



BLOOD TRANSFUSION

ANTIHUMAN GLOBULIN(COOMB) TEST

Sawa University

جامعة ساوة

College of health and medical
techniques

الاهلية

كلية التقنيات الصحية

Department of Medical

والطبية

Laboratories.....

قسم تقنيات المختبرات

Stage: forth

الطبية

Introduction

- The Coombs test is a laboratory test based on the principle that **antihuman globulin antibodies** combine with RBCs coated with **human immunoglobulin (IgG) or complement (C3d)**. The AHG acts as a bridge that causes RBC agglutination.

Types of Coombs Test

- - Direct Coombs Test (DCT): Detects antibodies or complement bound to RBCs .
- - Indirect Coombs Test (ICT): Detects free antibodies in the serum that may bind to RBCs.

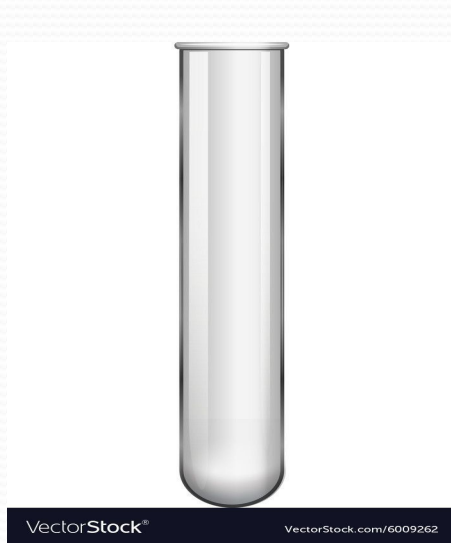
Clinical Applications

- - Diagnosing Autoimmune Hemolytic Anemia (AIH).
- - Pre-transfusion blood compatibility testing
- - Detecting Hemolytic Disease of the Newborn (HDN)

Requirments:

- Test tube (10 * 75 mm).
- Centrifuge.
- Normal saline.
- Micropipettes.
- Tips.
- Incubator.
- Anti human globulin reagent.
- Specimen.

● Test tube



incubator



centrifuge



Anti-Human Globulin ●



Specimen



- Blood sample collected in **EDTA** or **Na. citrate** tube.
- Type: —————→ **blood for DCT.**
- —————→ **serum for ICT.**
- The specimen should be tested as soon as possible after collection. If testing is delayed, the specimens should be stored in the refrigerator.
- Stored samples should be allowed to reach room temperature prior to testing.

● EDTA tube



Sodium citrate tube



Anti-Human Globulin reagent

- Anti-Human Globulin **Anti- IgG** acts as a link between the antibody coating of neighbouring red blood cells and induces agglutination. Uncoated red blood cells will not agglutinate.



Types of anti human globulin reagent

- **Polyspecific reagent**: mainly includes anti IgG and anti-complement antibody(C3d).
- **Monospecific reagent**: contains only one component such as anti_IgG or anti_C3d antibody.

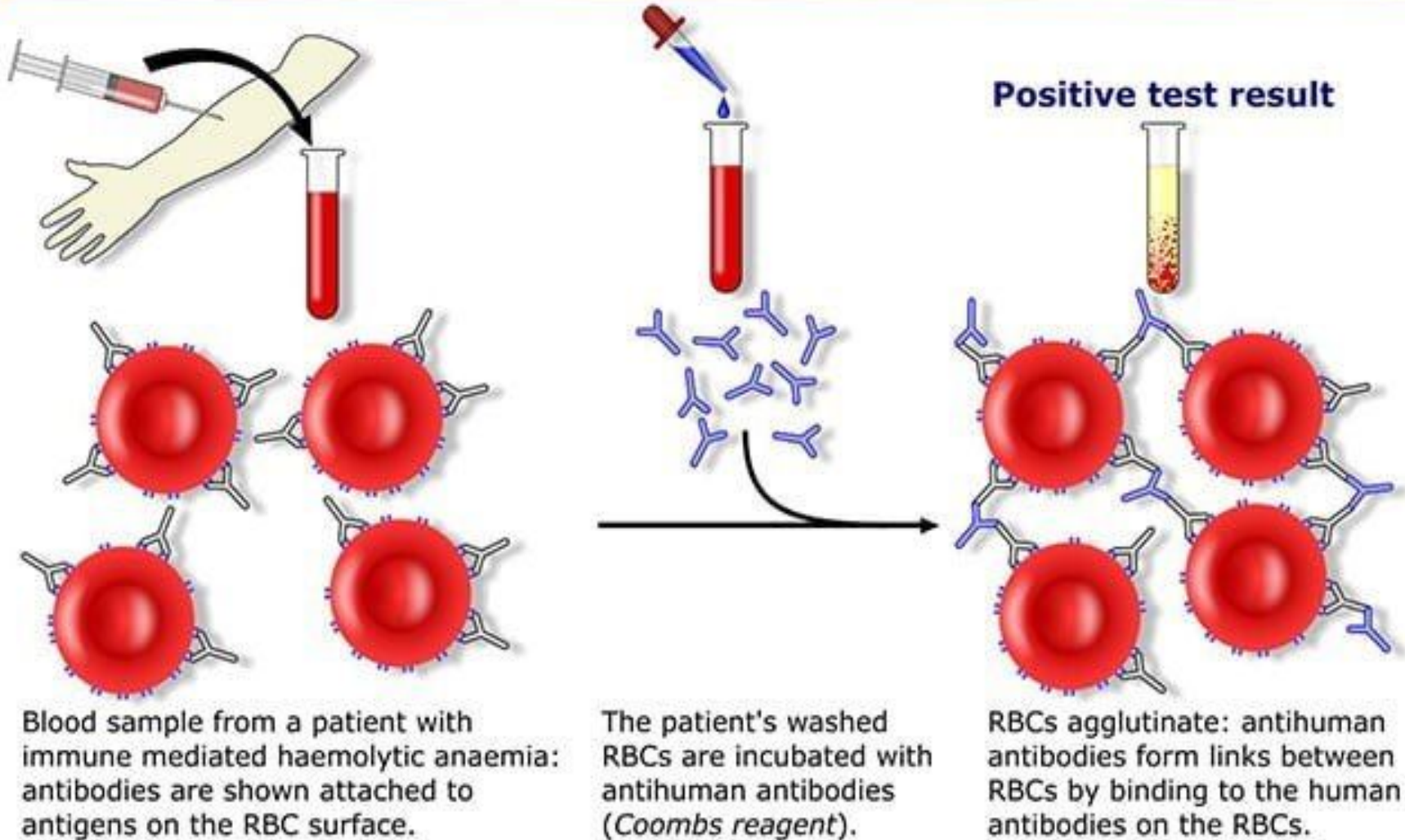
Precautions



- • For In-vitro diagnostic use.
- • Store at 2 to 8°C.
- • Do not use beyond the expiration date.
- • Discard reagents that are turbid.
- • Do not dilute.
- • Handle and dispose of reagents as potentially infectious
- • Caution: Do not pipette by mouth.

DCT

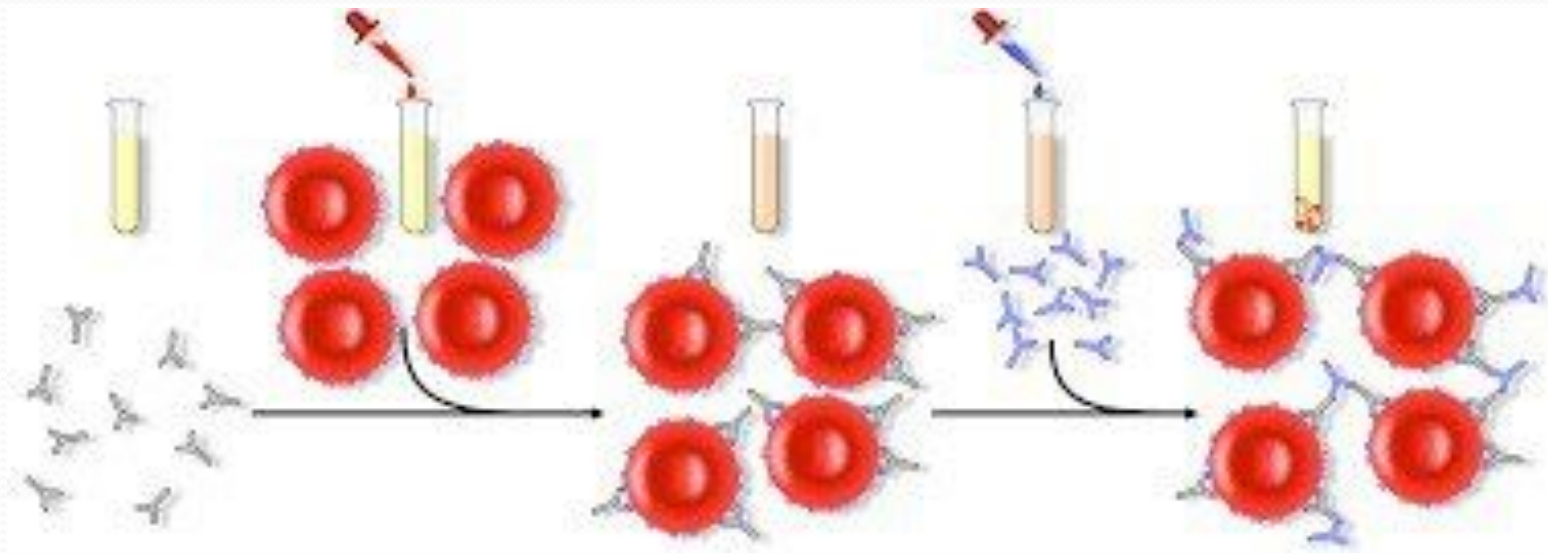
Direct Coombs test / Direct antiglobulin test



Procedure

1. Prepare a 5 % suspension in isotonic saline of the red blood cells to be tested.
2. With clean Pasture pipette add one drop of the prepared cell suspension to a small tube.
3. Wash three times with normal saline to remove all the traces of serum.
4. Decant completely after the last washing
5. Add two drops of Antihuman serum.
6. Mix well and centrifuge for one minute at 1500 RPM.
7. Resuspend the cells by gentle agitation and examine macroscopically and microscopically for agglutination.

ICT



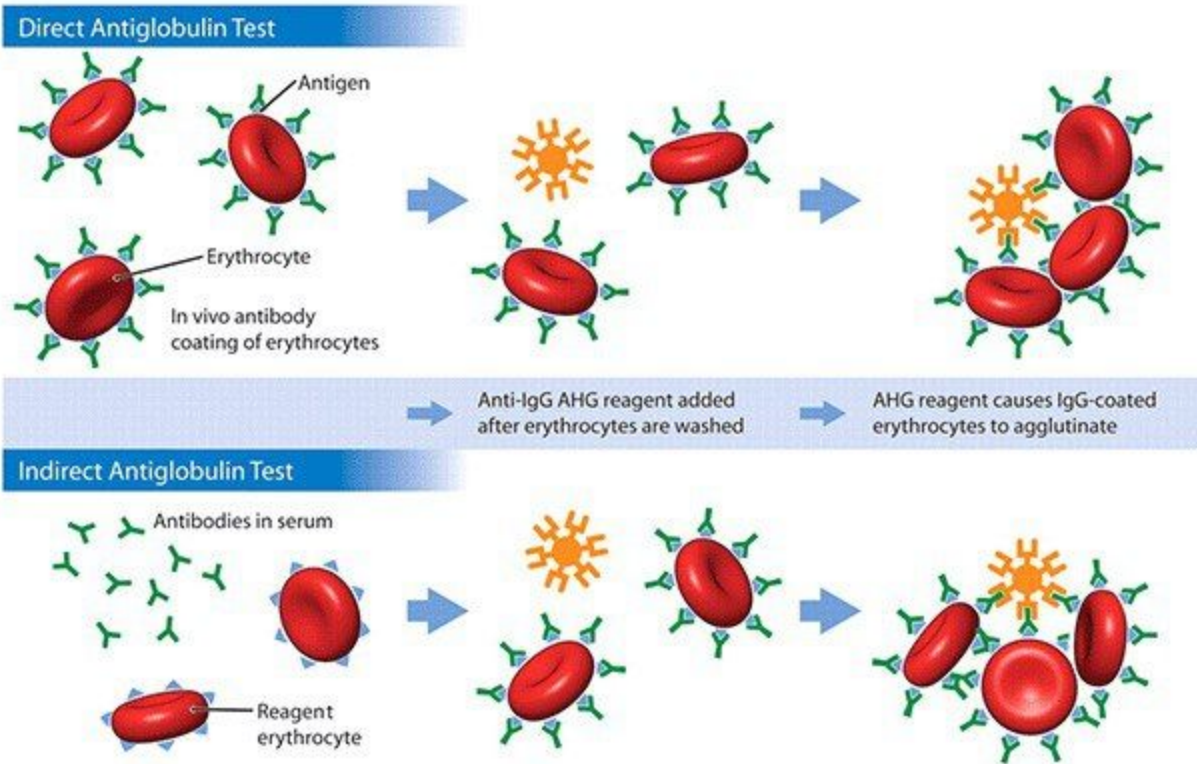
Coombs indirect procedure

- 1 2 to 3 drops of serum
(To be tested)
- 2 RBCs suspension 5%
Known O⁺
or donor /screening RBCs
- 3 Optional Bovine albumin → Increases the sensitivity
- 4 Mix well
- 5 Incubate for 30 minutes
or more at 37 °C
- 6 Centrifuge for 15 seconds
at 3400 rpm
- 7 Examine hemolysis
or agglutination
- 8 Wash RBCs with large amount
of saline 3 to 4 times
- 9 Add coombs serum 2 to 3 drops
- 10 Mix well
- 11 Centrifuge for 15 seconds at 3400 rpm
- 12 Examination for agglutination

Direct Coomb's Test

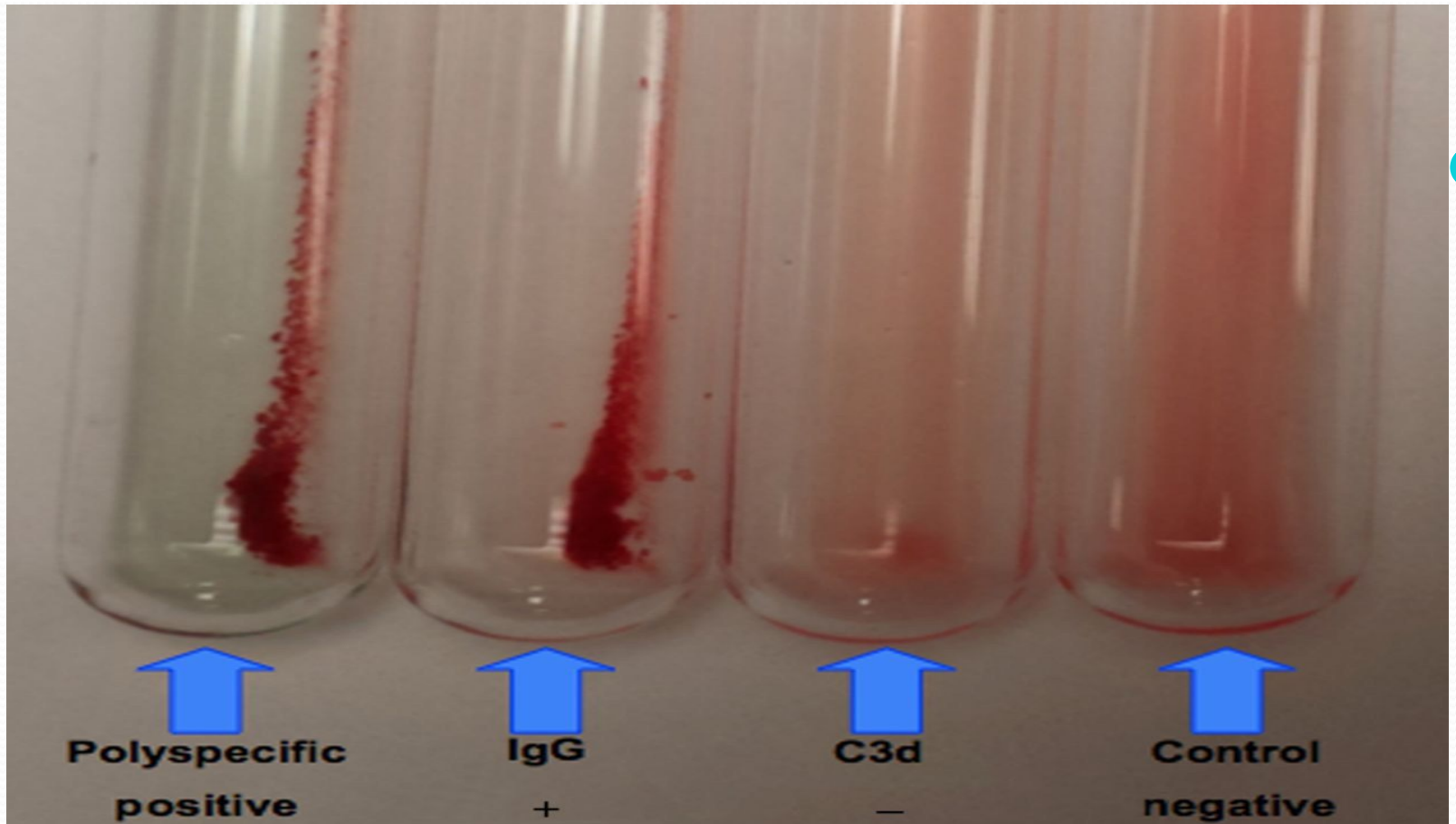
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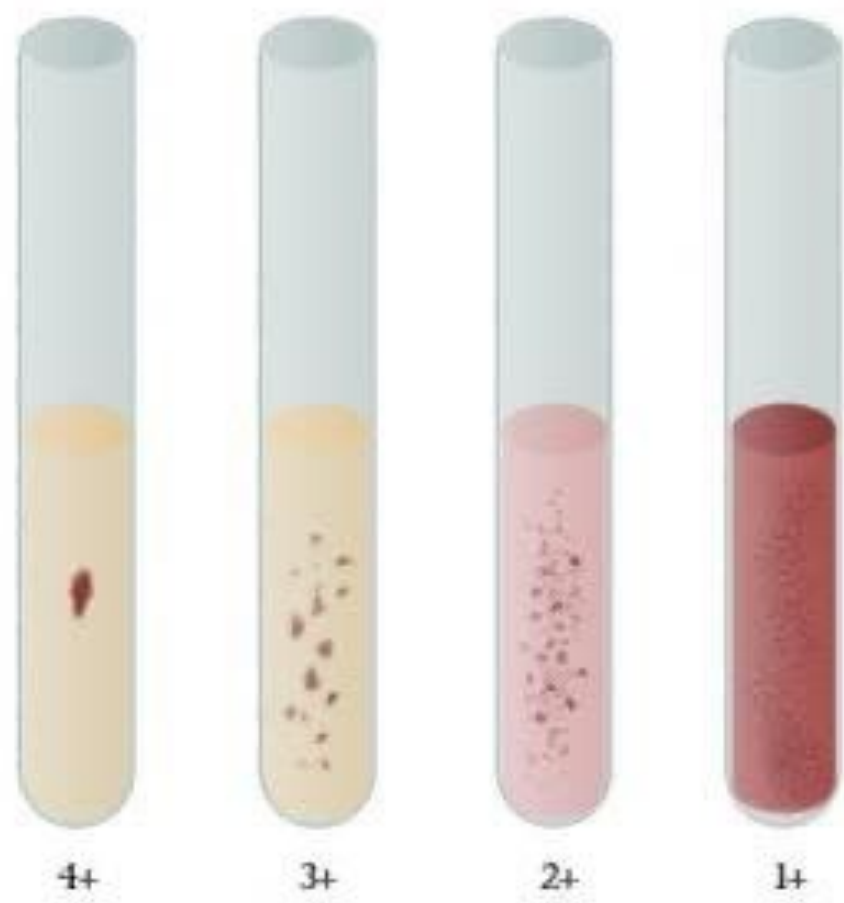
Indirect Coomb's Test



Interpretation of Results

- Positive Coombs Test: Indicates the presence of antibodies causing RBC destruction.
- Negative Coombs Test: No detectable antibodies against RBCs.





Positive coombs test

anti-D coated red blood cells (Fig. 1). Coombs and Poley

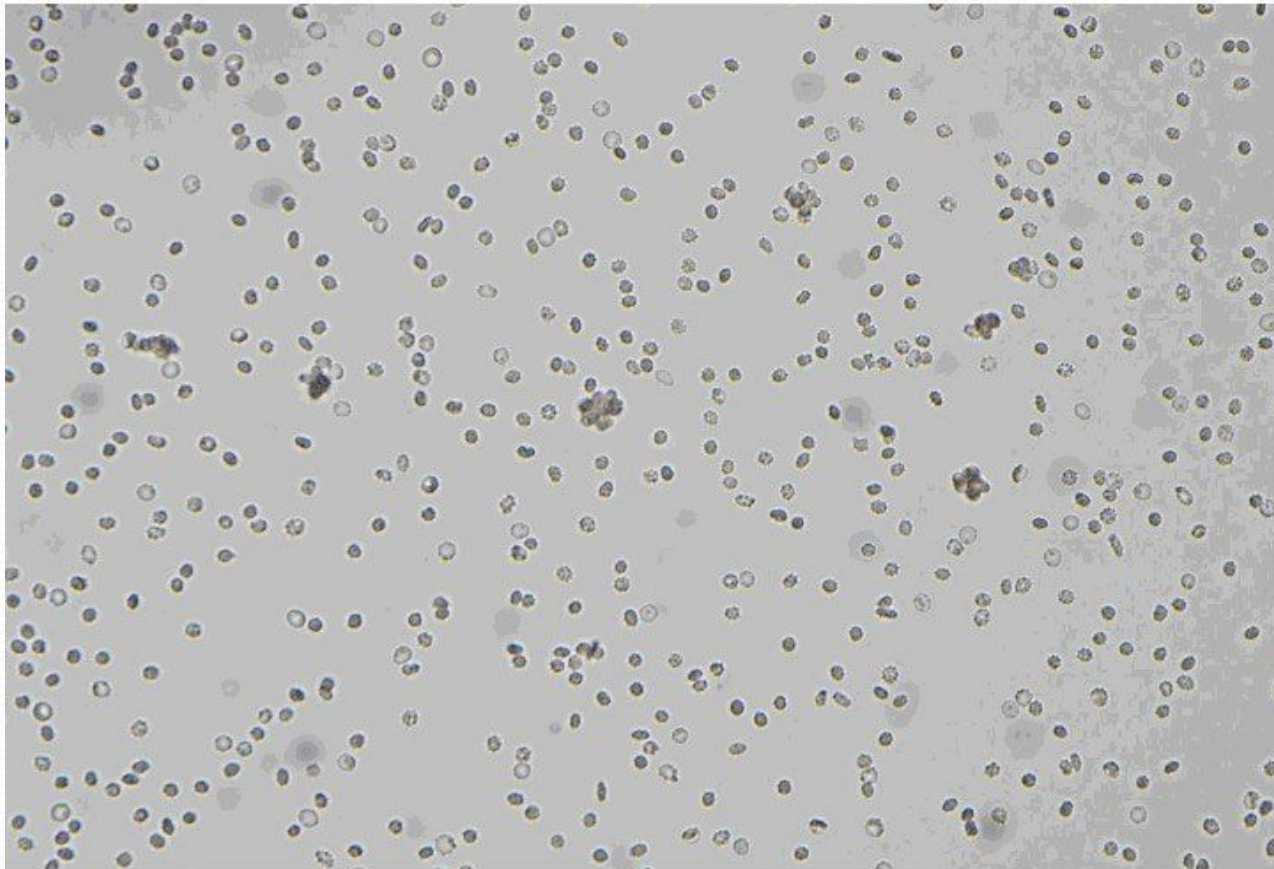
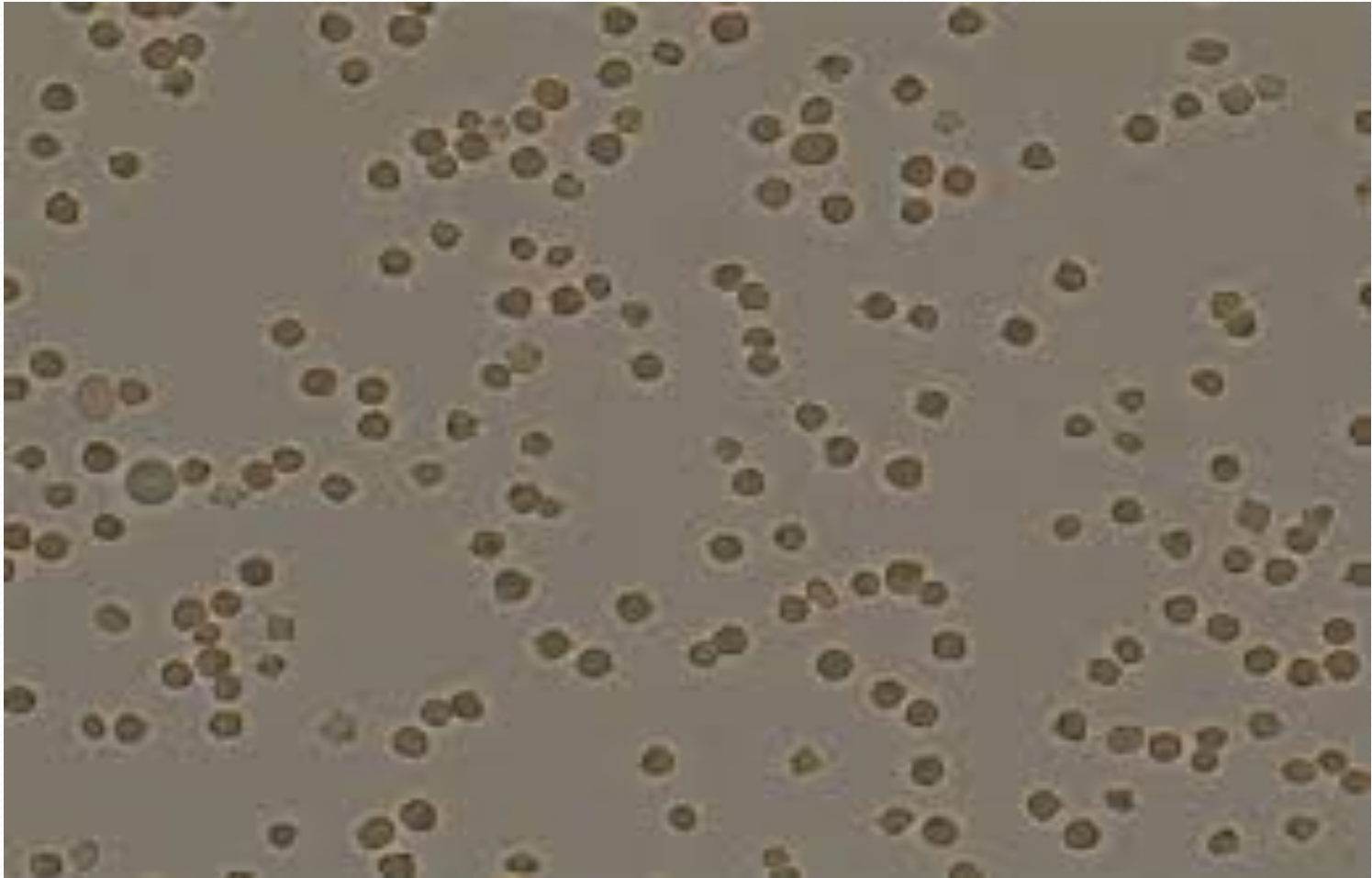


Fig. 1. Positive Coombs test result. Anti-D coated red blood cells (Fig. 1). Coombs and Poley

Negative coombs test



Factors affecting the coombs test

- Ratio of serum to cells.
- Temperature.
- Incubation time.
- Washing of RBCs.
- Saline for washing.
- Addition of AHG.
- Centrifugation.

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- What is the importance of washing of the RBCs in coombs test?
 - What is the importance of the incubation step in indirect coombs test?

THANKS

