TECHNICAL NOTE

Shirley A. Miller,[1] *MLT, ASCP, NCA; Clay M. Stern,*[1] *M.S.; and Moses S. Schanfield,*[1] *Ph.D.*

Simultaneous Subtyping of Group Specific Component and Esterase D by Isoelectric Focusing on Agarose Gels

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ABSTRACT: A quick, sensitive and economical technique has been developed to subtype GC and ESD simultaneously on the same agarose IEF gel. This method could be a useful tool for forensic application.

KEYWORDS: forensic science, group specific component, esterase D, immunoblotting, simultaneous subtyping, forensic application

A system that permits the typing of two genetic markers on the same agarose isoelectric focusing gel using a split gel method has been developed. One half of the gel is loaded with plasma or stain samples for subtyping of the vitamin D binding globulin, group specific component (GC). The other half of the gel is loaded with hemolysates for esterase D (ESD) subtyping. The run conditions have been optimized so that both systems focus in the allotted run time. After isoelectric focusing (IEF) has been completed, each half of the gel is then developed for its respective marker.

The modified gel formulation [*1*] consisted of 0.225 g IEF agarose (Pharmacia), 2.7 g sucrose, 0.16 g HEPES and 0.5 g MOPS in 20 mL H_2O. After boiling the agarose mixture and cooling to 75°C, 1.5 mL of Pharmalyte pH 4.5 to 5.4 (Pharmacia) was added and a 125 × 245 mm gel was cast onto GelBond Film (FMC) using the flap technique. Cathodal wick solution was 0.2N NaOH and anodal was $1MH_3PO_4$. GC sample wicks were 1 × 4 mm Whatman #1 filter paper saturated rated with a 1:5 dilution of plasma in 6 M urea. Stain samples were 2 × 4 mm wicks treated with a minimal amount of 6 M urea. ESD 4 × 4 mm wicks were saturated with hemolysate reduced with 2-mercaptoethanol. Both GC and ESD sample wicks were placed 3 cm from the cathodal wick.

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[1]Supervisor of DNA and Electrophoresis, Animal Forensic Specialist and Laboratory Director, respectively, Analytical Genetic Testing Center, Inc., Denver, CO.

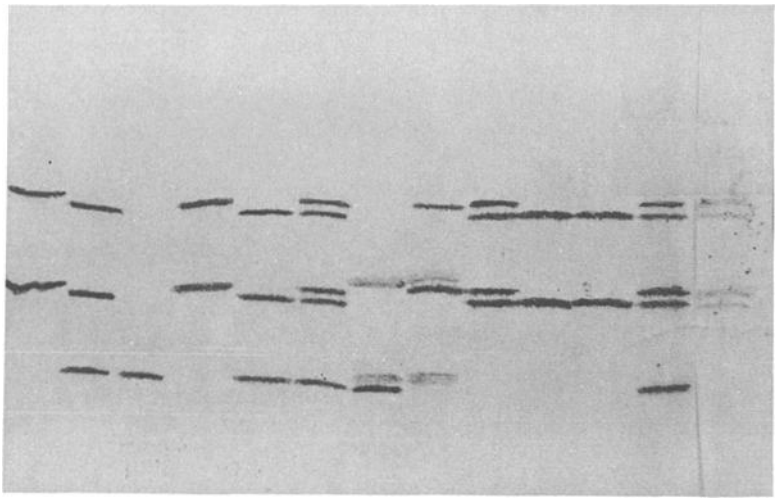

FIG. 1*a*—*From left to right are plasma samples: GC 1F, 1S2, 2, 1F, 1S2, 3 band control, 2-1C10, 1F-1C10, 1S1F, 1S, 1S, 3 band control, and 1S1F from a neat semen strain.*

An ICE chamber 1001 (E.C. Apparatus) was cooled to 9°C, power supply set to 2000 V, 20 mA and only the watts ramped as follows:

T 0	5 w	
T 15	8 w	Remove wicks
T 30	12 w	
T 45	15 w	
T 90	off	

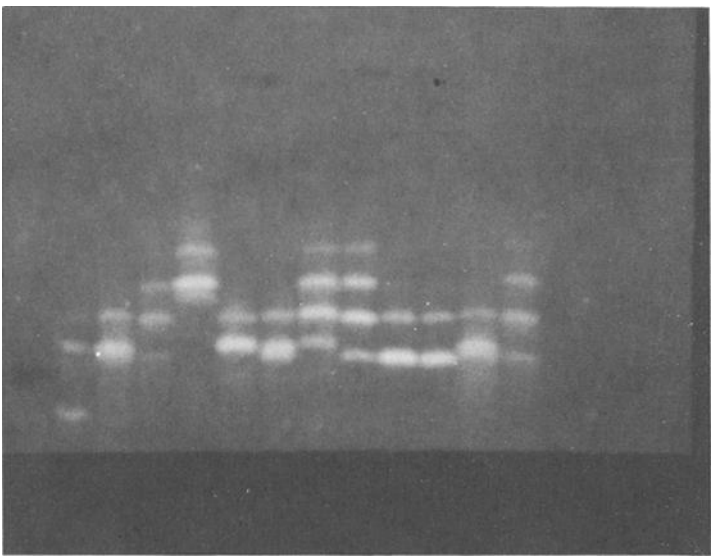

FIG. 1*b*—*From left to right are hemolysate samples: ESD 2-7, 1-2, 1-5, 5, 2, 1-2, 2-5, 1-5, 1, 1, 1-2, 1-5.*

After focusing, a sensitive immunoblotting method, as described in the literature [2], was performed on the GC half of the gel using a strip of Magna NT nylon membrane (MSI) and Uni-Block (AGTC) as blocking solution. Primary antibody was a 1:500 dilution of goat-anti-human GC (Incstar) in 0.5 × Uni-Block and secondary antibody was a 1:1000 dilution of rabbit-anti-goat immunoglobulin ALP conjugate (Incstar) in 0.5 × Uni-Block. Bands were detected using a BCIP / NBT substrate mixture (AGTC). The ESD half of the gel was developed using the conventional 4-methylumbelliferyl acetate filter paper overlay [3].

Figures 1*a* and 1*b* show band patterns obtained for GC and ESD on a simultaneous run.

This split gel technique has proven to be a quick, economical and sensitive way to subtype forensic evidence for two systems simultaneously. The immunoblot method uses approximately 20-fold less antibody and is significantly more sensitive than the immunoprint method as cited elsewhere [1]. GC has been detected in neat semen stains using this method. This entire procedure can be completed in one day.

References

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Address requests for reprints or additional information to
Shirley A. Miller
Analytical Genetic Testing Center, Inc.
7808 Cherry Creek South Drive #201
Denver, CO 80231