TECHNICAL NOTE

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An Improved High Resolution Single Method for Orosomucoid ORM1 and ORM2 Phenotyping

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ABSTRACT: An improved high resolution single method for orosomucoid ORM1 and ORM2 phenotyping using isoelectric focusing (IEF) in wide-scales ultrathin layer polyacrylamide gels of pH range 4.2 to 4.9 is presented. The method is reliable, simple, and provides an alternative for the three currently required ones for typing this genetic system.

KEYWORDS: forensic science, plasma protein, isoelectric focusing (IEF), genetic typing, orosomucoid (ORM1/ORM2), human identification

With the marked progress in the DNA technology, it is still necessary to use the conventional genetic markers in paternity testing and other cases require individuals identification in combination with the applied DNA systems.

Orosomucoid (ORM), also known as α_1-acid glycoprotein, is a plasma protein with molecular weight of 40,000 and the concentration of this protein is ranged between 0.36 to 1.46 mg/mL (1). Earlier studies on the genetic polymorphism of desialyzed ORM demonstrated three common electrophoretic patterns which are classified as a product of two codominant alleles at a single autosomal locus (2,3). Application of isoelectric focusing (IEF) to ORM phenotyping has revealed more complexity than expected in the initial studies, and the pattern obtained is explained in terms of two gene loci, ORM1 and ORM2, which are closely linked (4,5). The gene products of ORM1 and ORM2 loci are present in a molar ratio of 3:1, respectively (6). Subsequent investigations have shown that ORM is a very highly polymorphic plasma protein with more than 57 electrophoretic variants including four common alleles at the ORM1 locus; ORM1*F1, ORM1*F2, ORM1*S, and ORM1*dF1S, and one common allele at ORM2 locus, ORM2*M, are currently known, and this genetic system proved to be a very useful marker for paternity testing and other forensic cases as it is also detectable in blood stains, genetic linkage studies, and anthropological investigations (5,7–15). However, this system is still not widely used as the detection of the different genetic variants needs application of three electrofocusing methods, on the basis that the duplicated ORM1*dF1S as a heterozygote for ORM1*F1 or ORM1*S is detectable only if gels contain Triton X-100 (TX-100) used, whereas the ORM1*F2 is indistinguishable by this method and it is necessary to apply additional run in gels without TX-100. Besides, a third method for identification of the variants which are not detectable by the first two methods (9,10–14), which is time, labor, and money consuming.

This communication presents a single reliable, high resolution and rapid method using IEF in wide-scales ultrathin layer polyacrylamide gels for identification of different ORM1 and ORM2 variants.

Materials and Methods

Samples

Serum or plasma can be used. Pretreatment of the samples was carried out as described elsewhere (10).

IEF

Ultrathin layer polyacrylamide gels of 12 cm electrodes distance and 0.2 cm thickness, containing 2% pharmalyte pH range 4.2 to 4.9 (Pharmacia, Uppsala), and 12.5% sucrose, with gel concentration (T) = 5% and degree of cross linkage (C) = 6%, were photopolymerized using riboflavin and UV light. The electrode solutions were 1 M sodium hydroxide for the cathode and 1 M phosphoric acid for the anode. The power unit was adjusted to supply an initial voltage of 500 V and maximum voltage of 1200 V. The gel was prefocused for 30 min, then 5 μL of neuraminidase treated samples were applied at a distance of 1 cm from the cathodal electrode strip using 6 by 2 mm paper strips. The paper strips were removed after 30 min and the total time was 180 min at 2°C.

Immunofixation

Print immunofixation was performed using cellulose acetate membrane soaked in five times diluted monospecific ORM antiserum (DAKO). The membrane was placed in contact with the surface of the gel for 3 min at room temperature, then removed

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and washed with saline three times for 10 min each time, then stained with Acid Violet 49 for 3 to 4 min.

Results and Discussion

Figure 1 shows the band pattern of several ORM phenotypes including the ORM1 dFIS-F1, ORM1 F1-F2, and ORM1 F1-B10 on the same gel, which was previously required applying at least two different electrofocusing methods (9,11–14). Diagramatic representation of the common ORM1 and ORM2 phenotypes is given in Fig. 2. By the present method, the variant ORM2*H2 was located cathodal to ORM1*F2 as shown in Fig. 3 (lane 1), and its detectability became easier when compared with gels contain TX-100 where the ORM2*H2 is located in the narrow corridor between the ORM1*S and ORM2*M. To test for the reliability of our method, more than 850 samples; plasma and serum, which included 19 ORM alleles and 28 phenotypes were tested by previously recommended methods (13), then retyped by the present procedure, results were identical for all the sample, and the heterozygote phenotype ORM1 B10-S could be resolved into two clear bands by the present method as shown in Fig. 3 (lane 3), if compared with a single thick band by other methods. Besides, the new allele ORM2*H21 (Fig. 3, lane 5) was not always identifiable when it occurs in conjunction with the ORM1*S allele in the gels containing TX-100. These results confirmed that the present method had superior resolution not only with common alleles, but also with variants, even those which are difficult to detect by the other methods.

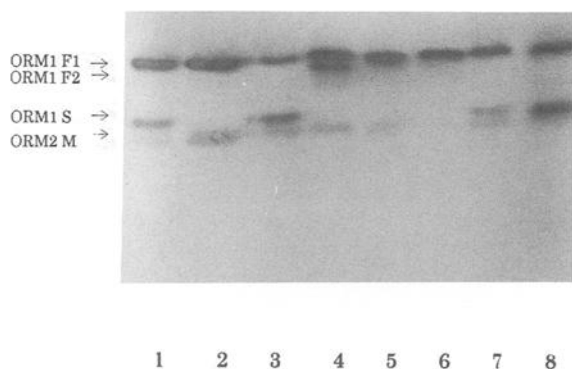

FIG. 1—*Immunoprinted band patterns of several ORM phenotypes. Lanes 1,7 = ORM1 dFIS-F1/ORM2 M; lanes 2,5,6 = ORM1 F1/ORM2 M; lane 3 = ORM1 F1-S/ORM2 M; lane 4 = ORM1 F1-F2/ORM2 M; and lane 8 = ORM1 F1-B10/ORM2 M. Anode is at the top.*

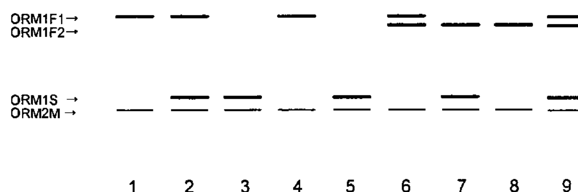

FIG. 2—*Diagrammatic representation of the common ORM1 and ORM2 phenotypes. Lane 1 = ORM1 F1/ORM2 M; lane 2 = ORM1 F1-S/ORM2 M; lane 3 = ORM1 S/ORM2 M; lane 4 = ORM1 dF1S-F1/ORM2 M; lane 5 = ORM1 dF1S-S/ORM2 M; lane 6 = ORM1 F1-F2/ORM2 M; lane 7 = ORM1 F2-S/ORM2 M; lane 8 = ORM1 F2/ORM2 M and lane 9 = ORM1 dF1S-F2/ORM2 M. Anode is at the top.*

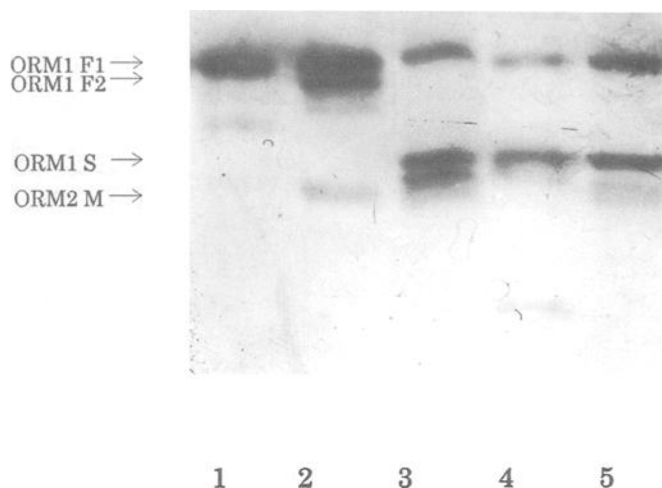

FIG. 3—*Immunoprinted band patterns of several ORM phenotypes. Lane 1 = ORM1 F1/ORM2 H2; lane 2 ORM1 F1-F2/ORM2 M; lane 3 = ORM1 dF1S-B10; lane 4 = ORM1 F1-S/ORM2 M-H10, and lane 5 = ORM1 F1-S/ORM2 M-H21. Anode is at the top. Triangles indicate the ORM variants.*

There are several precautions which should be taken in consideration to avoid missing of the duplicated ORM1*dF1S as its detectability depends upon the difference in the staining intensity of the two obtained bands: a) 5 μL of the neuraminadase treated plasma or serum will be sufficient, b) Dilution of the ORM antisera should be within 5 to 6 times, and c) Time of immunofixation should be limited to 3 to 4 min, with similar staining time.

In conclusion, the presented method offers reliability, superior resolution, and economic and labor-saving advantages over the three currently recommended ones.

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