criteria has to be made by the user. The performance of the system therefore depends largely on the skill and experience of the user. It should also be emphasised that use of the computerised cytogenetics database should always go hand in hand with the original data source, the Schinzel catalogue, since weighing of choices and comparison with photographs of patients are important steps in the diagnostic process.

Nevertheless, computer aided syndrome searching helps to ease the routine workload in a busy genetic counselling service. One of the major benefits of this approach, we feel, is in the initial stages of student training under the supervision of an experienced geneticist. Regular updates will certainly increase the value of the system for any potential user.

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Drs Winter and Baraitser comment on this paper on page 509 of this issue.

Detection of centromeric regions of chromosomes by immunofluorescence: procedure and application

Conventional techniques for the identification of centromeric regions can be time consuming and often produce inconsistent and variable results. The recent description of autoantibodies to centromere antigen in patients with the CREST variant of

progressive systemic sclerosis¹ has prompted us to use this antibody as a probe for identifying centromeric chromatin of chromosomes. We describe here a simple and rapid technique for the identification of centromeric regions of chromosomes by immunofluorescence microscopy. The clinical use of this technique is demonstrated by a case study.

Method

The anticentromere autoantibody used in this study was obtained from the frozen serum bank at E W Sparrow Hospital. The titre of the antibody was 1:5120 as determined by routine ANA screening on a Hep-2 cell substrate (Kallestad Laboratories, Austin, Texas). Chromosome spreads were prepared from peripheral blood cultures by the method of Yunis.² Briefly, methotrexate synchronised cultures were harvested, fixed, and dropped onto clean glass slides. The chromosome spreads were flooded with a 1:200 dilution of anticentromere antibody in phosphate buffered saline (PBS, pH 7.2) and incubated in a humidified chamber for 30 minutes at room temperature. The slides were then rinsed in two changes of PBS for five minutes each, then flooded with a 1:80 dilution of fluorescein conjugated antihuman IgG, Fab'2 (Kallestad), and incubated for 30 minutes at room temperature. The slides were washed twice in five minute changes of PBS and examined by fluorescence microscopy on a Zeiss epifluorescence microscope equipped with a 50 watt mercury-arc lamp.

Case report

A 38 year old couple was referred to this laboratory

FIGURE (Left) G banded microchromosome, (middle) banded microchromosome, (right) anticentromere (AC) banded microchromosome. Note the individual chromatid centromeres in the AC banded photograph.

for cytogenetic evaluation because of a history of spontaneous abortion. Routine G banding showed the man to have a normal 46,XY karyotype. The woman showed a small supernumerary chromosome in 23 of 30 cells analysed. Silver staining as described by Howell and Black³ showed an active NOR region on one end of this microchromosome. Anti-centromere immunostaining showed a positive centromere region proximal to the NOR⁺ region (figure). The presence of this centromeric material was confirmed by conventional C banding techniques.

Discussion

The identification of centromeric material by immunofluorescence microscopy using anticentromere autoantibody (anti-C technique) is rapid, simple, and inexpensive. In contrast to conventional C banding techniques, which require long incubation times (frequently up to 24 hours), this procedure can be performed in less than two hours. As shown by this case report, the anti-C technique can provide clinically useful information. We have since used this technique in several other cases and have found it to be reliable and sensitive. Our experience has shown that the anti-C technique gives superior

resolution when compared to Giemsa or Wright stained C banding techniques. We are at present evaluating the anti-C technique for its use in identifying the activity of centromeric material.

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