

Study of Rapid Determination of ABO Blood Group in Bloodstains by the Improved Dot-ELISA with Monoclonal Antibodies

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- In this paper, a determination of ABH blood group substances in bloodstains by the improved Dot-ELISA with horseradish peroxidase coupled monoclonal antibodies is described. The entire test can be performed in about 30 minutes by this method. This method is more simple, sensitive, correct and inexpensive than conventional methods and needs no complicated equipments. 1:1 dilution of bloodstains as low as 3200 fold (or 150 ng bloodstained cottonthread) is sufficient for detection of ABO blood groups by this method. ABH blood group substances from the bloodstains aged up to 2 years can be detected. The key of this method is an extraction reagent we prepared, which can rapidly dissolve ABH substances from red cell membranes without changing the activity of these antigens.

Key Words: ABO blood grouping, Bloodstains, Dot-ELISA, Monoclonal antibody, Extraction reagent

Bloodstain samples is one of the most important forensic biologic evidence. Determination of ABO blood group substances in bloodstains is a standard laboratory procedure. For a long time, methods of determination of ABO blood group were mainly the absorption and elution-absorption tests. These conventional methods are either time-consuming or requiring special equipment and are also cumbersome. It is difficult that these methods are used in the scene of crime.

Based on the method, we established for determination of ABO blood group in body fluids, in 1992. Now again we have established a method for determination of ABO blood group in bloodstains. Determination of ABH blood group substances by this method has been used in practical cases. This method is rapid, simple, sensitive and accurate. It produces clear results, and requires no equipment.

Materials and Methods

Materials

1. ABH enzyme-labeled monoclonal antibodies: produced by this institute
2. Extraction reagent: XT-100, NH_4OH , NaOH , PBS, Tris-HCl etc.
3. Substrate solution: 4-chloro-1-naphthol, 3, 3'-diaminobenzidine (DAB), 30% hydrogen peroxide
4. Nitrocellulose Paper: purchased from Beijing chemical-works School
5. Blocking solution: bovine serum albumin (BSA)
6. Washing buffer: 0.2M pH 7.4 phosphate buffered saline (PBS)
7. Bloodstain samples aged from 1 month up to 5 years

Dot-ELISA for determination of ABO blood group in bloodstains

1. Sample treatment: Bloodstain samples are soaked in a variety of extraction reagents, respectively.
2. Dot samples: About 1 μl extract were applied on three pieces of nitrocellulose paper, dried at room temperature.
3. Washing: put the papers in PBS-T and wash it two times for about two min/time.
4. H_2O_2 treatment: Soak the papers in 3% H_2O_2 for 1-10 min.
5. Washing: Repeat the washing (see step 3).

6. Blocking: Soak the papers in 1-2% BSA for 5 to 10 min.
7. Incubation: put the papers into the appropriate diluted (with blocking solution) enzyme-labeled monoclonal antibodies solution (A. B. H) for 5 to 60 min, respectively.
8. Washing: Repeat the washing (see step 3)
9. Development: soak the papers in developing solution for about 1 min.
10. Cease reaction: Development reaction was terminated by washing the paper in water, dried at room temperature.
11. Observe the result: If a visible blue brown color appears on the papers, the Ag-Ab reaction has occurred, if no color changes, no Ag-Ab reaction has occurred.

Selection of extract reagent

These reagents such as XT-100, NH_4OH , NaOH , PBS Tris-HCl etc. were made up into more than 10 extract reagents. Bloodstain samples were treated with these reagents respectively for determination of ABO blood group.

Selection of enzyme-labeled antibodies

Monoclonal anti-A antibodies from 3 cell lines, monoclonal anti-B antibodies from 3 cell lines, and monoclonal anti-H antibodies from 2 cell lines, were purified respectively and labeled with horseradish peroxidase (HRP) for determination of ABO blood group.

Blind study and sensitivity Test

200 samples were tested by Dot-ELISA. Their results were compared with known ABO blood group in bloodstains.

Sensitive test: bloodstains are determined by the Dot-ELISA at different dilutions.

Result

Dot-ELISA method for ABO blood group in bloodstains.

1. samples treatment: Bloodstain samples soak in extraction reagent.
2. Dot samples: About 1 μl extract was applied on three pieces of nitrocellulose paper, dried at room temperature.
3. Washing: put the papers in PBS-T and wash it two times for about two min/time.
4. H_2O_2 treatment: Soak the papers in 3% H_2O_2 for 3 min.
5. Washing: Repeat the washing (see step 3).
6. Blocking: Soak the papers in 1-2% BSA for 5 to 10 min.
7. Incubation: put the papers into the appropriate diluted (with blocking solution) enzyme-labeled monoclonal antibodies solution (A. B. H) for 10 to 20 min, respectively.
8. Washing: Repeat the washing (see step 3).
9. Development: Soak the papers in developing solution for about 1 min.
10. Cease reaction: Development reaction was terminated by washing the paper in

water, dried at room temperature.

11. Observe the result: If a visible blue brown color appears on the paper, the Ag-Ab reaction has occurred, if no color changes, no Ag-Ab reaction has occurred.

Selection of Extraction reagents

We select an extraction reagent that can rapidly dissolve ABH substances from red cell membrane without changing the activity of these antigens.

Selection of enzyme-labeled antibodies

Antibody that is IgG class can be used in determination of ABO blood group in bloodstains by Dot-ELISA. Antibody that is IgM class can not be used in determination of ABO blood group in bloodstains by Dot-ELISA.

Blind study and sensitivity test

200 samples were tested by Dot-ELISA. Their results are the same as known blood group.

Sensitivity test: 1 μ l extract of bloodstain diluted 3200 fold could still be correctly typed by Dot-ELISA. This amount corresponds to 150 ng bloodstain cotton thread. The color of the reaction becomes weaker and weaker as bloodstain extract concentration decreases.

Discussion

In 1987, J. Michael Cecka etc described determination of ABO blood group in bloodstains by Dot-ELISA. In 1992, Nu Ruiguang etc directly labeled the enzyme onto the anti-A, anti-B, and anti-H monoclonal antibody for ABO blood group by Dot-ELISA. The latter, sample treatment is time-consuming and requires special equipment. Extraction reagent we prepared can rapidly dissolve ABH substances from red cell membrane without changing the activity of these antigens. Sample treatment using the extraction reagent requires no special equipment. In the meantime, this method for determination of ABO blood group in bloodstains is the most sensitive, and takes only about 30 min. The extract reagent treatment takes time longer for the bloodstain aged up to 1.5 years.

Reference

1. J. Michael Cecka, et al. Direct Blood Group Typing of Forensic Samples Using A Simple Monoclonal Antibody Assay. *Forensic Science International*, 1987; 34: 205.
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