

Identification of ABO Blood Group Specific Substances from Human Epidermal Cells

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KEY WORDS ABO Blood Groups. Epidermal Cells. Forensic Cases.

ABSTRACT The study reports the successful detection of ABO blood groups specific substances from heel scrapings obtained from 123 'donors'. Both mixed agglutination and absorption - elution techniques were used.

INTRODUCTION

Landsteiner and Levine (1926), Kritschewski and Schwarzmann (1927) and Witebsky and Okabe (1927) were the first to report the detection of ABO blood group substances in various organ tissues with haemagglutination inhibition and the complement fixation techniques. Yoshida (1928) reported a method for detecting blood groups from tissues of parenchymal, digestive and genital organs with the absorption inhibition technique. Boyd and Boyd (1933, 1934, 1937), Matson (1934, 1936), Candela (1939), Hartman (1941) and Furuhashi et al. (1950) detected the ABO groups from dried human skin, muscle and mummified tissues with the absorption inhibition method. Kuno (1954), Sato (1954), Nakajima et al. (1957) and Nelken et al. (1957) also detected ABO blood groups from skin, tendon, nervous tissue and various mucosae using the same method.

Later Tsusumi (1981, 1982) employed the agglutination inhibition technique for the detection of ABO and Lewis groups from the extracts of decomposed gastric mucosae. In all these works, tissue homogenates and saline or ethanol extracts from the samples were used for blood typing. One major disadvantage with these methods was that it was not easy to group small samples. In order to overcome this difficulty direct detection methods on tissue cells without extracting blood group substances from

samples were investigated.

Glynn et al. (1957, 1959) made use of the fluorescein - labelled antibody technique to the detection of blood groups on various tissues. Subsequent to this, extensive studies on the distribution of ABH and Lewis blood group substances have been performed on various organs including digestive (Holborow et al., 1960; Szulman, 1960; Kent, 1964; Nakagami, 1967; Rouger et al., 1981; Takahashi and Kamiyama, 1982) respiratory (Holborow et al., 1960) urogenital (Szulman, 1962, 1977; Theide et al., 1965; Bariety et al., 1980), endocrine (Szulman, 1960) and dermal (Szulman, 1962; Dabelstein et al., 1984) tissues employing the fluorescein - labelled antibody technique.

The mixed agglutination technique introduced by Coombs and Bedford (1955) has proved to be very useful for the direct typing of ABO blood groups from dried blood adhering to stab weapons, bullets and clothings involved in varied forensic cases. There have been many reports on the determination of ABO types in tissue samples including skin epidermal cells (Coombs et al., 1956; Nakada, 1959; Swinburne, 1962; Yunis and Yunis, 1963; Poon and Dodd, 1964; Akaishi, 1965), spermatozoa (Yada, 1961) oral and vaginal epithelia (Holborow et al., 1960; Yada, 1961; Flory, 1966; Inuyama, 1976), dandruff (Swinburne, 1962), dirt particles (Ishiyama et al., 1975, 1977; Okada et al., 1975, 1978; Arima, 1979) and cells in amniotic fluid (Fuchs et al., 1956).

The mixed haemagglutination technique also has been used for the detection of blood group substances from formalin fixed, paraffin embedded tissues (Holborow et al., 1960; Kovarik et al., 1968; Stejskal et al., 1973; England et al., 1979; Ishiyama and Komuro, 1979; Lill et al., 1979;

Takatsu et al., 1980) and mummified tissues (Otten and Flory, 1964; Lippold, 1971).

Thus, it is clear that ABH substances are distributed throughout most organ tissue cells of the body and their detection can be of value in forensic investigations. In some accidental cases, such materials may be all that are available for any attempt to determine the blood group of an individual.

The problem of obtaining false positive and false negative results was investigated by Jenkins et al. (1972) and Pereira (1973) in which they discussed the possibility of bacterial contamination with *Escherichia coli* and related organisms which produce B-like, and less commonly, A-like substances. In their opinion the bodies that have been in water are particularly susceptible to this sort of contaminations.

MATERIALS AND METHODS

Tissue Samples: Heel scrapings were washed thrice in Sörensen phosphate buffered saline. Subsequently the tissue samples were divided into three parts for each technique and treated with one drop of anti-A, anti-B and anti-H (*Ulex europeus*), respectively.

Diluent: This was 22 per cent bovine serum albumin diluted 1.5 in 100 (v/v) in normal saline.

Papain Treatment of Red Cells: The papain was prepared according to Löws' (1955) method.

Mixed Agglutination Technique: The mixed agglutination technique was adapted from that of Ishiyama and Okada (1975).

- (i) Three microscope slides were prepared attaching adhesive tape to them.
- (ii) Tissue fragments were affixed directly on to the cellophane tape which was then placed on the microscope slide.
- (iii) One drop of anti-A, anti-B and anti-H sera was added on each specimen and incubated for 30 minutes at 20°C in a moist chamber.
- (iv) The slides were then washed thrice with isotonic saline to thoroughly remove the unattached antibodies.
- (v) One drop of 5 per cent papain treated appropriate indicator red cells suspended in albumin diluent was added and incubated for 20 minutes at room temperature.

- (vi) The slides were then reversed and immersed in normal saline to disassociate the free cells spontaneously.

- (vii) Agglutination reaction was observed under the microscope. Positive reaction was observed as botryoid clusters or red cells on the surface of the specimen.

Absorption Elution Technique: A modified absorption elution technique by Yada (1976) was employed.

- (i) The specimens were cut into equal sizes about 1-2 mm² and the pieces were divided into three small tubes.
- (ii) One drop of anti-A, anti-B and anti-H sera was added to respectively labelled tubes.
- (iii) The contents were then incubated for a minimum of 4 hours at 20°C.
- (iv) Uncombined antibodies were then decanted and the specimens were washed three times with sufficient amounts of ice-cold physiological saline.
- (v) After the final washing, the saline was pipetted off and one drop of albumin diluent added to each tube.
- (vi) The tubes were then placed in a hot water bath (56°C) for ten minutes; the tubes being agitated intermittently.
- (vii) One drop of papain treated 0.2 per cent appropriate indicator red cells suspended in albumin diluent was added to each tube with a gentle agitation.
- (viii) The tubes were then left at room temperature for one minute or so and then centrifuged at 1500 rpm for one minute.
- (ix) Presence or absence of agglutination was then observed using a concave mirror.

RESULTS AND DISCUSSION

A total of 123 specimens of known blood group and secretor/non-secretor status were analysed periodically every fortnight over a period of 8 months. The blood group antigens which can be detected from soft tissues are of the ABH and Lewis types but their detectability varies from tissue to tissue. The most useful tissues for this purpose are submaxillary gland, stomach, kidney, lung, muscle and skin. It is not easy to detect ABH substances in brain, uterus and testis. This variable detectability emanates from the differential

contents of blood group antigens among various tissues, as suggested by Hartmann (1941), Holborow et al. (1960) and Lill et al. (1979).

Moreover, many reports (Friedenrich and Hartmann; 1938, Hartmann, 1941; Glynn et al., 1959; Flory, 1966; Szulman, 1981) have shown that the contents of ABH substances in tissue cells are variable between secretors and non-secretors. This is understandable since mucus-secreting cells of secretors contain both alcohol soluble and water soluble substances but those of non-secretors contain water soluble antigens in much smaller amounts than do secretors. It is for this reason that the blood grouping of tissue cells of non-secretors, the mixed agglutination technique is generally suggested because of its sensitivity. At the same time in blood grouping technique, fixation by formalin solution or glutaraldehyde solution is needed for preventing blood group antigens from being eluted out of tissues (Yada, 1976; Mukoyama and Miyasaka, 1983).

Iseki (1962, 1977) discovered that in many bacteria there are enzymes which specifically decompose A,B,H, Le^a and Le^b substances. Jenkins et al. (1972), Pereira (1973) and Yuasa et al. (1977) conducted studies on the decomposition of blood group substances in soft tissues, especially muscles using the absorption elution technique and showed that muscle acquired blood group antigens different from the original one. Moreover, many bacteria possess antigens similar to human ABH antigens (Iseki, 1952, 1977; Springer, 1961). Therefore, it is apparent that in grouping decomposed tissue samples, especially the muscle, special care must be taken for evaluating the degree of decomposition and extent of contamination by bacteria, if any.

In the present study, satisfactory results could be obtained even after 8 months of storage at -20°C from heel scrapings belonging to both secretors and non-secretors. Mixed agglutination techniques proved to be more sensitive than absorption elution technique.

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