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- **Practical - 1 : Determination of human blood group**

Objective

To determine the blood group (A, B, AB or O) and Rh factor (positive or negative) of a human blood sample.

Material Required

1. Human blood sample [fresh finger-prick blood or stored blood]
2. Glass slides or a blood grouping tile
3. Anti-A, Anti-B and Anti-D antibodies [serum reagents]
4. Sterile lancets for blood collection
5. Cotton swabs
6. 70% alcohol for disinfection
7. Micropipettes or droppers
8. Distilled water [optional for dilution]
9. Mixing sticks [wooden or plastic applicators]
10. Marking pen [to label the slides or tile]
11. Disinfectant solution (e.g. 1% sodium hypochlorite]
12. Gloves and lab coat (for safety)

Preparation steps

1. Setting up :-
   - Label the slide or blood grouping tile with three regions Anti-A, Anti-B and Anti-D.

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- Arrange the reagents and tools on a clean, disinfed workbench.
- Ensure all participants wear proper personal protective equipment [PPE]

2. Blood sample collection:
- Sterlize the fingertip of the participant or subject with 70% alcohol.
- Use a sterile lancet to prick the fingertip and collect a drop of blood
- Wipe away the first drop of blood, then collect a fresh drop of testing.

Procedure:

1. Adding Blood and Reagents:
- Place a drop of blood in each of the three labeled region on the slide or tile.
- Add a drop of Anti-A antibody to the region labeled Anti-A
- Add a drop of Anti-B antibody to the region labeled Anti-B
- Add a drop of anti-D antibody to the region labeled Anti-D

2. Mixing:
- Use separate mixing sticks or applicators to gently mix the blood with each antibody reagent
- Avoid cross-contamination between the regions.

3. Observation:
- Observe the regions for agglutination [clumping] within 1-2 minutes
- Agglutination indicates a reaction between the blood cells and the antibody

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- Expected Result:-

Blood group	React^{n} with Anti-A	React^{n} with Anti-B	React^{n} with Anti-D
A^+	Agglutination	no agglutination	Agglutination
A-	Agglutination	no agglutination	No agglutination
B+	No agglutination	Agglution	Agglutination
B-	No agglutination	Agglution	No agglutination
AB+	Agglutination	Agglution	Agglutination
AB-	Agglutination	Agglutination	No agglutination
O+	No agglutination	No agglutination	Agglutination
O-	No agglutination	No agglutination	No agglutination

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- Interpreting Results:
- Based on the pattern of agglutination:
- Anti-A only: Blood group A
- Anti-B only: Blood group B
- Both Anti-A and Anti-B: Blood group AB
- Neither Anti-A nor Anti-B: Blood group O
- If there is agglutination with Anti-D, the blood group is Rh positive, if not, it is Rh negative

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- Practical 2

Objective To detect and identify specific bacterial antigens through visible agglutination using known antisera

Materials Required

- Bacterial culture A pure culture of the test bacterium
- Antisera specific antibodies targeting the bacterial antigens under investigation.
- Sterile saline [0.85% NaCl]: for preparing bacterial suspensions
- Glass slides or microtiter plates: For tube or microtiter agglutination assays
- Micropipettes or dropperes for precise liquid handling.
- Mixing sticks or applicators for combining reagents
- Incubator set at 37°C for incubation [primarily for tube agglutination tests]
- Personal protective equipment [PPE] Gloves, lab coat and safety goggles

Preparation steps:

1. Bacterial suspension preparation:
   - Harvest bacterial cells from a solid medium using a sterile loop.
   - Suspend the cells in sterile saline to achieve a uniform turbidity.

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approximately matching a 0.5 McFarland standard (equivalent to 1.5×10^8 CFU/mL)

2. Antisera preparation

Ensure antisera are at the appropriate working dilution as specified by the manufacturer.

Procedure

A. Slide Agglutination test :

1. Sample Application

• place a small drop (20-40μL) of the bacterial suspension onto a clean glass slide

2. Antisera Addition

• Add an equal volume of the specific antiserum to the bacterial suspension.

3. Control setup

• On the same slide, set up a control by mixing the bacterial suspension with sterile saline instead of antiserum

4. Mixing

• gently mix both preparations using separate applicators to avoid cross-contamination.

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5. Observation -

- Rock the slide gently for up to one minute and observe for visible clumping (agglutination) under adequate lighting.

Interpretation of Results

- Positive Agglutination - Visible clumping indicates the presence of specific antigens reacting with the corresponding antibodies.
- Negative Agglutination - Absence of clumping suggests that the specific antigens are not present, or the antibody does not recognize them.

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- Practical-3 Widal test

Objective

To perform and interpret the widal test for the diagnosis of enteric fever by detecting antibodies in the patient's serum.

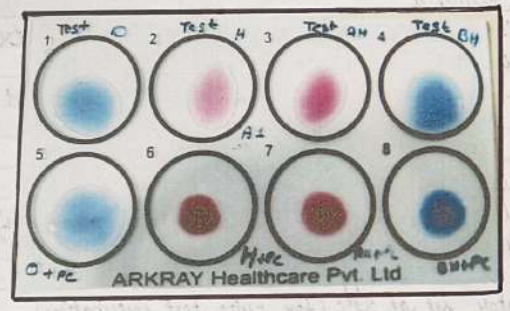
Material Required

1. Patient's serum (collected and separated after centrifugation of blood sample)
2. Antigen suspensions (commercially prepared O and H antigens of S. T
3. Isotonic saline (0.85% NaCl) for dilution.
4. Glass slides (for slide agglutination)
5. test tubes or microtiter plates (for tube agglutination)
6. Micropipettes (for accurate measurement of reagents)
7. Mixing sticks or applicators
8. Incubator set at 37°C (for tube test incubation)
9. control serum (positive and negative controls)
10. Personal protective Equipment (PPE) (gloves, lab coat, goggles)

Procedure

1. Preparation:
   - Place a drop (approximately 50µL) of the patient's

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serum on separate marked circles of a clean glass slide.

2. Antigen Addition
   - Add a drop of the respective antigen suspension (Salmonella typhi O, typhi H, paratyphi A, and Paratyphi B) next to the serum drops.

3. Mixing
   - Use a clean applicator stick to mix the serum and antigen in each circle thoroughly.

4. Observation
   - Gently rock the slide for 1-2 minutes to allow agglutination to occur.
   - Look for visible clumping under good lighting.

5. Control
   - Include a negative control by mixing antigen with isotonic saline instead of serum.

6. Interpretation
   - Agglutination indicates the presence of antibodies specific to the antigen tested.

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• Practical 4 - Antibiotic Sensitivity Test by Disc Diffusion Method.

Objective

To determine the sensitivity of a bacterial strain to four different antibiotics using the disc diffusion method.

Material required

1. Bacterial strain - A pure culture of the test organism (e.g. Escherichia coli or Staphylococcus aureus)
2. Antibiotic discs - Four commercially prepared antibiotics disc (e.g. Ampicillin, Ciprofloxacin, Gentamicin, Tetracycline)
3. Mueller-Hinton Agar (MHA): standard medium for antibiotic susceptibility testing.
4. Sterile cotton swabs for inoculation of the bacterial culture.
5. Sterile petri dishes containing solidified MHA medium.
6. Sterile saline (0.85% NaCl) to prepare the bacterial suspension.
7. 0.5 McFarland standard for adjusting the turbidity of the bacterial suspension.
8. Incubator: set at 37°C for incubation.
9. Ruler or vernier caliper: for measuring the zones of inhibition.
10. Personal protective equipment (PPE) Gloves, lab coat, goggles.

Procedure

A. Preparation of the Bacterial suspension

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• E coli

S. No	Antibiotics	conc.	Zone of Inhibition	reference
• 01 -	cloxicillin	0·2	7mm	Intermediate
		0·4	11mm	"
		0·6	12mm	"
		0·8	8mm	"
		1·0	9mm	"
• 02 -	Ofloxacino	0·2	13mm	Intermediate
		0·4	7mm	"
		0·6	6mm	"
		0·8	00mm	Resistant
		1·0	00mm	"
• 05 -	cefaclorit	0·2	0mm	Resistant
		0·4	Very large	Sensitive
		0·6	Very large	"
		0·8	"	"
		1·0	"	"
• 04 -	Amoxycillin	0·2	4mm	Intermediate
		0·4	5mm	"
		0·6	6m	"
		0·8	7mm	"
		1·0	4m	"

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1. Harvest Bacteria

• Using a sterile loop, pick a few colonies of the test organism from an overnight culture grown on a nutrients agar plate.

2. Prepare suspension

• Suspend the bacteria in sterile saline to achieve turbidity equivalent to a 0·5 McFarland standard ($1·5 \times 10^8$ CFU/ml). Compare visually with the standard.

B. Inoculation of the Agar plate

1. Label the Plate:

• Mark the bottom of the MHA plate to divide it into quadrants, labeling each quadrant with the name of the antibiotic.

2. Inoculate the plate

• Dip a sterile cotton swab into the prepared bacterial suspension, remove excess liquid by pressing it against the inside wall of the tube, and hold streak the entire surface of the MHA plate uniformly, Rotate the plate 60° and repeat the streaking twice to ensure even coverage. Finally, streak the edge of the plate.

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3. Allow to Dry

• Let the inoculated plate stand for 5-10 minutes to allow absorption of the inoculum.

C. Placement of Antibiotics Discs

1. Apply Discs:

• Using sterile forceps or a disc dispenser, place the antibiotic discs onto the surface of the inoculated MHA plate in the labeled quadrants. Ensure even spacing between the disc to avoid overlapping zones of inhibition.

2. Press Gently

• Lightly press the disc to ensure proper contant with the agar surface.

3. Incubate the Plate

• Invert the plate and incubate it at 37°C for 16-18 hours.

D. Observation and Measurement

1. Examine the plate:

• After incubation, observe the plate for clear zones of inhibition around each antibiotic disc.

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2. Measure Zones

- Using a ruler or vernier caliper, measure the diameter of the zone of inhibition (in millimeters) for each antibiotics.

Interpretation.

- Compare the measured zone sizes with the standard interpretive chart provided by the clinical and laboratory standards Institute (CLSI)
- Categorize the test organism as Sensitive (S), Intermediate (I), Resistant (R) to each antibiotic based on the zone size.

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- **Practical 5 - IMViC Test for Bacterial Identification.**

Objective:

To perform the IMViC tests for bacterial identification and differentiation based on their metabolic properties.

Material Required

1. Bacterial cultures Pure cultures of the test bacteria (e.g. Escherichia coli, Enterobacter aerogenes).
2. Sterile media and reagents
   - Tryptone broth (for Indole test)
   - MR-VP broth (for Methyl Red and Voges-Proskauer tests)
   - Simmons' citrate agar slants (for citrate test)
   - Kovac's reagent (for Indole test)
   - Methyl red indicator
   - Barritt's reagents A and B (for Voges-Proskauer test)
3. Sterile inoculating loop
4. Incubator set at 37°C
5. Test tubes
6. Personal protective equipment (PPE): (Gloves, lab coat, goggles)

Procedure

1. **Indole test**

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Bacteria	Indole Test	M-R Test	V-R Test	Citrate Test
E. coli	Positive	Positive	Negative	Positive
Bacillus cereus	Positive	Negative	Negative	Negative
Staphylococcus	Positive	Positive	Positive	Negative
Salmonella	Positive	Positive	Negative	Positive

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Principle:

Tests the ability of the bacteria to break down tryptophan into indole using the enzyme tryptophanase.

1. Inoculate the bacterial culture into sterile tryptone broth.
2. Incubate the tube at 37°C for 24-48 hours.
3. Add 5 drops of Kovac's reagent to the tube
4. Observation: A red or pink layer on the surface indicates a positive result. No colour change indiciates a negative result.

2. Methyl Red test

Principle:

Detects mixed acid fermentation of glucose by the bacteria, resulting in stable acid production.

1. Inoculate the bacterial culture into sterile MR-VP broth.
2. Incubate the tube at 37°C for 48 hours
3. Add 5 drops of methyl red indicator to the tube
4. Observation A red colour indicated a positive result (acidic pH). A yellow color indicated a negative result (neutral pH)

3. Voges-Proskauer Test

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Principle:

Detects the ability of bacteria to ferment glucose with the production of acetoin and 2,3- butanediol.

1. Use the same MR-VP broth from the Methyl Red test.
2. Add 10 drops of Barritt's reagent A (α-naphthol) and 10 drops of Barritt's reagent B (potassium hydroxide)
3. Gently mix the tube and allow it to stand for 15-30 minutes.
4. Observation - A red or pink color indicates a positive result (acetoin production). No color change or a copper color indicates a negative result.

iv. Citrate test -

Principle:

Test the ability of bacteria to use citrate as the sole carbon source, producing alkaline products.

1. Streak the bacterial culture onto a Simmon's citrate agar slant.
2. Incubate the tube at 37°C for 24-48 hours.
3. Observation - A blue color indicates a positive result (citrate utilization), Green color indicates a negative result (no citrate utilization).

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