Technical methods

Automated reagin test using a particle counter

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The automated reagin test (ART) was devised by McGrew et al. (1968) as an adaptation of the rapid plasma reagin (RPR) test (Portnoy et al., 1962) to a continuous flow technique. Results are obtained by the deposition of carbon particles onto a continuously moving filter paper strip. We describe a modification which provides an output for a conventional chart recorder from a particle counter.

Principle

Antigen and serum react as in the ART to the point of decantation to the filter paper strip. This decantation together with a second decantation is pulled to waste (Fig. 1). The remaining stream is resampled, diluted, and passed through a flow-cell in the particle counter (Autocounter; Technicon Instruments Co).

Received for publication 31 January 1977

Fig. 1  Flow diagram of modified ART.
Reagents and procedure

Altogether 3106 sera received for routine syphilis serology were tested. These included sera from VD and antenatal clinics and from general medical cases which had been inactivated by heating at 56°C for 30 minutes during the course of the routine testing for complement fixing antibody. Turbid sera were clarified by membrane filtration. ART antigen (Hynson, Westcott & Dunning) was obtained from Becton, Dickinson UK Ltd. The diluent was 0.05% (v/v) Tween 20 in physiological saline.

Samples were aspirated at the rate of 80 per hour. The antigen was continuously agitated by a magnetic stirrer. Optimal adjustment of the particle counter was achieved by dilution of the resampled stream (Fig. 1) and adjustment of the threshold setting. This setting was such as to eliminate noise while counting the maximum number of unagglutinated particles. Agglutination resulted in the removal of larger numbers of particles by decantation, a lower particle count, and a downward deflection of the recorder pen. Every tenth sample was a minimally reactive serum (MRS), as defined by the RPR card test, which provided a check on the sensitivity and reproducibility of the system. Samples giving a deflection equal to or greater than the MRS were titrated by dilution in ART-negative serum. Day-to-day reproducibility was checked by titrating a standard serum at the beginning of each run. The RPR was performed according to the manufacturer's instructions.

Results

Three thousand one hundred and six sera were examined by modified ART and by RPR card test (Table). Positive sera were diluted in negative serum for titration by the modified ART and titrated by the recommended method for the RPR. Figure 2 shows the correlation between the two methods. Sera positive by either method were examined by Treponema pallidum haemagglutination test (TPHA). All of the 24 sera which were ART positive, RPR negative showed a negative TPHA test. The one serum positive by RPR (titre 1/1), negative by ART, and positive by TPHA was from a West Indian man with no clinical evidence of syphilis.

Discussion

Agglutination is detected in this system by a particle counter which provides a recorder chart trace. This removes the risk of handling potentially infective filter paper on which results may in any case be difficult to interpret. It has a further advantage in the ease with which positive sera may be titrated to a
reproducible end point. Figure 3 shows the relation between ART titres and the percentage deflections from the baseline produced by the neat sera. The levelling off at higher titres is probably due to the total removal of reactive particles.

The results show that the modified ART produces more positive results than the RPR card test (Fig. 2). Of 49 sera positive by both methods, 18 (39%) showed higher titres with the modified ART. This may be due to the use of serum as a diluent for the modified ART or the increased sensitivity of the detection system.

Particle counting has demonstrated reaginic floculation reactions in a continuous flow system, and we have also carried out virus haemagglutination tests using similar apparatus. Further developments suggest that specific treponemal haemagglutination tests may be performed using this detector system.

References


A direct immunofluorescence method for the detection of hepatitis B core antigen in formalin-fixed and gelatin-embedded liver specimens

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Infection with hepatitis B virus (HBV) and the responses of the host to it are much more complicated than were previously thought. To date, at least three distinct antigen-antibody systems, that is, hepatitis B surface antigen (HBsAg)—anti-HBs, hepatitis B core antigen (HBcAg)—anti-HBc, and e antigen—anti-e, have been identified in the serum of individuals infected with HBV. The diagnostic and prognostic values of determining these markers in the serum have already been established (Almeida et al., 1971; Magnius and Espmark, 1972; Hoofnagle et al., 1973).

HBsAg was demonstrated in the cytoplasm, and HBcAg in the nucleus, of hepatocytes by immunofluorescence in unfixed frozen sections (Brzosko et al., 1973). The immunofluorescence method has been successfully extended to formalin-fixed liver specimens for the demonstration of HBsAg, and this has opened the way for retrospective studies of necropsy material (Huang et al., 1976). We now report a simple method for detecting HBcAg in formalin-fixed liver specimens by a direct immunofluorescence method.

Material and methods

Liver tissue obtained at necropsy from patients with various hepatic disease and which had been fixed and stored in 10% formalin was used. Three blocks of about 1 cm³ size were cut, one from the left lobe, another from the right lobe, and the third from the centre of the liver. They were washed free of formalin in tap water for 12 hours. The specimens were then incubated in distilled water containing 0.01% (w/v) NaN₃, and 5% (w/v) gelatin (Difco Laboratories, Detroit, Mich.) at 37°C for 12 hours, and successively in water supplemented with 10% gelatin at 37°C for 4 hours. The specimens were then transferred onto a Petri dish filled with 10% gelatin at 37°C, and the dish was allowed to consolidate at room temperature. The liver specimens were then cut from the gelatin plate and snap-frozen in n-hexane cooled in a dry ice/acetone bath.

Anti-HBc was obtained from the serum samples of asymptomatic carriers of HBV containing a high titre of anti-HBc (immune adherence haemagglutination titre of 2¹⁶ or higher). The gamma-globulin fraction was prepared by precipitation in 33%—saturated (NH₄)₂SO₄ solution followed by chromatography on DEAE cellulose. Anti-HBc γ-globulin was then labelled with fluorescein isothiocyanate (FITC). Similarly, γ-globulin fractions of rabbit and human anti-HBc antisera (passive haemagglutination titre of 1:2000 or higher) were isolated and labelled with FITC, and fluoresceinated anti-HBs reagents were obtained.

Cryostat sections were cut at 4 μ and mounted on a slide glass with egg-white/glycerol (1:1). They were fixed by immersion in acetone for 1 minute at
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*J Clin Pathol* 1977 30: 774-776
doi: 10.1136/jcp.30.8.774