Acidification of plasma for detection of pH-dependent antibodies

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Most antibodies to blood group antigens react optimally at a pH range of 6.5–7.5, similar to that of normal plasma or serum. Some antibodies, however, including anti-M, react preferentially or exclusively in an acidic environment with a pH below 6.5. Antibodies with anti-M specificity often show dosage. They can be weakly reactive and even look like nonspecific reactivity at the antihuman globulin phase especially when immediate spin and/or room temperature testing is not part of routine screening. Acidification of serum or plasma may help to identify these antibodies as clinically insignificant versus as an unidentified antibody for which clinical significance is unknown. ***Immunohematology* 2019;35:116–118.**

Key Words: Acidification, pH enhanced, antibody detection, anti-M

Reagents/Supplies

Reagents	Supplies
▪ Plasma or serum containing antibody of interest ▪ 0.2 N HCL (1.0 N HCL diluted with distilled H_2O) ▪ Isotonic saline ▪ Reagent RBCs ▪ Anti-IgG (optional) ▪ IgG–coated RBCs (optional)	▪ Test tubes ▪ pH meter or pH strips ▪ Transfer pipettes ▪ Centrifuge ▪ Cell washer ▪ Agglutination viewer ▪ Microscope ▪ 37°C incubator or water bath ▪ 4°C refrigerator or ice bath

HCL = hydrochloric acid; RBCs = red blood cells.

Procedural Steps

1. Mix one volume of 0.2 N HCL with nine volumes of plasma (or serum) for each sample to be tested.
2. Verify that the pH of each sample is between 6.0 and 6.5 with a pH meter or pH paper.
3. Select RBCs for testing; wash RBCs at least one time with isotonic saline.
4. Prepare a 3–4 percent RBC suspension in isotonic saline.
5. Add one drop of prepared RBCs to two drops of plasma to a properly labeled test tube.
6. Incubate for a time and temperature of interest.
7. Centrifuge for 15 seconds at 3400 rpm (or as otherwise indicated).
8. Examine tube for agglutination.
9. Indirect antiglobulin testing (IAT) may be included, if desired, using standard methods.

HCL = hydrochloric acid; RBCs = red blood cells;
PBS = phosphate-buffered saline.

Principle

The MNS blood group system antigens are structurally similar polypeptides that transverse the cell membrane once with a carboxyl cytoplasmic region and an extracellular amino terminal domain.[1,2] The extracellular domain of both molecules has many sialic acid–rich O-glycans. The majority of these O-linked carbohydrate structures are branched tetrasaccharides containing one *N*-acetylgalactosamine (GalNAc), one galactose (Gal), and two NeuNAc (sialic acid).[3] The culmination of the carboxyl group charges of these sialic acids results in a negatively charged red blood cell (RBC) membrane.[4] The pH dependency or enhancement of some antibodies with M specificity may be due to the charged carboxyl groups present on the glycophorin A that carry the M antigen.[5]

The first naturally occurring pH-dependent anti-M was discovered in 1964 when a saline agglutinin was present in a donor's acid citrate dextrose plasma that was not demonstrable in the serum from the same donation. After screening more than 1000 random NN donors, Kathryn M. Beattie and Wolf W. Zuelzer found an additional 23 examples of this pH-dependent anti-M. The data presented by Beattie and Zuelzer support the hypothesis that the acidification of the serum, and therefore the addition of hydrogen ions, activates or enhances the charge of the antibody molecule facilitating agglutination. Subsequent alkalization and a decrease in hydrogen ions result in the reduction of the antibody charge and the disassociation of the antigen–antibody complex.[6]

Indications

Most antibodies to blood group antigens react optimally at a pH range of 6.5–7.5, similar to that of normal plasma

or serum. Yet some antibodies, including anti-M, react preferentially or exclusively in an acidic pH below 6.5. Anti-M is generally considered insignificant and not causative of decreased RBC survival or hemolytic disease of the fetus and newborn (HDFN).[2] However, 50–80 percent of these antibodies are IgG or have an IgG component that is capable of direct agglutination.[7,8] Antibodies reactive at 37°C and/or the antihuman globulin (AHG) phase may be IgM, IgG, or both and should be considered clinically significant unless testing to confirm significance is performed. Although rare, anti-M has the potential to cause HDFN if present in maternal plasma as IgG.[9]

Limitations

The acidification of plasma (or serum) to detect pH-dependent antibodies should only be used in conjunction with approved routine blood bank methods and procedures for the screening and/or identification of RBC antibodies. This procedure is designed to enhance or detect pH-dependent antibodies and serves only as one of many tools to aid in resolution of routine and/or complex serologic problems.

As with all serologic procedures, failure to obtain the correct or expected results may be caused by contamination or improper use and storage of reagents. In addition, failure to follow the procedure, the use of incorrect incubation temperature or time, improper centrifugation time or rpm, and failure to add samples or reagents will all result in invalid results. Specific limitations for this procedure include failure to use the proper concentration of hydrochloric acid (0.2 N HCL), improper washing of test RBCs, not adjusting the pH of the test samples properly, and the resuspension of RBCs in phosphate-buffered saline (PBS) rather than isotonic saline. Note: PBS can buffer the acidified plasma and should not be used.

Quality Control

Routine blood bank daily quality control should be followed depending on the type of testing for which the acidified plasma will be used. The RBCs used for testing should be confirmed to be M+N– and M+N+, if testing for dosage. An M– RBC should be considered if reactivity is present in all samples. The pH of the acidified plasma should be checked with a pH meter or pH paper (litmus paper) and should be between 6.0 and 6.5.

Summary/Conclusions

Antibodies with M specificity often show dosage. In addition, they can be weak and look like nonspecific reactivity at the AHG phase, especially when immediate spin or room temperature testing is not part of routine antibody detection or identification. Anti-M is usually clinically insignificant if reactivity is confined to low temperatures and not present at 37°C or at the AHG phase. However, identifying an anti-M reacting only at cooler temperatures may be important for patients undergoing procedures that induce hypothermia, including some cardiac surgeries. Additionally, identifying weak examples of anti-M, even those typically considered insignificant, might be beneficial for patients in sickle cell crisis who have a history of hyperhemolysis.[5] In these cases, it may be desired to provide M– RBC units to prevent further immunization or exacerbation of hemolysis.

Acidification of plasma to detect pH-dependent antibodies seems to be used to detect naturally occurring cold-reacting pH-dependent M antibodies. Still, for some transfusion-dependent known antibody producers susceptible to hemolytic transfusion reactions, this procedure and the identification of these weak latent antibodies may be beneficial.

Like anti-M, anti-i present in cold hemagglutinin disease[10] and anti-Pr can show pH dependency[3,5] but are more likely to be clinically significant. Other antibodies usually considered clinically significant have also been reported, including anti-s[2] and anti-f.[11] Acidification of serum may aid in identification of these antibodies. The pH dependency of some antibodies may explain discrepant reactivity sometimes seen between fresh plasma and serum samples from the same patient or between antibody detection reagents. Little research has been done to determine the clinical significance of these pH-dependent antibodies and, because this procedure is rarely used, it is difficult to say how many pH-dependent antibodies remain undiscovered.

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