

ABO Blood Grouping of Old Blood Stains by Ultra Micro Reverse Agglutination with Monoclonal Anti-A, -B Antibodies

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Introduction

In the practice of forensic medicine, especially in the case of identification, it is very important that the personal identification include the collection of bloodstains and blood grouping. Blood grouping from old bloodstains as well as hemolytic blood is a very complicated process and is difficult to identify. The authors have already introduced the use of a simple, practical methods by ultra micro passive agglutination with monoclonal anti-A,-B,-H antibodies for the ABO blood grouping of hemolytic blood. The old bloodstains were collected through the months of October and November of 1987, during the medico-legal autopsy. The blood type was identified, and then, the blood was spread out on to filter paper. These were stored for five years at room temperature. Recently, the gelatin particles were presented to us by Fuji Rebio Inc. Three types of gelatin particles were investigated to the comparison of specificities under the same condition with one known-identity.

Materials and Methods

1. Materials.

1) Gelatin particles; Three types of gelatin particles of previously unknown-identity were used, along with one known type*, totaling four types, these are mentioned below.

	Electrophoretic ratio	Particle diameter
Lot.082	0.763 $\mu\text{m}/\text{cm}/\text{sec}/\text{v}/\text{cm}$	3.09 μm
Lot.088	0.751 $\mu\text{m}/\text{cm}/\text{sec}/\text{v}/\text{cm}$	3.04 μm
Lot.091	0.739 $\mu\text{m}/\text{cm}/\text{sec}/\text{v}/\text{cm}$	3.05 μm
Lot.042*	0.730 $\mu\text{m}/\text{cm}/\text{sec}/\text{v}/\text{cm}$	3.75 μm

These are artificial particle carrier, that construct from soluble polysaccharide include gelatin and metaphosphoric acid, are treated to bridging and unsolvability.

2) Monoclonal antibodies:

Anti-A antibody ; agglutination titer is x256 or more(saline)

Seraclone Anti-A (mouse) Lot.790581 Biotest AG

Anti-B antibody ; agglutination titer is x256 or more(saline)

Seraclone Anti-B (mouse) Lot.790591 Biotest AG

3) Samples: Bloodstains that were collected during the medico-legal autopsy, a through of October and November of 1987 were spread on the filter paper, dried at room temperature, then stored in individual envelopes marked with autopsy number and blood types.

2. Methods.

1) Treatment of tanned acid to gelatin particles.

The medium is 0.15 mol, pH.6.5 Phosphate Buffered Saline include 30 ppm tanned acid, this is optimum condition for treatment of tanned acid to the particles at this investigation.

The gelatin particles is suspended with pH.6.5, P.B.S. to 1.25 v/v%, and added equal volume of the above tanned acid solution. Stored at 37 °C, 30 min. in water bath. Then, wash with cold saline at 1 time, and after, this tanned acid treated particles is resuspended with the same P.B.S. to 1.25v/v%.

2) Sensitization of monoclonal anti-A,-B antibodies.

To begin with divide in three of the above tanned acid treated gelatin particles suspension.

The first divided suspension is sensitized with monoclonal anti-A antibody.

The second divided suspension is sensitized with monoclonal anti-B antibody.

The third divided suspension is not sensitized for negative controls. The first and second suspension, severally suspension is mix with equal volume Anti-A or Anti-B antibody. Each mixed suspension is stored 37 °C, 1 hour in water bath, sometimes shake to mixture. And after that, the each sensitized gelatin particle suspension is wash with cold saline at 2 times to eliminate of surplus antibody. Then, each sensitized suspension is resuspended with reaction medium, that is composed of 0.15 mol, pH.7.2, Phosphate Buffered Saline include 1.0 % Bovine Serum Albumin, 0.01 % Tween 80 and 0.01 % NaN_3 as an antiseptic.

3) Prepare of sample

In this paper, ABO blood groups of old bloodstains on filter paper was performed by the two methods.

In one method, each sample was cut to a suitable size, and fixed with methanol on a slide glass. Then, the fixed sample is scratched by a needle like a pin, and got a some fine fibrils of old bloodstains.

In other method, A, B and O(H) antigens were transferred from old bloodstains on the filter papers to wet gauze by rubbing. The gauze was fixed with methanol and unbound' by a needle. So that got a some fine fibrils.

4) Reaction

1. Fill the each holes of Terasaki's microplate with the correct amount of liquid paraffin. We shows the Lot. No. of gelatin particles, and was sensitized with monoclonal anti-A antibody at the upper row and anti-B at under row in the Table 1 and 2. Then, each sample put into the liquid paraffin of the each holes to vertical line as the Tables.

2. Each 3 μl of the sensitized gelatin particle suspension add into the hole, already include the sample, to horizontal line as the Tables, by Terasaki's dispenser.

These are mixed during about 10 minutes by micromixer and reacted at room temperature, and after, the agglutination read under a substance microscopy.

Results and Discussion

1. The result of ABO blood grouping from old bloodstains spread on the filter papers were shown in Table 1.

These were weaker reaction than Table 2. The gelatin particles suspension of Lot. No.082 and 088 were not reacted. These are caused by not difference of the particle diameter but the electric ratio, because it is same condition, that was treated with tanned acid and sensitized with antibody concentrations. Lot.No.091 and already used particles suspension were well specific reaction.

2. The result of A, B and O(H) antigens were transferred from old bloodstains to wet gauze by rubbing were shown in Table 2.

This method is most suitable for the practice of forensic medicine. Accordingly, either fresh or old detained bloodstains were simply collected and identified by this method at the scene.

The authors were identified the ABO blood groups from old bloodstains, that were collected five years ago. It is used very simple and useful method, that is developed and invented by one of the authors. The method is ultra micro reverse passive agglutination. In generally, the method of ABO groups identity from old bloodstains is used by heat elution technique. However, this technique is simple but need some hours. Use the method of this investigation, that was successfully performed in more short time and could be detected correctly and simply.

Table 1 ABO Blood Grouping from Old Bloodstains Spread on the Filter Papers

	A Group	B Group	AB Group	O Group
082-Anti A	-	-	-	-
082-Anti B	-	-	-	-
088-Anti A	-	-	-	-
088-Anti B	-	-	-	-
091-Anti A	+	-	+	-
091-Anti B	-	+	+	-
042-Anti A	+	-	+	-
042-Anti B	-	+	+	-

Table 2 Old Bloodstains were Transferred from Filter Paper to Wet Gauze by Rubbing

	A Group	B Group	AB Group	O Group
082-Anti A	-	-	-	-
082-Anti B	-	-	-	-
088-Anti A	-	-	-	-
088-Anti B	-	-	-	-
091-Anti A	++	-	++	-
091-Anti B	-	++	++	-
042-Anti A	++	-	++	-
042-Anti B	-	++	++	-

Conclusion

The results obtained in this study show that the method of ultra micro reverse passive agglutination was successfully performed in a short time and could be detected correctly and simply in comparison with others.

Reference

H.Kashiwade and Y.Sato : ABO Blood Grouping of Hemolytic Blood by Reverse Passive Agglutination with Monoclonal Anti-A,-B,-H Antibodies. Res. Pract. Forensic Med. 33:27-30(1990)