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A METHOD FOR SEXING 90-DAY-OLD BLOOD STAINS IN ABSORBENT AND NONABSORBENT MATERIALS

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Summary

Cells from blood stains in absorbent and nonabsorbent materials like stainless steel, unpolished wood, cloth, glass, and paper, can be correctly sexed using fluorescent stains even after 90 days, during which the stains had been exposed to light, unfixed.

The application of fluorescence staining techniques (Caspersson et al. 1969) to the study of interphase nuclei in man permitted the identification of a male sex-chromatin, or Y body (Pearson et al. 1970), and of a female sex-chromatin, or Barr body (Greensher et al. 1971, Goday et al. 1971, François et al. 1971).

The possibility of sexing old blood stains in everyday materials like cloth, paper, wood, etc., is of interest in police work and forensic medicine. Schwinger (1971) described a technique that allowed him to correctly sex blood stains as old as 30 days. However, when the method was used in our laboratory, it was found that the preparations contained excessive amounts of paper, wood, or cloth fibers. Since these fibers stain with fluorescent agents, and the blood cells concentrate mainly on the remainings of the material analyzed, they have to be studied on a highly fluorescent background that makes sexing difficult even if the preparations are examined in a dark room.

We describe here two methods that completely eliminate the remainings of the material studied from the preparations, and provide sufficient cells for a correct sexing of samples as old as 90 days, even using a single drop of blood.

METHODS

From one drop up to several ml of blood from 10 male and 10 female normal donors were placed on stainless steel, cotton fabric, unpolished wood, glass, and bond paper, coded and left at room temperature and exposed to light for 90 days.

Method for Samples on Metal, Wood, Glass, and Paper

Carefully lift small fragments of the dried blood with a sharp scalpel. Transfer to 6-10 ml of 25% acetic acid. Stir in a magnetic stirrer until a homogeneous suspension is obtained (about 20 min.).

Centrifuge 8 min. at 800 rpm. Decant supernatant. Resuspend the cells. Add 2 ml of a 3:1 methanol:acetic-acid mixture. Repeat the procedure 3-5 times.

To prepare slides, resuspend the cells in 4 drops of 3:1 methanol:acetic acid. With a Pasteur pipet place 3 drops of the suspension on a clean slide. Blow gently to help spreading. Air dry.

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Method for Samples on Cloth

Cut out a small piece of the sample (1-2 cm^2). Dip cloth in hydrogen peroxyde and let stand for 24 h. Discard cloth, and centrifuge the cell suspension for 8 min. at 800 rpm. Decant supernatant. Resuspend the cells. Add 2 ml of a 3:1 methanol: acetic-acid mixture, and proceed as described for stains in other materials.

The dry preparations are stained in a 0.5% aqueous solution of quinacrine dihydrochloride for 10 min., washed 4 min. in distilled water, air dried, mounted in McIlvaine's buffer pH 4.5, and sealed.

Observations were made in a Leitz photomicroscope equipped with an HB 200 lamp, using a BG-12, 5 mm excitor filter and a 510-530 barrier filter.

COMMENTS

The preparations obtained with the methods described are practically free of remainings from the materials used, and contain a large number of well preserved blood-cell nuclei (Fig. 1), even when made from 90-day-old single-drop samples. In each sample, 100 nuclei were observed.

Cloth is the most difficult material to work with. The old homemaker's recipe to remove blood stains, namely, hydrogen peroxyde, proved most effective to extract the cells from the

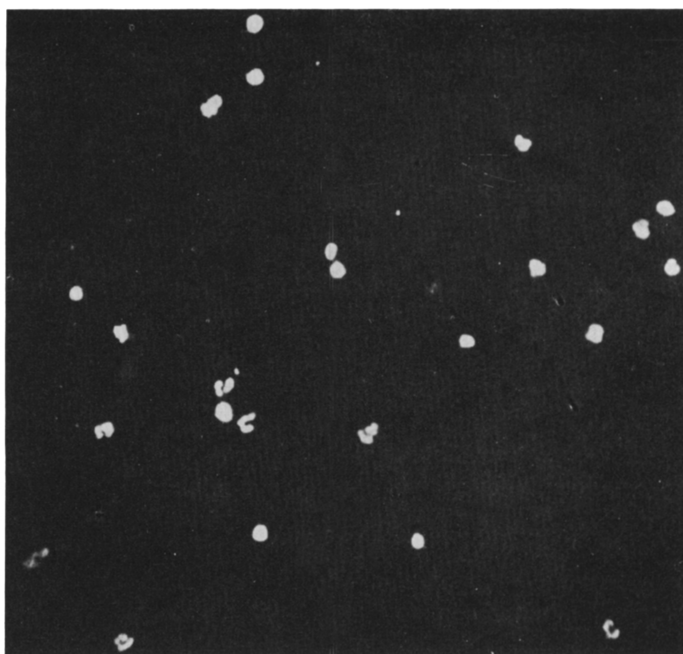

FIG. 1. *Low-power view of a blood preparation. Note clean background and large number of lymphocytes and polymorphs.* [100 ×]

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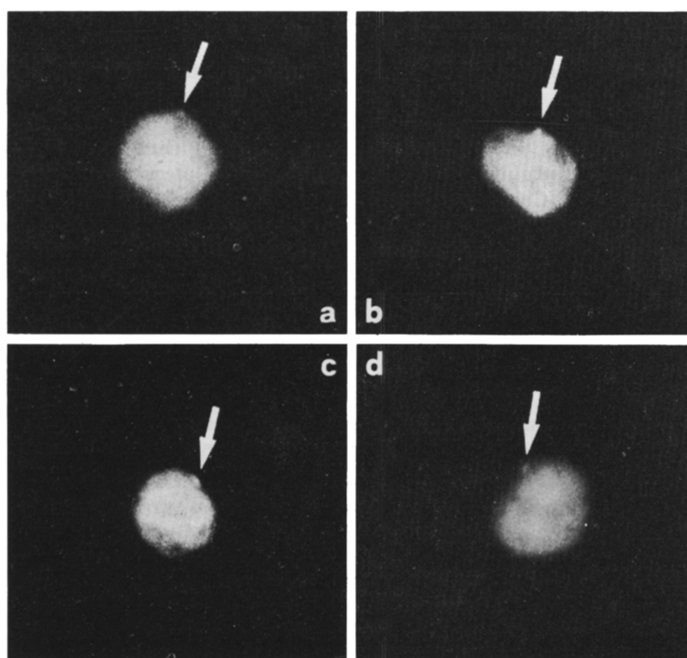

FIG. 2. *Y body in blood cells from 90-day-old samples on:* (a) *wood,* (b) *steel,* (c) *paper, and* (d) *cloth.* [700 ×]

sample. However, if the stain is older than about 30 days, the final cell concentration decreases considerably and sexing of the sample becomes more laborious.

When using the technique described for metal, wood, glass, and paper, care should be taken not to scrape off the cells. Clean preparations can only be obtained if the dried blood is carefully lifted in small fragments.

The Y body is easily identifiable in blood cells (Fig. 2). In all male samples, a mean of 30% of the cells contained one Y body.

In female samples, the Barr body is extremely difficult to detect. This is probably due to the fact that the Barr body has a more diffuse type of fluorescence and is lost on the highly fluorescent background of the dense chromatin of blood-cell nuclei. However, female samples can be easily identified by exclusion, that is, by the absence of a Y body.

It must be kept in mind that some rare cases of normal males, lacking the highly fluorescent region in the long arms of the Y chromosome, have been described (Borgaonkar 1971). Such individuals would be erroneously identified as females with the methods described.

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RIASSUNTO

Il sesso di cellule di macchie di sangue su materiali assorbenti e non assorbenti, quali acciaio inossidabile, legno grezzo, stoffa, vetro e carta, può essere correttamente determinato con colorazioni fluorescenti fin dopo 90 giorni di esposizione alla luce.

RÉSUMÉ

Le sexe des cellules de taches de sang sur des matériaux absorbants et non absorbants, tels que acier inoxydable, bois raboteux, étoffe, verre et papier, peut être correctement détérminé jusqu'à après 90 jours d'exposition à la lumière.

ZUSAMMENFASSUNG

Das Geschlecht von Blutfleckenzellen auf absorbierendem und nicht-absorbierendem Material (rostfreier Stahl, Grünholz, Textilien, Glas, Papier) lässt sich bis nach 90 Tagen Lichtaussetzung durch fluoreszente Färbung korrekt feststellen.

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