluorescence polarisation method was performed on an Abbott TD_x analyser using Abbott reagents, according to the manufacturer's instructions, with an appropriate control run for each protocol during each eight hour period. Samples were initially run using Protocol A of the manufacturer's procedure, giving results between 1.5 and 26.0 mg/dl. For greater precision, samples yielding results less than 6.5 mg/dl were repeated using Protocol C. Samples giving results greater than 26.0 mg/dl were repeated using a 1 in 2 dilution protocol.

Turbidimetry was carried out on a Roche Cobas Fara II using Roche reagents, protocols, and controls. Routine calibration enabled readings to be taken within the range 0.5–16.0 mg/dl. Samples yielding results higher than 16.0 mg/dl were re-analysed using a 1 in 5 dilution protocol with saline and the CRP standard (16.0 mg/dl) was used as a control, according to the manufacturer's instructions.

The results obtained on both methods were compared using Pearson's linear correlation coefficient. The correlation of the two methods (figure) was highly significant ($r^2 = 0.99$, X coefficient = 1.01, constant = 0.11).

Discussion

The results suggest that the turbidimetric method is a useful and an acceptable alternative to polarised fluorimetry for the measurement of CRP concentrations. Both methods permit the rapid, accurate, automated, and quantitative determination of CRP concentrations in the routine diagnostic laboratory.

The choice of method will be determined by a number of factors in any given situation. Clearly, if a laboratory already uses either instrument for performing other analyses, capital or maintenance costs, or both, may be reduced by using existing hardware. Equally, the use of familiar analysers may reduce the amount of staff training required for implementation of the test. These analysers may already be connected to departmental computer systems. The cost of consumables will be an important additional factor, particularly if further economies can be made by bulk buying.

There can be little doubt that with the availability of rapid, automated assays, CRP determination will play a greater role in the management of some patient groups, and there will be a growing demand for laboratories to provide this service. We recommend the turbidimetric assay as an acceptable alternative to the widely used polarised fluorimetric method.

- 1 Ballou SP, Kushner I. C-reactive protein and the acute phase response. *Adv Intern Med* 1992;37:313–36.
- 2 Young B, Gleeson M, Cripps AW. C-reactive protein: a critical review. *Pathology* 1991;23:118–24.
- 3 Johnson AM. A new international reference preparation for proteins in human serum. *Arch Pathol Lab Med* 1993;117:29–31.
- 4 Benattar C, Francoual J, Magny JF, Lindenbaum A. Development of a rapid method of immunoassay of C-reactive protein by polarisation of fluorescence. *Ann Biol Clin* 1989;47:181–5.
- 5 Otsuji S, Shibata H, Umeda M. Turbidimetric immunoassay of serum C-reactive protein. *Clin Chem* 1982;28:2121–4.