

Division of Blood Transfusion Service

Ministry of Health and Family Welfare



Quality Control In Immunohematology



Teaching Aim

To be able to evaluate and select the proper reagents to ensure Quality.



Serological tests in blood banks

- ABO grouping and Rh typing
- Weak D (or Du as earlier named) testing
- Antibody detection and identification
- Cross matching
- Antibody titration
- Direct antiglobulin test
- Haemagglutination inhibition test (for secretor status in saliva)



Blood Bank Reagents

- Type of blood bank reagents
 - Detect an antigen present or absent on RBC (donor/patient red cells) eg.- antisera
 - Detect antibody present or absent in serum (donor/patient serum) eg.- reagent red cells
- You need to have a known source of antigen to detect antibody or known antibody to detect antigen.



ANTIBODIES

POLYCLONAL

Derived from different B Lymphocytes cell lines

Batch to Batch variation affecting Ab reactivity & titre

NOT Powerful tools for clinical diagnostic tests



MONOCLONAL

Derived from a single B cell clone

- **Reproducible**
- **Predictable**
- **Potentially inexhaustible supply of Ab with exquisite specificity**

Enable the development of secure immunoassay systems.



Monoclonal Antibody

Advantages

- Not contaminated with other proteins
- Consistently reproducible affinity & specificity
- Can be produced indefinitely in unlimited quantities

Disadvantages

- Difficult preparation
- High cost



Selection of Antisera

- Antisera must be of high quality with a shelf life of at-least one year of use and should be received in cold chain
- Should contain a preservative to minimize contamination.
- Should be stored in the refrigerator at 2-8°C
- Should be used according to manufacturer's instructions



Selection of Antisera (contd...)

- Must comply with the standards laid down for potency (titer and avidity) and specificity
- New reagents should not be introduced into routine work until internal QC testing have confirmed that they are satisfactory
- Should be clearly labeled with :
 - Batch number
 - Expiry date
 - Storage temperature



What specifications need to be considered?

Appearance

- Reagent must be clear.
- No turbidity, precipitate, particles on visual inspection

Specificity

- Clear-cut reaction with RBC bearing the corresponding antigen(s)
- Do not contain any other antibody specificity



What specifications need to be considered? (contd...)

Potency: it is measured by

A] Titer

- It is the highest dilution of the antisera at which the macroscopic agglutination is seen at strength of 1+

B] Avidity

- Avidity means the overall strength of reaction between antigen and antibody
- It is measured by the time duration in seconds for the appearance of macroscopic agglutination



Specificity

- Label clean three test tubes for each antisera to be used
- Add 2 drops of antisera to be tested
- Put one drop of 2-5% red cell suspension of known ABO group red cells in respective tubes
- For eg add corresponding red cells suspension in three glass test tubes for testing the specificity of anti A antisera.



Anti-A
+
A red cells



Anti-A
+
B red cells



Anti-A
+
O red cells

Avidity

- Label a clean glass slide for each antisera to be used
- Put one drop of 10% red cell suspension of respective ABO group.
- Put 1 drop of respective antisera adjacent to the drop of red cell suspension
- Mix both the drops using disposable applicator stick
- Start the stop watch simultaneously
- Observe and note the time required for visible agglutination over the view box



Titer – doubling dilution

- Label 10 test tubes
- Add one volume of saline to all test tubes except the first tube
- Add an equal amount of antiserum to each of the first two tube
- Using a clean pipette mix the contents of the 1 in 2 dilution several times and transfer one volume into the next tube
- Continue the same process for all the dilutions, using a clean pipette to mix and transfer each dilution



Titer – doubling dilution (contd...)

- Add 1 drop of the corresponding red cell suspension (5%) into each test tube. Mix well and keep these test tubes at room temperature for at least 15min
- Centrifuge all these test tubes at 1000 rpm for 1min.
- Examine test results macroscopically; grade and record the reactions



Dilution and Titer

- Dilution is expressed as: 1 in 16
which means that the dilution factor is 16
- Titer is simply the inverse of dilution. So, it is the number at which the end point agglutination (1+) is achieved.
e.g. At titer of 16 is recorded for end point agglutination at a dilution of 1 in 16.

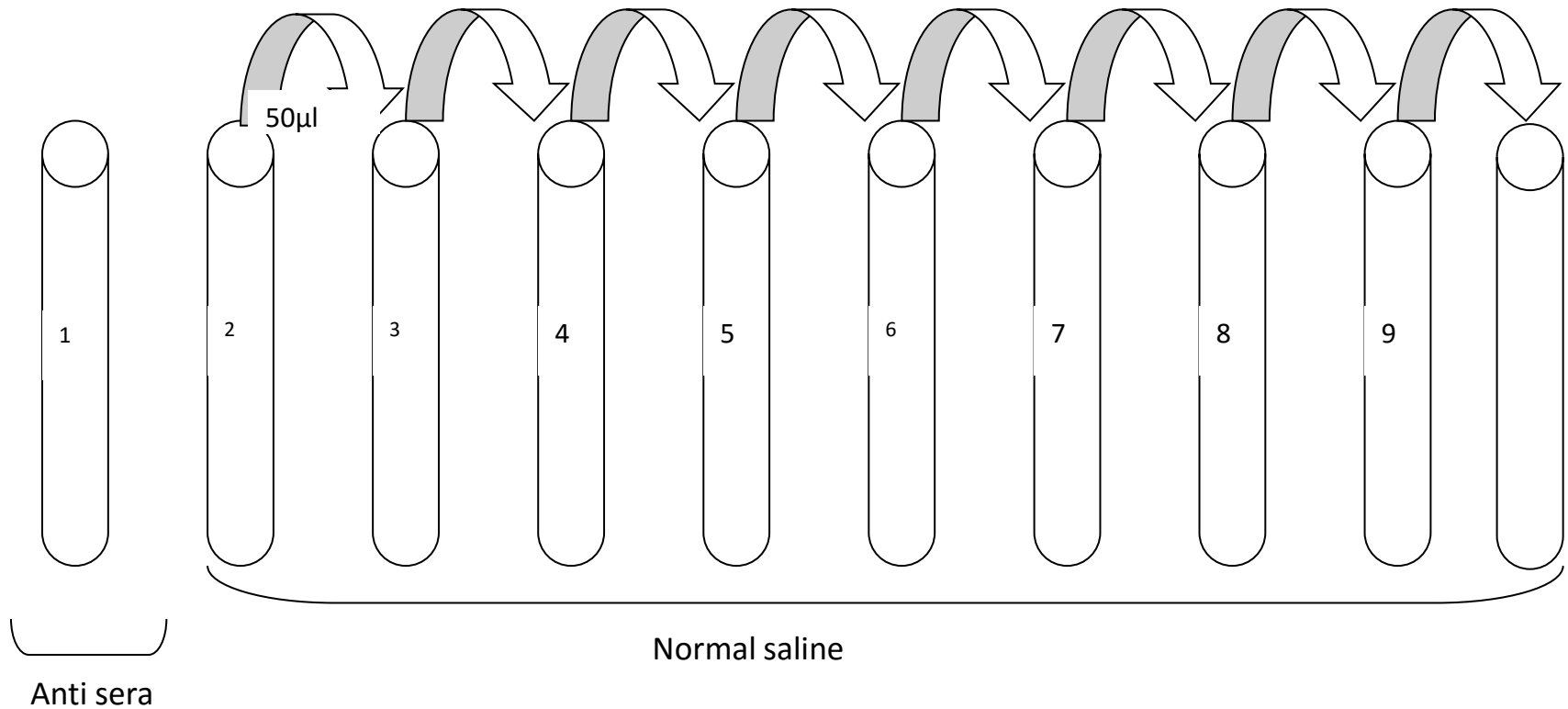


Dilution and Titer (contd...)

- The first tube is a NEAT on i.e. without any dilution
- The second tube contains one part serum and one part of normal saline.
- Hence, it becomes 1 in 2 dilution
(It can be written as 1 : 1 and read as 1 is to 1)



Titer – doubling dilution (contd...)



Interpretation

- Observe the highest dilution that produces macroscopic agglutination
- The titer is reciprocal of the dilution level for e.g 32 for 1:32
- If there is agglutination in the tube containing the most diluted serum, the end point has not been reached, and the additional dilution should be prepared and tested



Strength of agglutination

4+	One large agglutinate with clear background
3+	Several large agglutinates with clear background
2+	Medium size agglutinates with clear background
1+	Small agglutinates with turbid background
0	No agglutination
Mf	Mixture of agglutinated & unagglutinated RBCs
H	Haemolysis

Various areas for QC in serology

■ Reagents

- Antisera anti-A, anti-B, anti-AB, anti-D, AHG
- Red cells A1, B, O cells
- Medium normal saline
- Potentiator LISS / **Albumin** / PEG

■ Equipment

■ Personnel

■ Techniques



ABO ANTISERA



Anti-A, Anti-B, Anti-A,B (**Monoclonal Antibodies**)

- Anti-A, Anti-B
 - blends of 2-3 MoAbs to optimize the intensity of agglutination for a slide tests & the potency for detection of the weaker sub groups e.g. Ax & Bw
- Anti-A,B
 - blends of at least 2 MoAbs to optimize both A & B reactions
- Anti-A+B
 - blends of Anti-A & Anti-B MoAbs



Quality Assurance in Blood Grouping

- Use of standardized reagents
- Daily QC of reagents
- Tubes should be clean & dry to avoid false positives
- Serum should be added first followed by red cells
- Hemolysis during antigen antibody reaction is considered as positive reaction
- Macroscopic readings may require agglutination viewer
- Negative reactions can be confirmed microscopically



Quality Assurance in ABO Grouping

- Ideally, test should be done using test tubes
- Test should be done at room temperature (22⁰C)
- Tubes should be clean and properly labeled
- Both, Cell & Serum grouping should be performed
- Anti- AB may be included for confirmation of group O and weak variants of A and B
- Serum grouping using pooled red cells
- Pooled cells should be prepared daily and check for specificity
- Serum grouping helps detect irregular antibodies and Bombay phenotype



DGHS CRITERIA

TRANSFUSION MEDICINE - TECHNICAL MANUAL

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CENTRAL DRUGS STANDARD CONTROL

ORGANISATION



Quality Control of ABO Antisera

Parameter	Quality Requirements	Frequency
Appearance	No turbidity , precipitate, particles or gel formation by visual inspection	Each day
Specificity	Clear reaction with red cells having corresponding antigens and no reaction with negative control	Daily and each new lot
Avidity	Macroscopic agglutination with 10% red cells suspension using slide test	Daily and each new lot
Reactivity	No immune hemolysis , rouleaux formation or prozone	Each new lot
Potency	Sera should give 3+ reaction in saline tube test using a 3% red cell suspension at R.T.	Each new lot

Quality control - antisera

Antisera	Titer	Avidity
Anti-A		
A ₁ cells	>256	3 -6 Sec
A ₂ cells	>128	5 – 6 sec
A ₂ B cells	> 64	5 – 6 sec
Anti-B		
B cells	>256	3 – 4 Sec
A ₂ B cells	>128	5 – 6 sec
Anti – AB		
A ₁ cells	>256	3 – 4 sec
B cells	>256	3 – 4 sec
A ₂ cells	>128	5 – 6 sec

Worksheet for QC of Anti-A antisera

Parameters	Required quality control criteria (DGHS)	Name of the firm		
	Date of Manufacture			
	Date of Expiry			
	Lot No.			
	Quantity			
Appearance ▪ Turbidity ▪ Precipitate ▪ Particles	No No No			
Specificity ▪ A ₁ cells ▪ A ₂ cells ▪ B cells ▪ O cells	3 – 4+ 3 – 4+ Negative Negative			
Reactivity ▪ Immune hemolysis ▪ Rouleaux ▪ Prozone	No No No			
Avidity ▪ A ₁ cells ▪ A ₂ cells ▪ A ₂ B cells	3 – 4 sec 5 – 6 sec 5 – 6 sec			
Potency (titre) ▪ A ₁ cells ▪ A ₂ cells ▪ A ₂ B cells	1:256 1:128 1:64			
Fulfilling DGHS criteria				



Lectins

- Lectin is a seed extract that has antibody specificity
- Lectins do not contain antibodies, instead they contain proteins that react similar to antibodies
- Used to identify certain types of blood group antigens by binding to the carbohydrate determinant of the antigen, resulting in agglutination
- Other use of Lectin is to investigate red cell polyagglutination
- Some examples
 - Dolichos biflorus (binds A₁ antigen)
 - Ulex europaeus (binds H antigen)



QC of anti-A₁ and anti-H lectins

Reagent	Red cells	Titer	Avidity (s)
Anti-A ₁	A ₁	1:16	15-20
	A ₂	neg	
	O	neg	
Anti-H	O	1:16	15-20
	A ₁	1:1	
	A ₂	1:8	
	Oh	neg	

Pooled Red Cells

- Pools of red cells from 3–5 blood donors
 - Represent all clinically significant antigens
- Prepared daily
 - Identify and record donor unit number
 - Confirm the group
 - Wash 3 times with saline
 - Add equal volume of washed red cells in a tube
- Prepare working solution (5%)
 - Add 1 drop of pooled cells to 19 drop of saline
- Check specificity
 - Example: B cells should react with anti-B only



QC of reagent red cells - specificity

Known red cells	Anti-A	Anti-B
A	4+	Neg
B	Neg	4+
O	Neg	Neg
O Rh D neg	Neg	Neg

Inclusion of O cells & autocontrol is must in reverse grouping to rule out

- ✓ Bombay blood group
- ✓ Auto antibodies
- ✓ Allo antibodies
- ✓ Rouleaux formation



Everyday QC of antisera & reagent red cells

Reagent Red cells	Anti-A Lot /batch	Anti-B Lot / batch	Anti-AB Lot / batch	Anti-D Lot / batch
A1 3 donor Unit no	4+	Neg	4+	Not Applicable
B 3 donor unit no	Neg	4+	4+	Not Applicable
O pos 3 donor unit no	Neg	Neg	Neg	4+
O neg 3 donor unit no	-	-	-	Neg



Anti – D Reagents



Quality Assurance in Rh Grouping

- Anti-D in duplicate for confirmation of Rh D negatives
- Use of one IgM and one blend of IgG + IgM preferable
- If Rh D negative, Weak D test to be done in case of donors



Quality Control of anti-D Antisera

Parameter	Quality Requirements	Frequency
Appearance	No turbidity , precipitate, particles or gel formation by visual inspection	Each day
Specificity	Clear reaction with O positive red cells and no reaction with O negative cells	Daily and each new lot
Avidity	Macroscopic agglutination with 40% red cells suspension using slide test	Daily and each new lot
Reactivity	No immune hemolysis , Rouleaux formation or prozone	Each new lot
Potency	Sera should give 3+ reaction in saline tube test using a 3% red cell suspension at R.T.	Each new lot

Acceptable Titer & Avidity

Type of reagent	Type of red cells	Titer Immediate spin	Titer 30 min incubation	Avidity time (s)	Strength
IgM monoclonal	O positive	1:64-1:128	1:64-1:128 (at RT)	5-10	3+
Blend of IgM+IgG monoclonal	O positive	1:32-1:64	1:128-1:256 (at 37°C)	10-20	3+

Worksheet for QC of anti-D (IgM+IgG) antisera

Parameters	Required quality control criteria (DGHS)	Name of the firm			
	Date of Manufacture				
	Date of Expiry				
	Lot No.				
	Quantity				
Appearance ▪ Turbidity ▪ Precipitate ▪ Particles	No No No				
Specificity ▪ O Positive cells ▪ O Negative cells	3 – 4+ Negative				
Reactivity • Immune hemolysis ▪ Rouleaux ▪ Prozone	No No No				
Avidity • O Positive cells (R ₁ R ₁)	10 – 20 sec				
Potency (titre) O Positive cells (R ₁ R ₁) • Immediate spin • 30 to 45 minutes incubation	1:32 – 1:64 1:128 – 1:256				
Fulfilling DGHS criteria					

Antihuman Globulin Reagents



Anti Human Globulin Reagents

- Detects IgG antibodies and Complement protein that have attached to RBC.
- 2 Types
 - Polyspecific
 - Monospecific



Polyspecific AHG Reagent

- Used in AGT to detect in vivo attachment of IgG and/or complement on the surface of the red cell or in serum
- Usually available as combination of Anti-IgG and Anti-C3d

Monospecific AHG Reagents

- Used in the investigation of positive DAT to determine the nature of molecules attached to the red blood cells
- Differential DAT with monospecific AHG can detect IgG or C3 on the red blood cell surface
- Several formulations exist:
 1. Anti IgG
 2. Anti-C3d



Quality Control of AHG Antisera

- Each vial of a new batch tested for its specificity and sensitivity with IgG coated red cells as positive control and non-sensitized red cells as negative control.
- The potency of anti-IgG of AHG reagents can be estimated by titration using IgG (anti D) sensitized red cells
- Minimum requirements for quality product of AHG :

Anti IgG	1:64
Anti C ₃ /C ₄	1:4



Quality Control Of AHG Antisera

Parameter	Quality Requirements	Frequency
Appearance	No turbidity , precipitate, particles or gel formation by visual inspection	Each day
Reactivity & Specificity	No Prozone phenomenon	Each new lot
	No hemolysis or agglutination of unsensitized red cells	Each new lot
	Agglutination of red cells sensitized with anti-D sera	Daily
	Agglutination of red cells sensitized with complement binding antibody	Each new lot
	Agglutination of red cells sensitized with C3b and C3d	Each new lot

Worksheet for QC of AHG (anti IgG + anti C3d)

Parameters	Required quality control criteria (DGHS)	Name of the firm			
	Date of Manufacture				
	Date of Expiry				
	Lot No.				
	Quantity				
Appearance ▪ Turbidity ▪ Precipitate ▪ Particles	No No No				
Specificity Agglutination with ▪ O Positive unsensitized cells ▪ O Positive sensitized cells	No Yes				
Reactivity ▪ Prozone	No				
Potency (titre) ▪ O+ve anti D (IgG) sensitized cells	1:64				
Fulfilling DGHS criteria					



QC of Normal Saline

Parameter	Quality Requirement	Frequency
Appearance	No turbidity / particles	Daily
pH	6.8 to 7.4	New batch
1 ml saline + 1 ml 5% RBC, centrifuge for 10 min. Observe for hemolysis	No hemolysis	New batch

Reagent Red Cell Panels



What are reagent red cell panels?

- Red cell suspensions used in tests employing the principles of hemagglutination and hemolysis for the detection and identification of blood group antibodies.

- **Sources**
 - Commercial
 - In-house
 - Regular local donors
 - Staff members/volunteers



Applications of Reagent Red Cells

- Reverse ABO grouping
- Antibody screening
- Antibody identification
- Antibody titration
- Allogeneic adsorption
- Preparation of Check cells



Learning Outcomes

- You now understand the difference between monoclonal and polyclonal reagents
- You will know how to evaluate and select a reagent
- You will be able to carry out quality assessment of reagent available and in use in your laboratory