A Comparative Study of Cross Match With and Without Centrifugation in Covid-19 Positive Blood Samples in Blood Bank of a Tertiary Care Center in North India

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ABSTRACT
Centrifugation is a frequently employed technique in transfusion medicine used for compatibility testing, ABO and Rh typing and cross-matching. However, there is considerable evidence that it can lead to the generation of aerosol particles of size up to 5 μm. If the fluid being processed contains an infectious micro-organism like 2019 novel coronavirus (2019-nCoV), there could be a hazard due to the aerosols in the laboratory with particles capable of penetrating and being retained in the lower respiratory tract. Considering this, we wanted to determine if reliable cross-match testing can be done without the use of a centrifuge and if there are safer alternative testing methodologies available to perform a compatibility test. This was a cross-sectional study that included blood samples received in the blood bank along with requisition of blood components from 30 patients with coronavirus disease who tested 2019-nCoV positive by PCR and were admitted at BPS GMC, Sonepat and SGT Medical College Gurgaon from May 2020 to June 2020. Of the 30 samples processed, ten samples were subjected to cross-match by Immediate Spin Cross Match Method. Rest 20 samples were cross-matched by Saline Cross Match Method. However, in comparison to a standard saline cross-match where the incubation period is generally of 30 min, a prolonged incubation period of 45 min was adopted followed by addition of 22% Albumin. After that, the sample was observed for hemolysis and agglutination. The results obtained from both the methods were noted, compared and statistically analysed.

INTRODUCTION
Centrifugation is the use of the centrifugal forces generated in a spinning rotor to separate biological particles, such as cells, viruses, subcellular organelles, macromolecules (principally proteins and nucleic acids) and macromolecular complexes (such as ribonucleoproteins and lipoproteins) (Rickwood and Graham, 2015). It is one of the most useful and frequently employed techniques in transfusion medicine where it is used for ABO and Rh typing and cross-matching.
Red cells have a net negative charge at their surface and therefore repel one another. Negatively charged molecules on the red cell membrane cause mutual repulsion of red cells. This repulsion may be decreased by various laboratory manipulations and by inherent or altered red cell membrane characteristics.

Multiple strategies are used to overcome this repulsion and to enhance agglutination. Centrifugation physically forces the cells closer together (Funk et al., 2014). Some of the most commonly used methods which employ centrifugation during compatibility testing and cross match are the conventional spin tube method, indirect antiglobulin test (IAT), solid-phase red cell adherence tests (Rollih et al., 1985), automated column agglutination technology (Walker, 1997) and automated testing platforms.

While these allow for simultaneous performance of several criteria, the decreased workload on lab personnel, reduced sample volume required for testing and rapid results, they have one potential severe disadvantage. There is considerable evidence (Stern et al., 1974; Pottage et al., 2014; Bennett and Parks, 2006) that manipulation of body fluids in various laboratory procedures, one of them being centrifugation, can generate airborne particles.

If the fluid being processed contains an infectious micro-organism like 2019 novel coronavirus (2019-nCoV), there could be a hazard due to the aerosols in the laboratory (Harper, 1981). The average diameter of the virus particles is around 125 nm (.125 μm), where the diameter of the envelope is 85 nm, and the spikes are 20 nm long (Neuman et al., 2006).

In a study conducted by Harper (1981), estimates were made of the size distribution of the airborne particles generated by cell washing centrifuges. Results showed that sizable proportions of these particles were below five μm in diameter and therefore in size range capable of penetrating and being retained in the lower respiratory tract.

Use of centrifugation techniques poses considerable risk to the health of lab technicians working in the blood transfusion department. Considering this, we wanted to determine if reliable cross-match testing can be done without the use of a centrifuge and if there are safer and cost-effective, alternative testing methodologies available to perform a compatibility test.

**Material & Methods**

This was a cross-sectional study that included blood samples received in the blood bank along with requisition of blood components from 30 patients with coronavirus disease who tested 2019-nCoV positive by PCR and were admitted at Bhagat Phool Singh Government Medical College for Women, Khanpur Kalan, Sonipat during May 2020 to June 2020. Samples were received and processed under the interim laboratory biosafety guidelines by WHO.

**Inclusion and Exclusion Criteria**

Inclusion criteria included blood samples which were collected no more than three days before the intended transfusion unless the patient was pregnant or transfused within the preceding three months. If the patient’s transfusion or pregnancy history was uncertain or unavailable, blood samples collected within three days of RBC transfusions were processed. Exclusion criteria included hemolysis samples.

**Cross Match Procedure**

Of the 30 samples processed, ten samples were subjected to cross-match by Immediate Spin cross-match method (Method A). For each donor sample to be tested, a tube was labelled. Then two drops of patient’s serum were put in the tube followed by addition of one drop of 2-4% of saline suspended red cells of the donor. The tube was then incubated for 10 minutes followed by centrifugation at 1000rpm for 1 minute. Rest 20 samples were cross-matched by saline cross-match technique (Method B).

However, in comparison to a standard saline cross-match where incubation time is generally of 30 min, a prolonged incubation time of 45 min was adopted. In method B, the tube was incubated for 45 minutes so that the cells were separated from plasma by gravity. After that, the sample was observed for hemolysis and agglutination. The results obtained from both the methods were noted, compared and statistically analysed. Patients were monitored after the start of each transfusion for 24 hours for any adverse transfusion reactions.

**Results**

Of the ten samples which were cross-matched by immediate spin cross-match method, there was successful compatibility testing in all samples within the first attempt, and no adverse reaction (both major and minor) occurred post-transfusion of blood components as shown in Table 1. The 20 samples which were subjected to compatibility testing by saline cross-match technique with a prolonged incubation time of 45 min also showed successful significant and minor cross match except for one sample as shown in Table 2.

Adequate history was taken from the patient regard-
Table 1: Compatibility testing by Method A: Immediate Spin Crossmatch by Centrifugation

| S.No | Major Cross Match | Minor Cross Match | Compatibility | Transfusion Reaction |
|------|-------------------|-------------------|---------------|---------------------|
| 1    | ✓                 | ✓                 | Compatible    | None                |
| 2    | ✓                 | ✓                 | Compatible    | None                |
| 3    | ✓                 | ✓                 | Compatible    | None                |
| 4    | ✓                 | ✓                 | Compatible    | None                |
| 5    | ✓                 | ✓                 | Compatible    | None                |
| 6    | ✓                 | ✓                 | Compatible    | None                |
| 7    | ✓                 | ✓                 | Compatible    | None                |
| 8    | ✓                 | ✓                 | Compatible    | None                |
| 9    | ✓                 | ✓                 | Compatible    | None                |
| 10   | ✓                 | ✓                 | Compatible    | None                |

Table 2: Compatibility testing by Method B: Saline Crossmatch

| S.No | Major Cross Match | Minor Cross Match | Compatibility | Transfusion Reaction |
|------|-------------------|-------------------|---------------|---------------------|
| 1    | ✓                 | ✓                 | Compatible    | None                |
| 2    | ✓                 | ✓                 | Compatible    | None                |
| 3    | ✓                 | ✓                 | Compatible    | None                |
| 4    | ✓                 | ✓                 | Compatible    | None                |
| 5    | ✓                 | ✓                 | Compatible    | None                |
| 6    | ✓                 | ✓                 | Compatible    | None                |
| 7    | ✓                 | ✓                 | Compatible    | None                |
| 8    | ✓                 | ✓                 | Compatible    | None                |
| 9    | ✓                 | ✓                 | Compatible    | None                |
| 10   | ✓                 | ✓                 | Compatible    | None                |
| 11   | ✓                 | ✓                 | Compatible    | None                |
| 12   | ✓                 | ✓                 | Compatible    | None                |
| 13   | ✓                 | ✓                 | Compatible    | None                |
| 14   | ✓                 | ✓                 | Compatible    | None                |
| 15   | ✓                 | ✓                 | Compatible    | None                |
| 16   | x                 | ✓                 | Incompatible   | None                |
| 17   | ✓                 | ✓                 | Compatible    | None                |
| 18   | ✓                 | ✓                 | Compatible    | None                |
| 19   | ✓                 | ✓                 | Compatible    | None                |
| 20   | ✓                 | ✓                 | Compatible    | None                |

ing previous pregnancies and prior blood transfusions and a second cross match were performed on the same sample from the second unit. No significant adverse transfusion reactions were reported from this group as well. A mild febrile response was observed in one patient, which was adequately managed with antipyretic. Hence, the results of the cross-match obtained by both Method A and Method B were reliable for reporting.

DISCUSSION

Cross-matching or compatibility tests are done to ensure that a particular unit of blood can be safely transfused to a patient. Cross-matching detects the presence of unexpected antibodies in the recipient’s serum that can react with the donor’s red cells. This is known as a major cross match. Detection of antibodies in the donor’s serum that can react with the recipient’s red cells is known as a minor cross match. During the process of doing cross-matching, various factors can affect the binding of antigen with an anti-
body.

These include antigen-antibody ratio, pH, temperature, type of immunoglobulin, incubation time, the freshness of serum and red cells, zeta potential, centrifugation and use of antibody potentiators or enhancement media. Among these, centrifugation is one of the essential laboratory techniques responsible for the production of aerosols. So in case of samples received from patients suffering from infections that spread through aerosols like in COVID-19, centrifugation will increase the biohazard risk of spread through aerosols for the laboratory personals.

Hence it would be better to avoid the process of centrifugation in such cases and its place, various other safer methods can be adopted which will not result in the generation of aerosols and at the same time, minimally affect the reliability of cross match results. Reagents that act as potentiators include 22% Albumin (Reckel and Harris, 1978), Low Ionic Strength Saline (LISS) (Lown et al., 1979), enzymes (e.g. papain, ficin, bromelain), positively charged molecules (e.g. polybrene, PEG) and Anti Human Globulin (Armstrong et al., 2008).

Other factors which increase antigen and antibody binding can be used, like in our study. We increased the incubation time from 30 minutes with centrifugation to 45 minutes without centrifugation. Also, we have seen in our research that the results in the cross-match were not affected even without centrifugation.

So in the case of biohazard samples, it will be safer to avoid the technique of centrifugation that creates aerosols and increase the risk for laboratory personnel.

One limitation of our study is small sample size due to the defined study population and limited literature on the presence of other antibodies generated by the immune system in response to a disease process which can affect the cross match results. More and more studies need to be carried out on a larger scale and including other factors that influence antigen and antibody binding. So that the results can be validated and methods to reduce biohazard risk for health care workers can be followed in COVID-19 pandemic or any other pandemic that can occur in future.

CONCLUSION

Centrifugation is an essential part of the cross-matching of blood samples. But in the case of COVID-19 positive samples, it can be avoided taking care of all the other factors that can enhance antigen and antibody binding, thereby reducing the risk for health care workers of blood transfusion department.

Ethics

The research was approved by the Institute's Ethics committee.

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Conflicts of interest

The authors declare that they have no conflict of interest for this study.

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