CMC EQAS
TRANSFUSION MEDICINE MODULE

WEBINAR ON ANTI-HUMAN GLOBULIN TESTING

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ANTIGLOBULIN TEST

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TEACHING OBJECTIVES

To **state** the principle of Antiglobulin test

To **recall** the steps in performing Antiglobulin test (DAT & IAT)

To **perform root cause analysis** when the test result is unacceptable

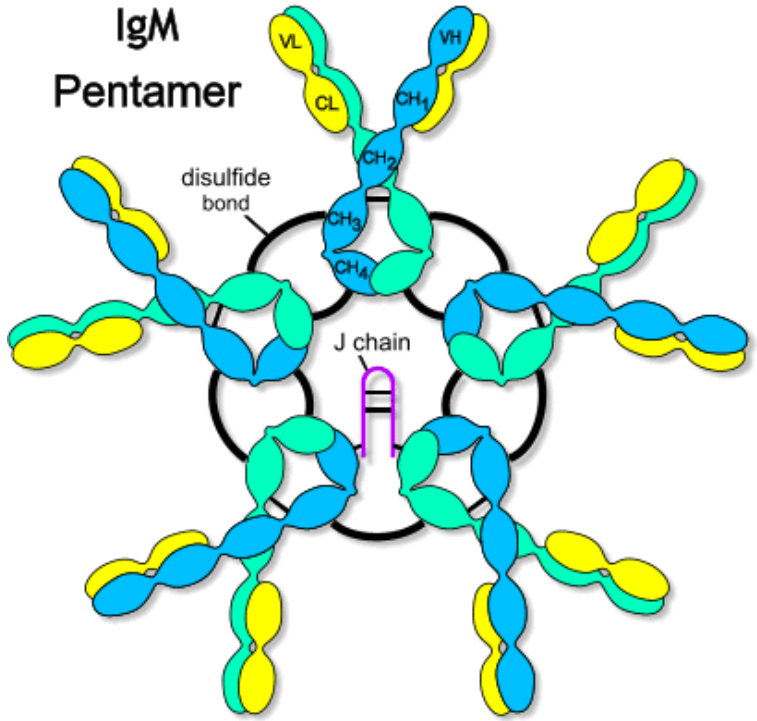
4 PRINCIPLE

Detection of antibodies in the sample

- Coated on red cells
- Present in the plasma/serum

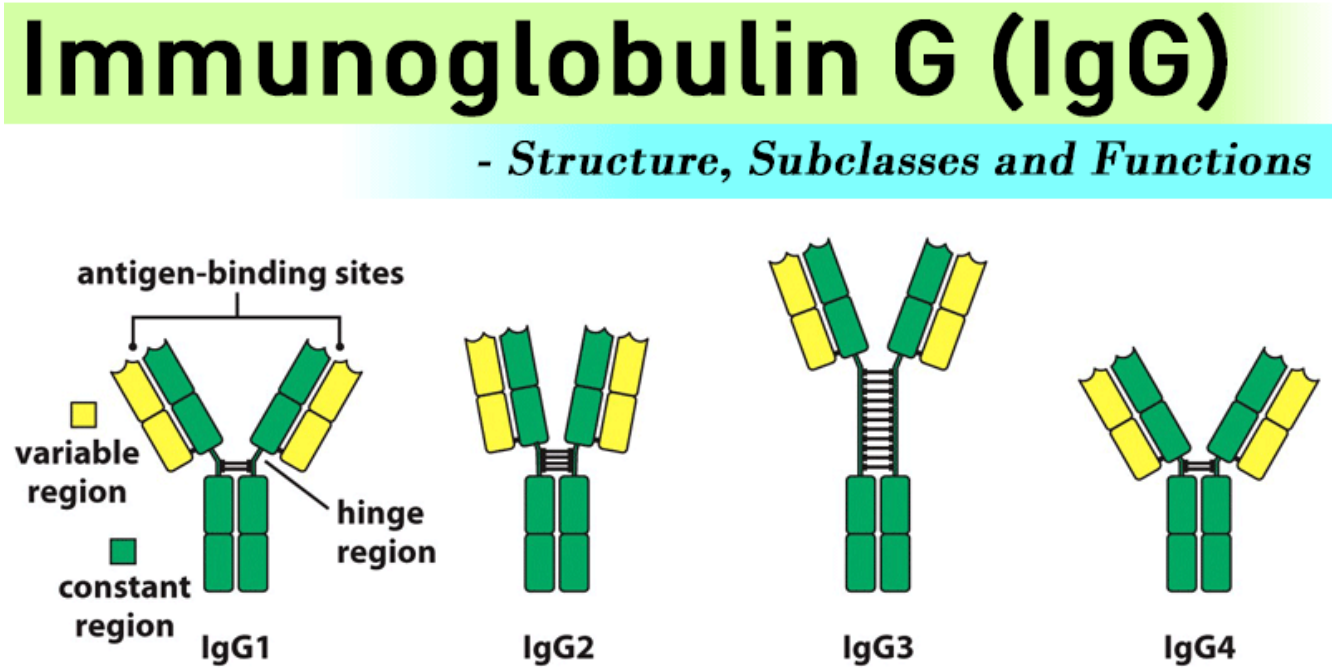
IGM ANTIBODIES (COMPLETE)

- Agglutinate in saline phase
- Pentavalent
- Usually naturally occurring
- Do not cross placenta
- React at temperature varying from 4 - 20°C
- Example: ABO antibodies



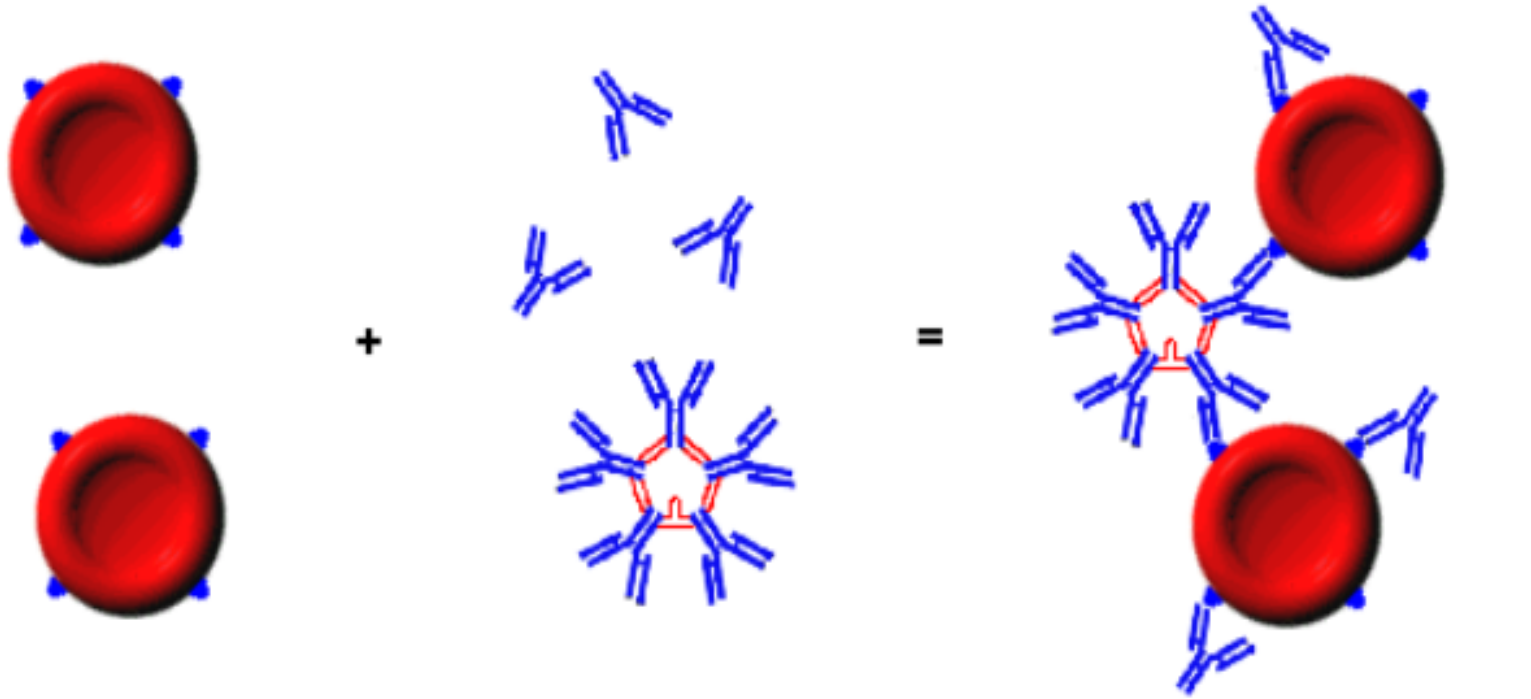
IGG ANTIBODIES (INCOMPLETE)

- Agglutinate in Coomb's phase
- Monovalent
- Usually immune in nature
- Can cross placenta
- React at 37°C
- Example: Rh antibodies



Antigen-Antibody Reaction

AGGLUTINATION

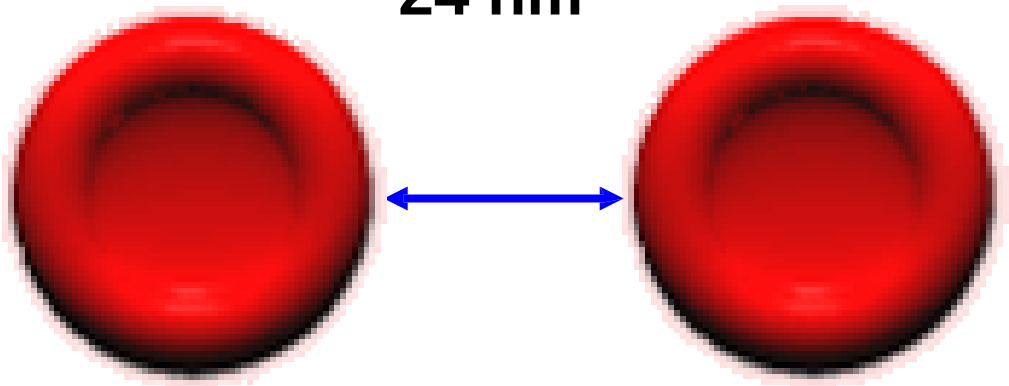


Erythrocytes with antigen "blue"

Antibody against antigen "blue"

- Binding to erythrocytes
- Agglutination of erythrocytes
- Eventually complement activaton

24 nm



Intracellular distance

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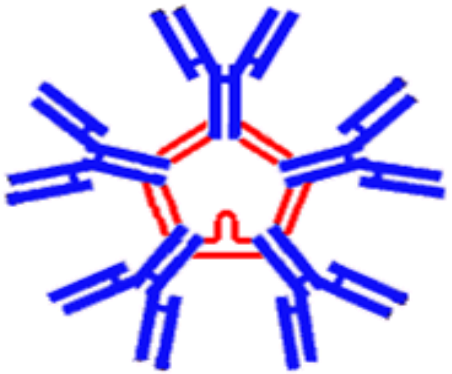
14 nm

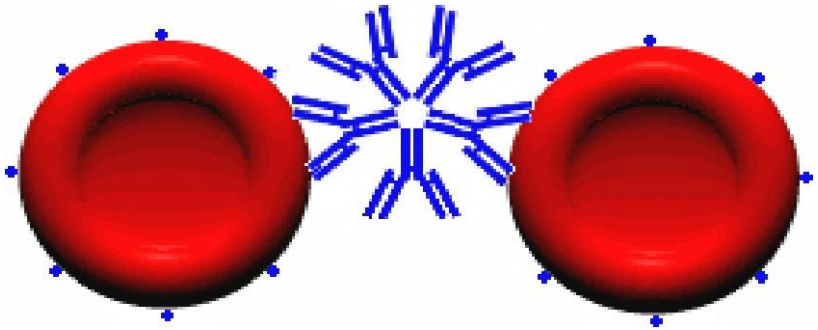
IgG

35 nm

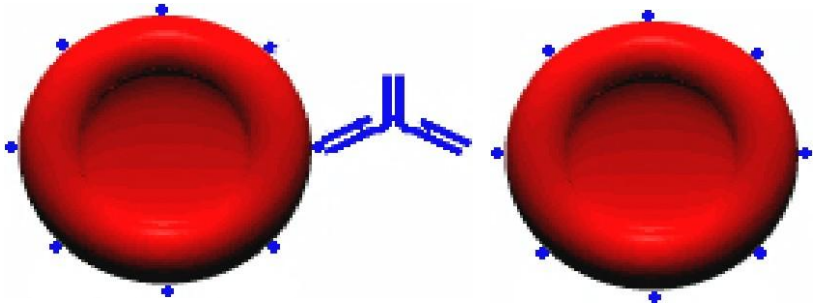


IgM

COMPLETE VS INCOMPLETE ANTIBODY



Complete antibody - IgM

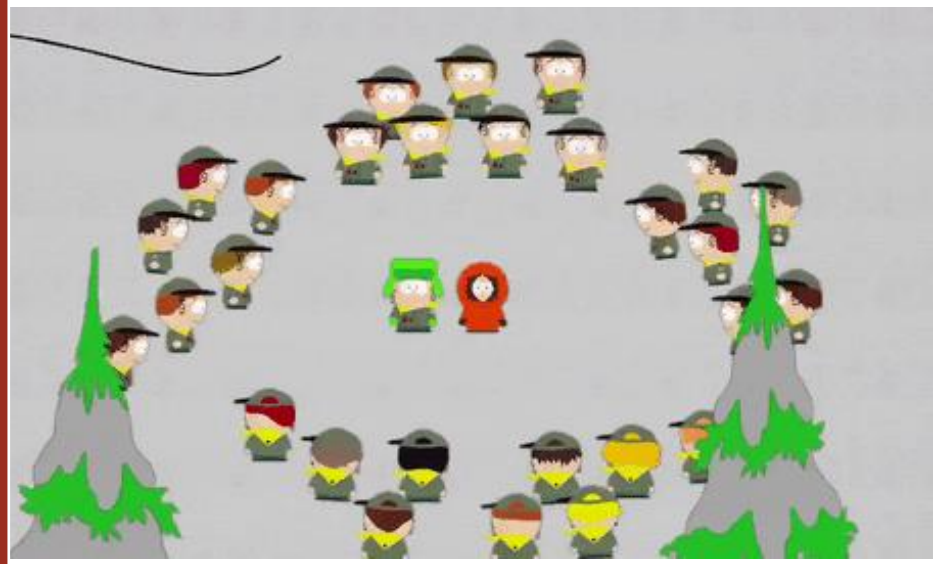


Incomplete antibody -IgG

PRINCIPLE - ANTIHUMAN GLOBULIN TEST

Detects incomplete antibodies

- **Principle of AGT**
  - Antibodies and complement components are globulins.
  - AHG forms bridges between antibody-coated red cells.



ANTIHUMAN GLOBULIN (AHG)

- Antihuman:
  - Antibodies against human antigens
- Globulin:
  - All antibody molecules are globulins
- Antihuman Globulin is an antibody directed against the Fc portion of human antibodies and/or complement components

TYPES

- Monospecific – IgG or C3d
- Polyspecific – IgG and C3d (Blend)

- Importance of using Polyspecific blend

- Preparation
  - Monoclonal (hybridoma)
  - Polyclonal (small animal)

SELECTION OF AHG REAGENT

- **RECOMMENDED Titre – 64**
  - Using 1+ Sensitized O R_1r Positive Cells
  - Serial dilutions
  - Last tube that gives 1+ reaction is the selected titre

- **Specificity**
- No reaction with unsensitized cells
- Ensure no prozone phenomenon
- **Reliable supplier/Cold chain/Cost**

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SAMPLE FOR AHG TESTING

SAMPLE FOR TESTING

- Correctly identified
- EDTA or Serum sample
- Fresh sample as recent as possible
- Should be less than 24 hrs
  - (Old serum sample can result in complement being reduced)

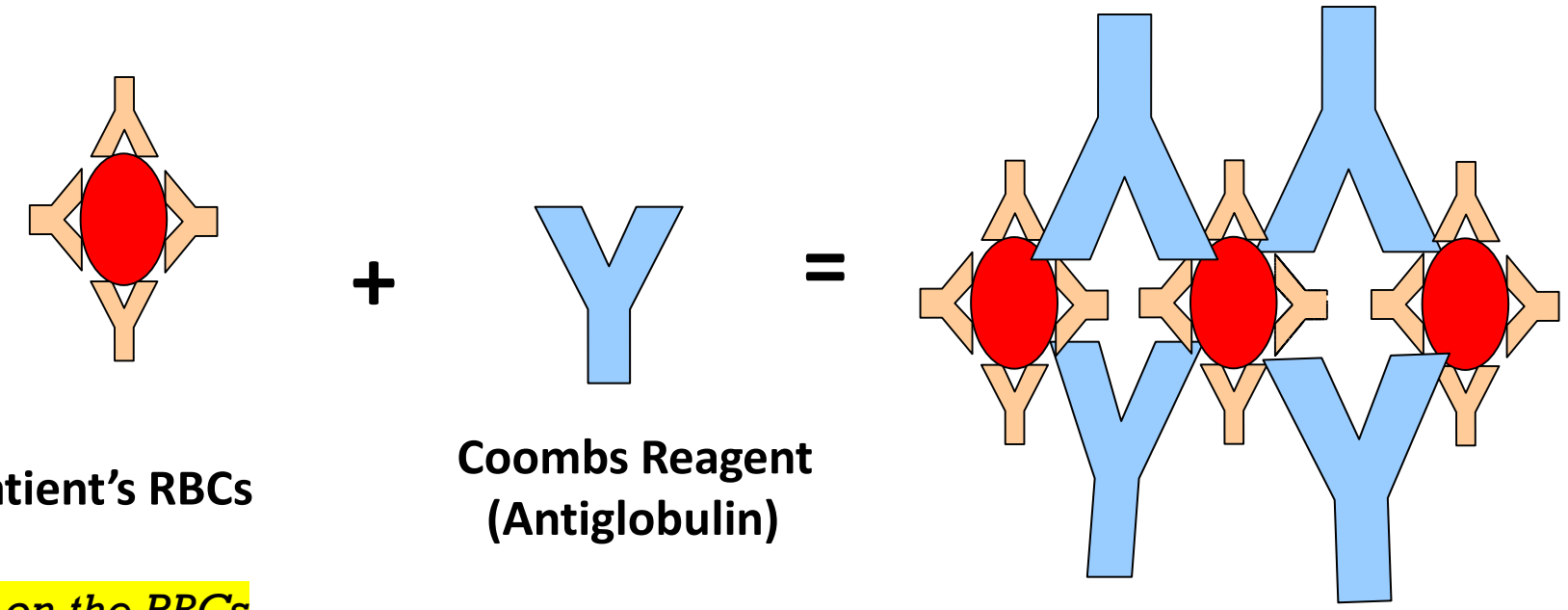
METHODS

- Tube method
- Column agglutination method

- Cannot be done on slides

DIRECT ANTIGLOBULIN TEST

Detects the in vivo sensitization of red cells



Antibodies coated on the RBCs

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PROCEDURE - DAT

Having a standardized operation procedure (SOP) is mandatory

It should be up-to-date

It should reflect your laboratory process

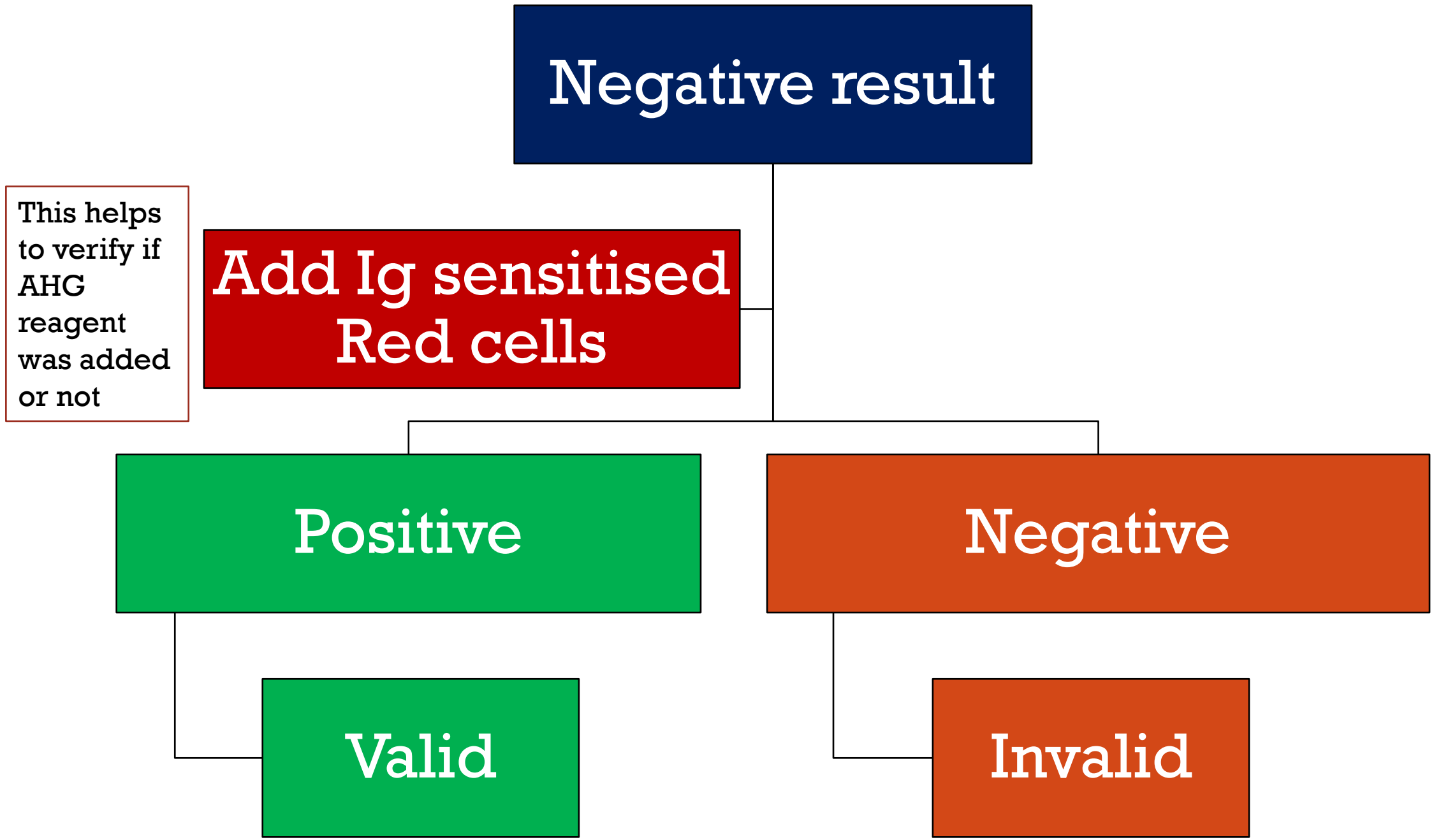
All equipment should be calibrated

MATERIALS REQUIRED

- AHG reagent-Polyspecific (anti-IgG & C3d)
- Sensitized red cells / Check cells – O Positive cells sensitized with Anti D
- Negative control - unsensitized O Rh positive cells
- Normal saline (0.9%)
- 75 x 12mm glass tubes
- Pasture pipette
- Tabletop centrifuge - Calibrated

PROCEDURE

- Follow your laboratory SOP


Negative result
This helps to verify if AHG reagent was added or not
Add Ig sensitised Red cells
Positive
Negative
Valid
Invalid

CONTROLS

- Positive Control – Diluted Anti D
- Negative Control – Fresh AB serum or 0.9% Saline

APPLICATIONS OF DAT

Review patient history and do relevant additional testing

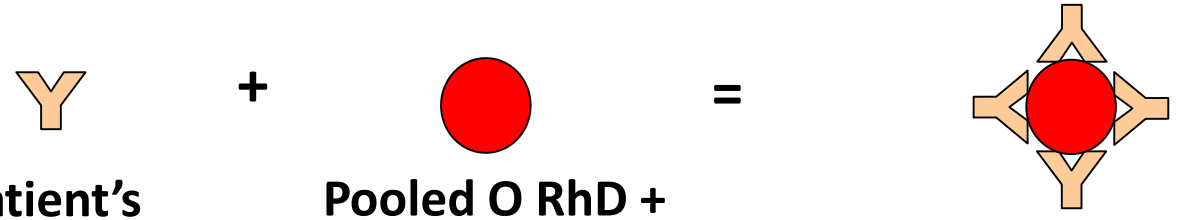
Medical diagnosis
Historical blood group
Transfusion history
Transplantation history
Current medications

- Diagnosis of Hemolytic disease of fetus and newborn
- Diagnosis of autoimmune hemolytic anemia
- Diagnosis of drug induced immune hemolytic anemia
- Investigation of hemolytic transfusion reaction

INDIRECT ANTIGLOBULIN TEST

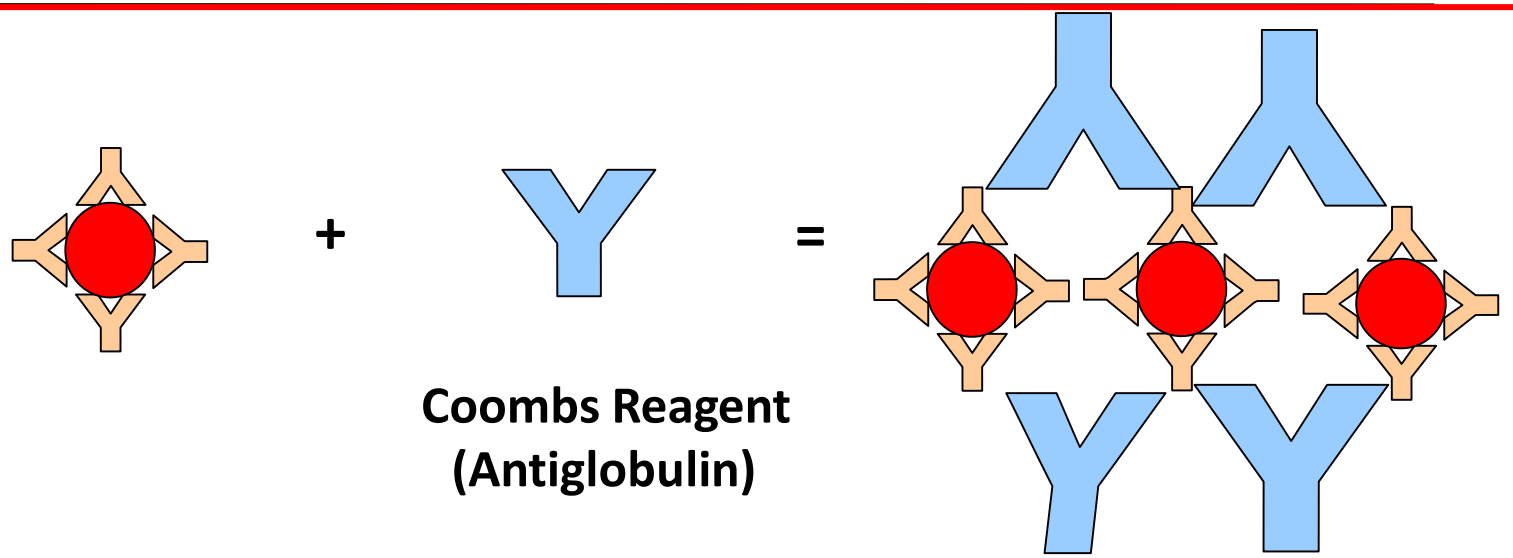
Detects the free antibodies in the serum

Step 1



Patient's Serum + **Pooled O RhD + RBCs** =

Step 2



Coombs Reagent (Antiglobulin)

PROCEDURE - IAT

Having a standardized operation procedure (SOP) is mandatory

It should be up-to-date

It should reflect your laboratory process

All equipment should be calibrated

MATERIALS REQUIRED

75 x 12mm glass tubes and Test tube racks
Pasteur pipettes
Normal Saline (0.9 %)
LISS (optional)
AHG reagent - Polyspecific (anti-IgG & C3d)
Pooled ‘O’ cells (minimum 2 ‘O’ cells, preferably 3 ‘O’ cells)
Sensitized red cells / Check cells
Tabletop centrifuge
Serologic Incubator

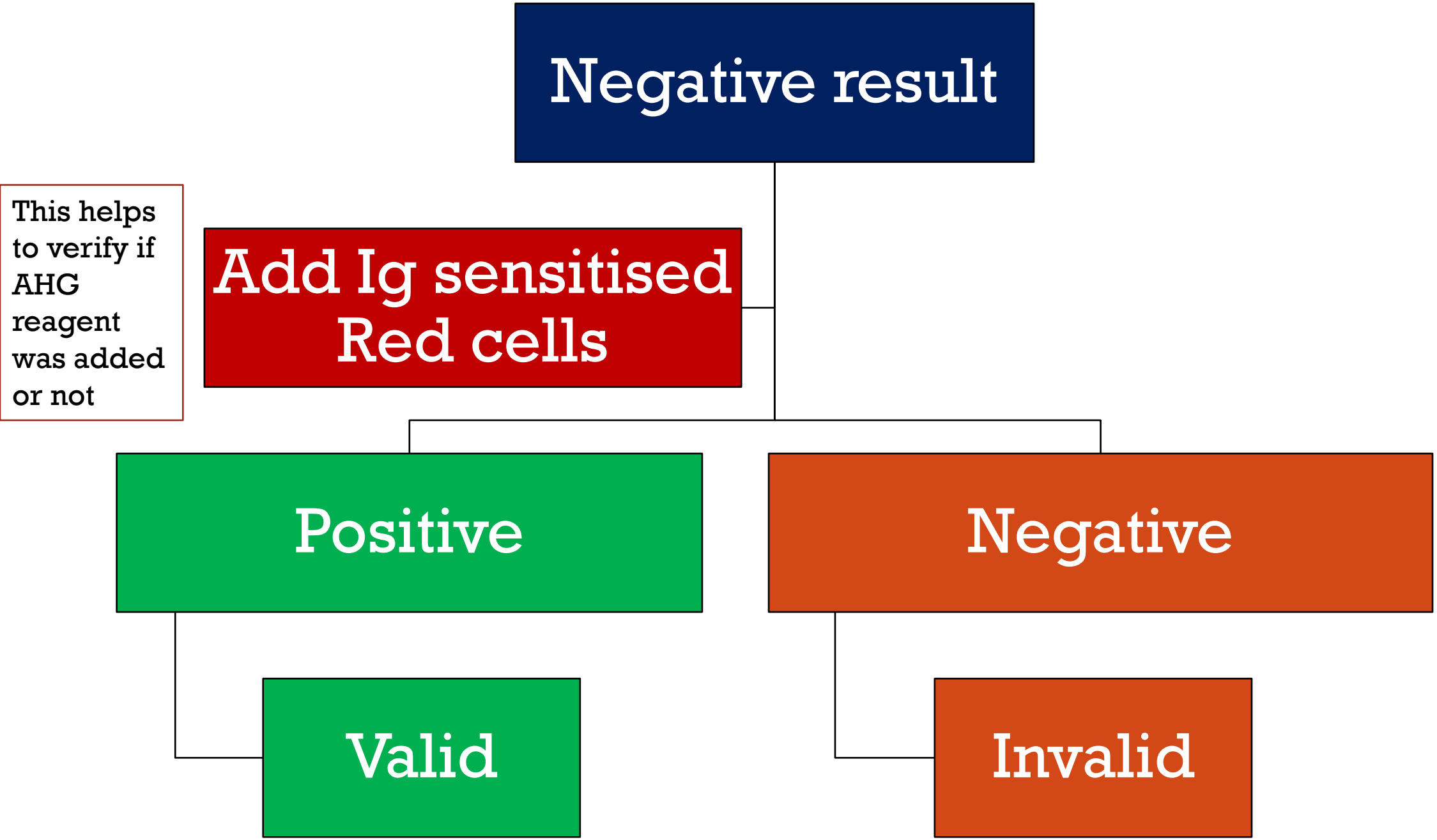
REAGENT RED CELLS

- For Donor testing - Pooled O Rh Positive cells
- Patient testing use unpooled O cells
- Preferably phenotyped (R1R1 or R2R2 cells)
  - NOT random O Pos samples pooled
- Commercial
- Inhouse

AABB. (2023). Technical Manual (21st ed.). AABB, Bethesda, MD

PROCEDURE

- Follow your laboratory SOP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Add Ig sensitised Red cells
Positive
Negative
Valid
Invalid

CONTROLS

- Positive Control – Diluted Anti D
- Negative Control – Fresh AB serum or 0.9% Saline

APPLICATIONS OF IAT

Review patient history and do relevant additional testing

Medical diagnosis
Historical blood group
Transfusion history
Transplantation history
Current medications

Detection and identification of unexpected antibodies in the serum

Cross matching

Typing of minor red cell antigens

Detection of weak D (earlier Du test)

Titration of antibodies

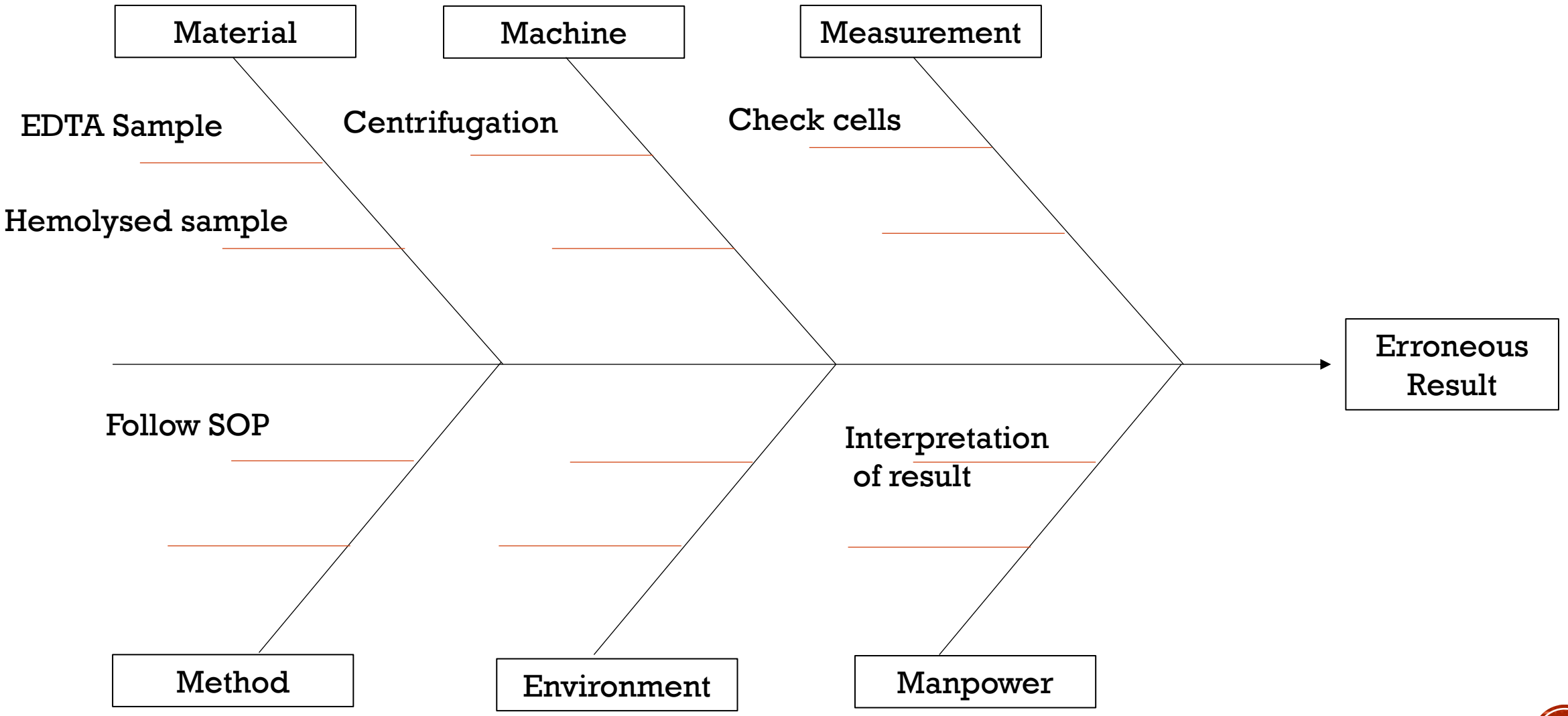
Anti-D in maternal serum in HDN

ROOT CAUSE ANALYSIS APPROACH

Root Cause Analysis (RCA) is a comprehensive term encompassing a collection of problem-solving methods used to identify the real cause of a non-conformance or quality problem.

ROOT CAUSE ANALYSIS

Identify the Problem



GATHER DATA AND INVESTIGATE POSSIBLE CAUSES

Issue Observed	Possible Causes	Investigative Approach
False negative DAT/IAT in suspected hemolysis case	▪ Insufficient sensitization of RBCs ▪ Improper washing of RBCs ▪ Neutralization of AHG reagent ▪ Low-affinity antibodies ▪ Improper centrifugation ▪ Variability in reagent sensitivity	▪ Following SOP ▪ Ensure proper washing (≥3 washes) ▪ Use check cells to validate negative reaction ▪ Repeat test with different AHG reagent ▪ Use Polyspecific and monospecific reagents separately ▪ Incubate at 4°C if low-affinity antibodies are suspected

GATHER DATA AND INVESTIGATE POSSIBLE CAUSES

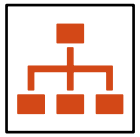
Issue Observed	Possible Causes	Investigative Approach
False positive DAT/IAT	▪ Inadequate washing (residual plasma proteins) ▪ Over-centrifugation ▪ Clotted or old sample ▪ Contaminated glassware	▪ Repeat with properly washed RBCs ▪ Ensure proper centrifugation settings ▪ Use fresh, anticoagulated sample
Hemolysis in test tube	▪ Strong complement activation ▪ Autoimmune hemolysis ▪ In vitro hemolysis due to sample handling	▪ Check for hemolysis in patient sample before testing ▪ Use fresh sample if hemolysis is present

IMPLEMENT CORRECTIVE AND PREVENTIVE ACTIONS

Root Cause	Corrective Action	Preventive Action
Inadequate washing of RBCs	Repeat test with properly washed RBCs	Ensure proper training on washing steps
Improper centrifugation	Adjust centrifuge settings and retest	Calibrate centrifuge regularly
Contaminated or expired reagents	Use fresh reagents and document disposal	Implement reagent inventory tracking
Weak antibody reactions	Use polyspecific and monospecific reagents separately	Consider alternative testing if needed
Sample-related issues (e.g., clotted sample)	Request a fresh anticoagulated sample	Reinforce proper sample collection protocols

DOCUMENTATION & REPORTING

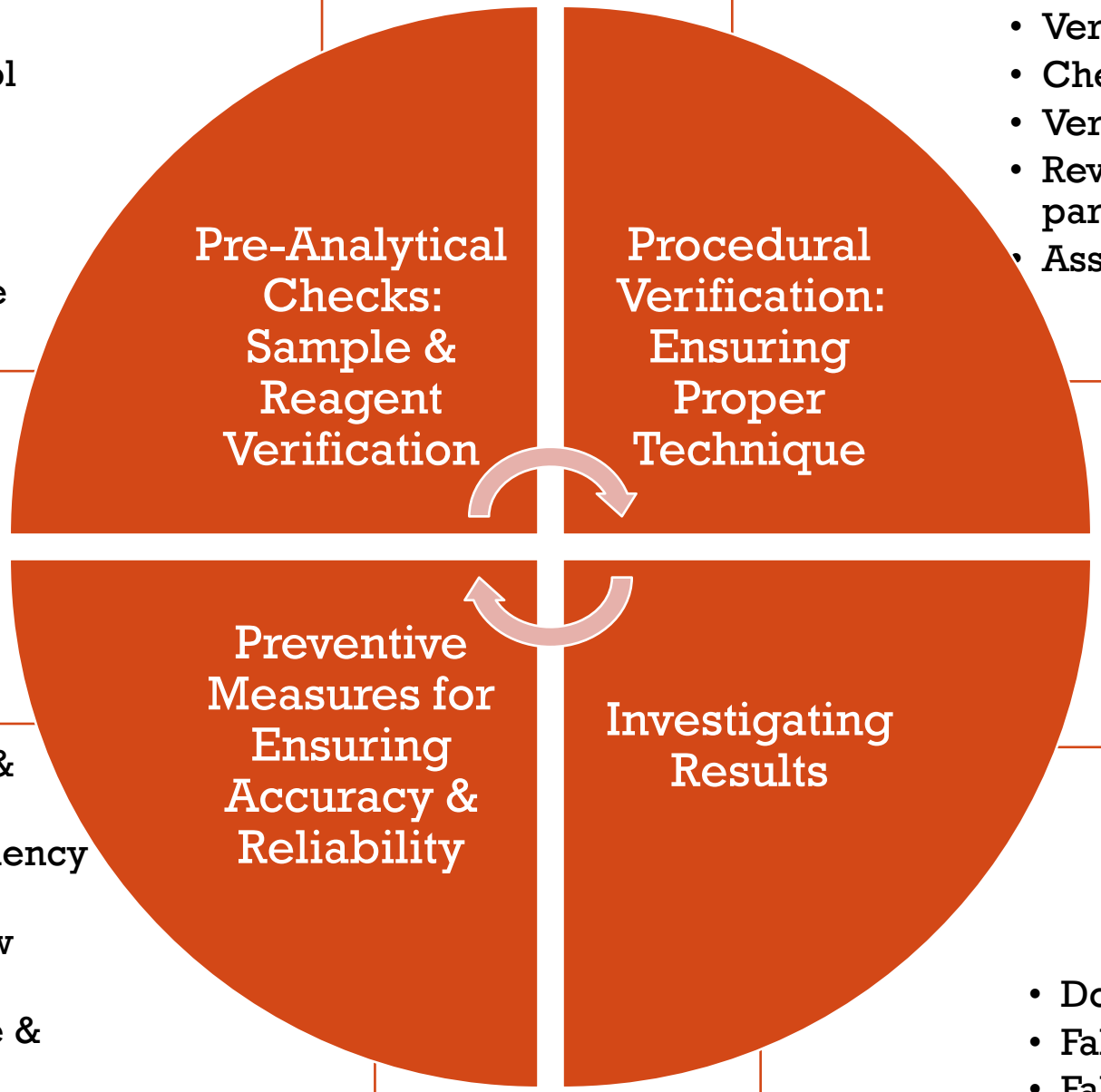
Log discrepancies in the Blood Centre Incident Report.



Report unresolved issues to the Quality Assurance (QA) team.

Monitor trends in errors for process improvement.

CHECKLIST



- Verify sample integrity
- Confirm reagent quality & storage conditions
- Check for proper control use
- Validate antiglobulin reagent (AHG) activity
- Ensure proper saline & enhancement media use

- Check sample-to-reagent ratio
- Verify incubation conditions
- Check washing technique
- Verify reagent addition
- Review centrifugation parameters
- Assess reading technique

- Standardized Protocols & Training
- Quality Control & Proficiency Testing
- Documentation & Review Process
- Equipment Maintenance & Calibration
- Correlate with Clinical Findings

- Document the result
- False positive
- False Negative
- Hemolysis

SUMMARY

To **state** the principle of Antiglobulin test ✓

To **recall** the steps in performing Antiglobulin test (DAT & IAT) ✓

To perform **root cause analysis** when the test result is unacceptable ✓

POINTS TO JOT DOWN

- Even 10 mL of AHG can be neutralized by a tiny amount of free serum protein.
- A couple of drops of residual saline can dilute the AHG reagent below detectable levels.
- A technologist can forget to add AHG to a test tube.
- Normally people do NOT produce unexpected antibodies.
- Therefore, in the blood centre - the test should usually be **negative.**
- Read - Manufacturer's instructions carefully
- Follow Standard Operation Procedure

THANK YOU

For information email us at : teqas@cmcvellore.ac.in

Our website: https://cmceqas.org/