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A New Method in Criminology: Use of ELISA to Detect AFP on Different Materials with Monoclonal Anti-α-fetoprotein

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Summary. A new method has been introduced to distinguish normal adult serum stains from fetal or newborn serum and amniotic fluid stains with ELISA in cases of criminal abortion and infanticide. The method is based on the sensitive detection of α-fetoprotein (AFP) by a two-site enzyme immunoassay (EIA) following its elution with high efficiency from different materials (e.g., cotton, paper, synthetic fabric, or glass) by phosphate-buffered 0.5 *M* NaCl solution

Key words: Monoclonal antibody – ELISA technique – Fetal umbilical cord blood-stain – Amniotic fluid stain

Zusammenfassung: Es wurde eine Methode erarbeitet, um in Fällen von Abtreibung bzw. Kindestötung (Tötung des Neugeborenen) zwischen den vom Neugeborenen herrührenden biologischen Spuren (Blut- und Fruchtwasserflecke) und den von anderen Individuen stammenden Blutflecken unterscheiden zu können. Von der Oberfläche der verschiedenen Spurenträger (Baumwollstoff, Papier, Kunstfaser und Glas) wurden die eingetrockneten Flecke mit 0,5 *M* NaCl enthaltendem Phosphatpuffer eluiert und dann aus den Eluaten das Alpha-Fetoprotein mit monoklonalen Antikörpern mit Hilfe der ELISA-Technik nachgewiesen.

Schlüsselwörter: Monoklonale Antikörper – ELISA-Technik – Nabelblutflecke – Fruchtwasserflecke

In special cases of homicide, i.e., criminal abortion and infanticide, the demonstration of findings relating to the fetus or infant at the site of the criminal act is of great importance in medicolegal laboratory investigations. If the origin of a blood stain is unknown, the distinction between fetal umbilical cord serum or amniotic fluid and adult serum would provide and additional possibility for evidence in medicolegal practice.

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AFP is a glycoprotein that has been detected at very low concentration (10 ng/ml) in normal adult serum and in higher concentrations [1, 4, 5, 8] in maternal and fetal sera and amniotic fluid. It is synthesized by the yolk sac and fetal liver. After birth it decreases gradually, reaching the level of normal healthy adults with in 1 month [2, 6, 9]. In standard clinical practice measurement of the AFP level in maternal serum and in amniotic fluid is used for the detection of neural tube defects [3, 11].

Fetal and adult blood stains may be distinguished via the detection of AFP. To detect AFP from dried and eluted samples on different trace supports (e.g., cotton, paper, synthetic fabric or glass), we have used a sandwich ELISA technique [2, 10].

Reagents

The two monoclonal antibodies used for ELISA were described by Monostori et al. [7]. Anti-AFP-1 antibody was used to coat the plate. Anti-AFP-33 antibody was conjugated with horseradish peroxidase according to Wilson and Nakane [12]. The AFP standard was obtained from DAKO.

Material and Methods

Preparation and processing of samples: 50, 100, 500, and 1,000 µl fetal umbilical cord serum and blood, amniotic fluid, normal adult serum and whole blood were applied to different solid supports, such as cotton, paper, synthetic fabric, and glass. The samples were eluted for 1 h at room temperature with 1 ml phosphate-buffered 0.5 *M* NaCl at pH 7.2. The protein concentrations of the eluates were measured at 280 n*m* with a Spectromom 195 spectrophotometer. The extinction coefficient was $E_{280}^{1\,mg/ml}$ 1.65. Sandwich ELISA is based on the method of Uotila et al. [10]. Briefly, the wells of polystyrene microtiter plates (DYNATECH) were coated with 100 µl anti-AFP-1 monoclonal antibody solution (2.5 µg/ml) diluted with carbonate buffer of pH 9.6. The plates were incubated at 4° C overnight. After incubation, the wells were washed three times with phosphate-buffered saline of pH 7.2 containing 0.05% Tween 20 (PBS-Tween). One hundred microliters standard AFP solutions or eluate samples were added to the wells. The standards and sample dilutions were made in PBS-Tween buffer containing 0.5% bovine serum albumin (Sigma). After three washing steps and the addition of 100 µl anti-AFP-33 HRPO, a dilution of 1:2,500 was incubated in each well at 37°C for 1 h. After three washing steps, 200 µl substrate solution (20 mg o-phenylenediamine and 10 µl 30% H_2O_2 in 60 ml phosphate-citric acid buffer of pH 6.0) was added to each well. After a 30-min incubation at 37°C, the reaction was stopped with 50 µl 4 N H_2SO_4, and the absorbance was measured at 492 n*m* with a Gilford spectrophotometer.

Results

The dried samples could be stored at room temperature for at least 1 week without change. The concentrations of the recovered proteins were measured spectrophotometrically at 280 n*m*.

Figure 1 shows the amount of total protein in fetal umbilical cord serum (Fig. 1a), amniotic fluid (Fig. 1b) and adult serum (Fig. 1c), and in their eluates from cotton, paper, synthetic fabric, and glass supports. Of the total protein

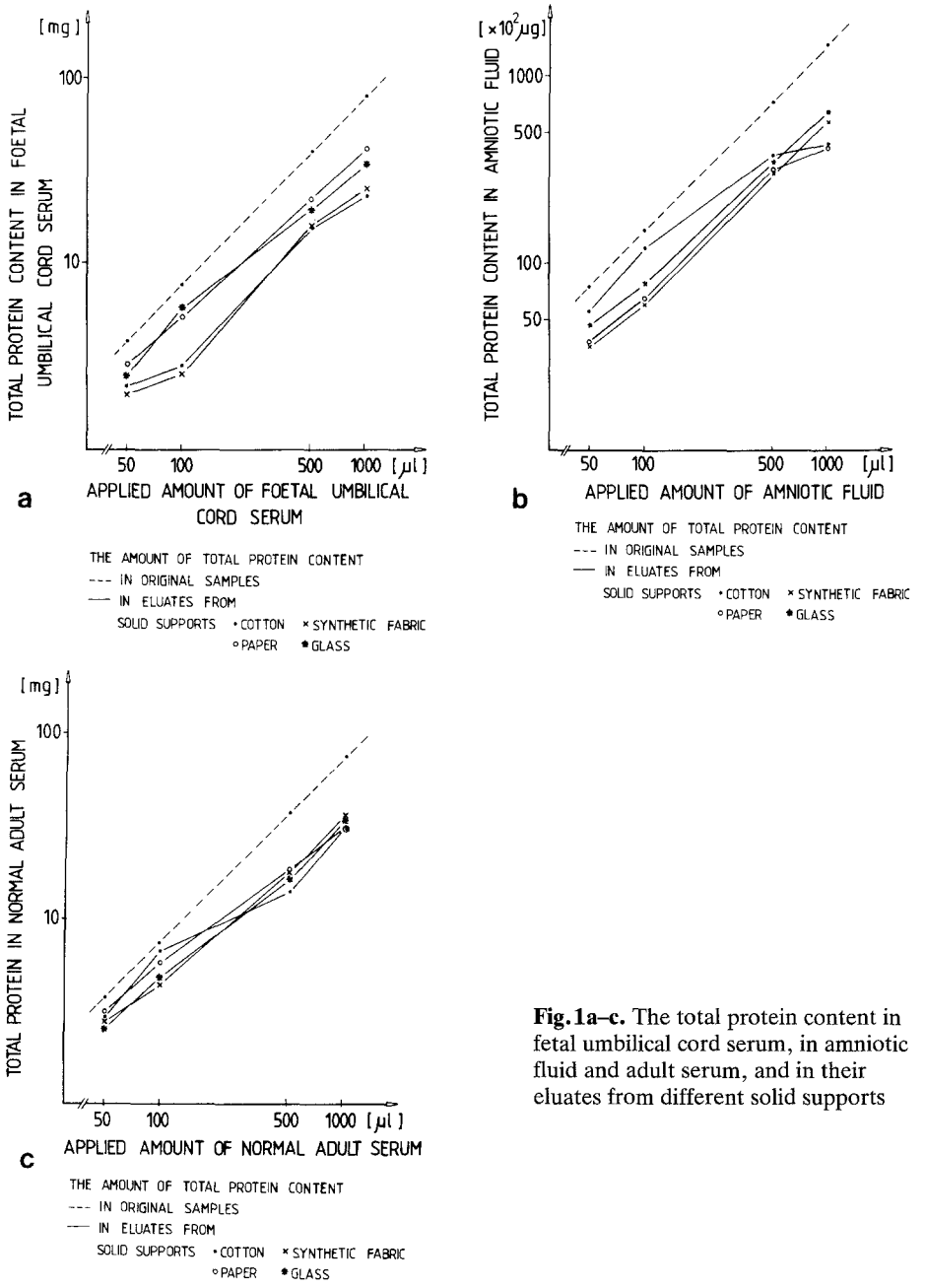

Fig. 1a–c. The total protein content in fetal umbilical cord serum, in amniotic fluid and adult serum, and in their eluates from different solid supports

content 30%–90% were eluted from the fetal and adult blood stains and amniotic fluid stains dried on cotton, paper, synthetic fabric, and glass supports. To calculate the unabsorbed and eluted AFP from the samples, AFP standards were used in the range of 1–100 ng/ml. Figure 2 shows the calibration curve for these AFP standards. The AFP contents of the original and eluted samples of fetal umbilical cord serum and amniotic fluid are presented in Fig. 3a and b. The

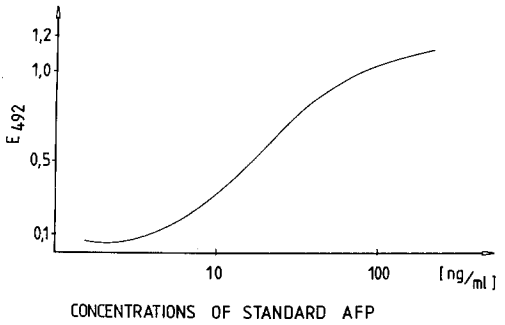

Fig. 2. Calibration curve to calculate the unabsorbed and eluted AFP from the samples with the ELISA method

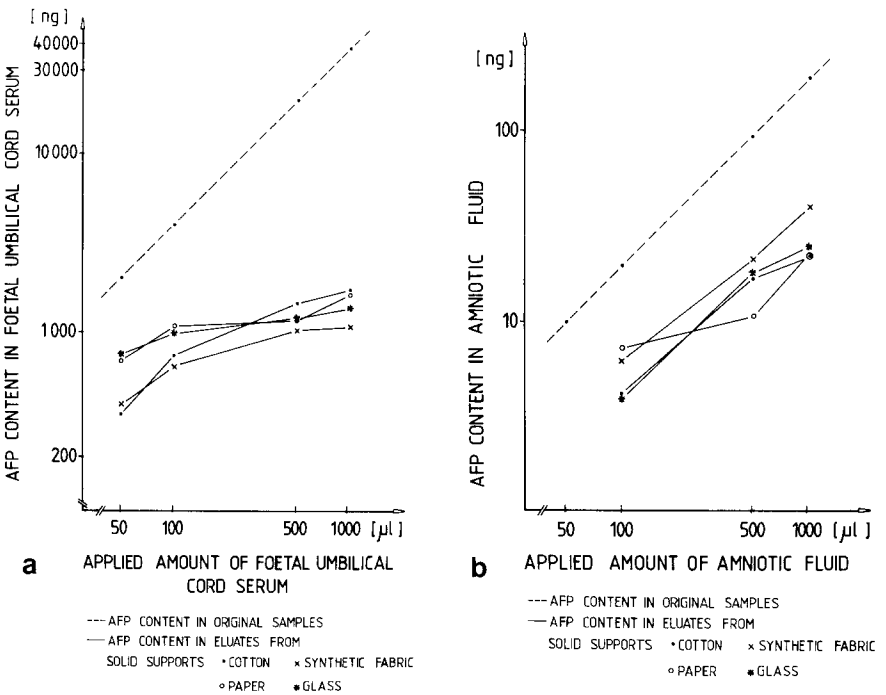

Fig. 3a, b. The AFP contents of the original and eluted samples of fetal umbilical cord serum and amniotic fluid

extent of AFP recovery from the solid supports was 10%–40%. Similar results were obtained when whole blood was used instead of serum (data not shown). There was no detectable AFP in either the original or the eluted normal adult serum and whole blood.

Discussion

In medicolegal practice, cases of criminal abortion and infanticide often necessitate the detection of traces relating to the fetus or the infant at the site of the criminal act and the distinction between fetal and adult blood-stains. We have developed a technique for the differentiation of fetal and adult dried serum and

amniotic fluid samples mimicking traces of a criminal event. The distinction is based on the detection of AFP. This is performed with two monoclonal anti-AFP antibodies, which react with two distinct antigenic determinants on the molecule. The advantages of using monoclonal antibodies are their strict specificity and high affinity, and the ready availability of standard antibodies, these properties comply with the requirements of medicolegal investigations. The blood, serum and amniotic fluid samples were dried on different solid supports. Of the total protein content 30%–90% and 10%–40% of the total AFP were eluted from the solid supports.

The sensitivity of the applied sandwich ELISA technique allows the quantitative determination of AFP from as little as 50 µl fetal blood and 100 µl amniotic fluid on a support, but there is no detectable AFP in the stains of adult blood or serum.

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Abbreviations

AFP, α-fetoprotein; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; PBS-Tween, phosphate-buffered saline containing 0.05% Tween 20; HRPO, horseradish peroxidase; anti-AFP-33 HRPO, antibody conjugated with horseradish peroxidase

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