# Rouleaux and saline replacement

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Rouleaux is a phenomenon that commonly occurs in patients who have an increased number of circulating protein macromolecules. It is a benign, in vitro reaction that appears microscopically as red blood cells (RBCs) line up against each other; many liken the RBC aggregation to "stacked coins." This unexpected reactivity may cause confusion in direct agglutination testing such as reverse blood typing and crossmatching. Saline replacement is the established method to resolve rouleaux. True agglutination will remain when plasma is replaced with saline for resuspension of the RBC button. Rouleaux will no longer be seen when the plasma proteins are removed. ***Immunohematology* 2018;34:91–92.**

**Key Words:** rouleaux, stacked coins, saline replacement, macromolecule

## Principle

Rouleaux is a type of red blood cell (RBC) aggregation with a distinct "stacked coins" appearance when observed microscopically. These aggregates frequently have a more refractive appearance than classic RBC agglutination. There are typically many "stacks" in the sample, and their size can vary greatly. The rouleaux aggregates disperse differently from the main RBC button during resuspension, such that experienced technologists can sometimes recognize this phenomenon even before confirming it microscopically. It is most typically seen in patients with disease states that cause an increase in plasma protein macromolecules, such as multiple myeloma, diabetes mellitus, and many infections.[1] The macromolecules are thought to cause rouleaux by two unrelated mechanisms: the bridging model or the depletion model. One or both may occur in an individual. In the bridging model, the macromolecules partially adsorb into the RBC and bridge to the RBC closest to it. In the depletion model, the macromolecules move away from the RBCs and change the osmotic pressure between RBCs causing them to aggregate (Fig. 1).[2] Once recognized, rouleaux can be resolved using the saline replacement method. By replacing the patient's plasma/serum with saline, the macromolecules are removed, and the RBCs dissociate.

## Reagents/Supplies

| Reagents | Supplies |
|---|---|
| ▪ 0.9% saline or PBS (pH 6.5–7.5) | ▪ Transfer pipettes<br>▪ 10 × 75 or 12 × 75 mm tubes<br>▪ Calibrated serologic centrifuge<br>▪ Microscope |

PBS = phosphate-buffered saline.

## Procedural Steps

- Confirm the presence of rouleaux microscopically following initial RBC resuspension.
- Re-centrifuge the test tube.
- Remove patient's plasma/serum using transfer pipettes.
- Replace with saline.
- Resuspend. Confirm.

RBC = red blood cell.

## Indications

Rouleaux may be present in any direct agglutination test containing plasma or serum. Examples are reverse ABO typing, immediate spin crossmatch, and immediate spin and/or 37°C reading of antibody detection and/or identification testing. If unexpected positive results present after routine incubation and centrifugation, rouleaux should be considered. Once confirmed, the saline replacement method[3] should be performed using the tube method. It is important to remember that rouleaux can only be found in the presence of patient plasma or serum, since this is where the interfering macromolecules are found. Rouleaux is not seen in the antiglobulin phase of testing because the wash step rids the test system of the patient's plasma/serum.

## Procedure

Examine suspected reactivity microscopically. Rouleaux will appear as stacked coins, whereas true agglutinates are

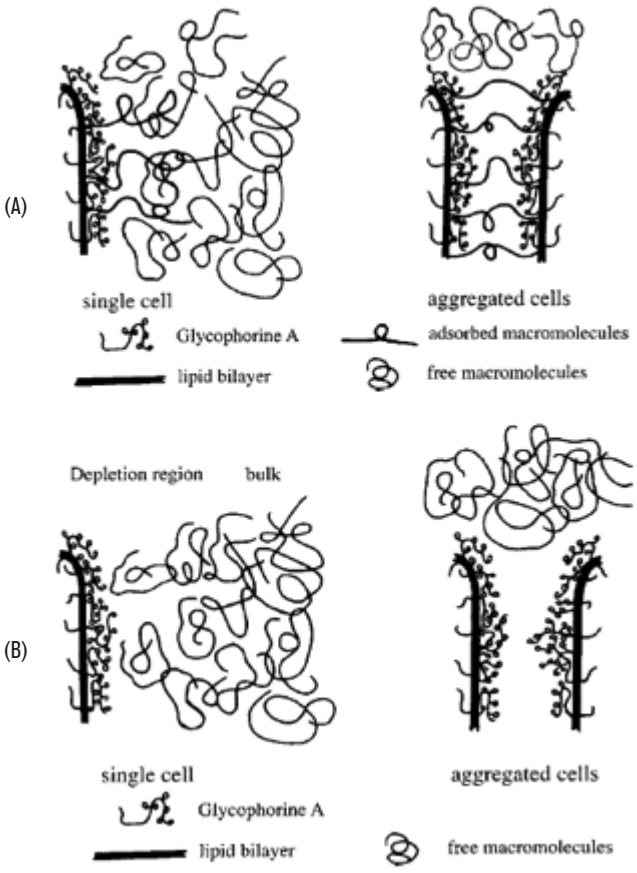


**Fig. 1** (A) Schematic drawing of the bridging model. The adsorption of dextran into single cells and the formation of bridges during the absence of shear flow lead to rouleaux formation of cells. (B) Schematic drawing of the depletion model. Depletion of macromolecules from the interface (with or without a weak adsorption) leads to rouleaux formation if the cells come into close proximity due to the osmotic pressure difference between the pressure in the bulk phase and in the gap. (Reprinted from Bäumler et al.[2] with permission from *Biorheology.*)

amorphous clumps. After confirming the presence of rouleaux, re-centrifuge the plasma (or serum) and cell mixture. Carefully remove the plasma using transfer pipettes, leaving the RBC button undisturbed. This effect can be achieved by tilting the tube so that the plasma is away from the cell button, allowing the pipette to aspirate it without touching the RBCs. Gently replace the plasma with an equal volume of saline without disturbing the RBC button. Resuspend the RBC button, and observe for agglutination. Rouleaux will disperse when suspended in saline, but true agglutination is stable in the presence of saline and will remain.

Once saline replacement has been performed, only direct agglutination testing may be interpreted. Serum/plasma may not be reintroduced to the test system. If test reading at additional phases are desired, the test must be repeated using a separate tube for each phase where a reading will occur.

## Limitations

Rouleaux does not typically cause interference in column testing, although high protein levels can lead to false positives or hazy reactions in this method.[4] Confirmation of rouleaux and resolution must be performed with the tube method.

## Acknowledgments

The author would like to thank Jan Hamilton for her guidance while working on this manuscript.

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