SER: Species Determination - Ouchterlony

Principle

Immuno-double diffusion is used to determine the species of origin for body fluids and tissue.

The method uses agarose gels with wells into which the antibodies and antigens are placed. The antibodies and antigens diffuse into the gel. When antigen and antibody meet at the point of equivalence, they bind to each other, cross-link, and precipitate, leaving a white line of precipitation that can be visualized with a protein stain.

Equipment and supplies

This procedure uses the following laboratory equipment and supplies:

- Immuno diffusion plates (commercially available)
- oven
- pipettes
- glass or plastic support medium
- Gelbond
- filter paper
- blotting paper
- weight
- stereomicroscope

Reagents

This procedure uses the following reagents:

- 1 M NaCl
  - Dissolve 58.4 grams of NaCl in deionized water and bring up to a volume of 1 liter.
- 5% Ammonium Hydroxide
  - Combine 25 mL of deionized water with 5 mL of 30% ammonium hydroxide.
- Destain Solution
  - Mix the following: methanol/acetic acid/deionized water (50/10/50)
- Stain Solution
  - Dissolve 0.2 grams of Brilliant Blue in 100 mL of *Destain Solution*
- human and animal antiserums
- animal normal serums

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Serum and antiserum quality control

Each lot of normal serum and antiserum received by the laboratory must be quality control tested prior to use in casework.

- The normal serum is titered against the antiserum using a serial dilution of normal serum (up to 1:1024) to determine the optimum concentration.
  - The host animal that produced the antiserum should also be run against the antiserum at its optimum concentration.
- The antiserum is screened against a panel of common animal serums (diluted 1:100) to evaluate specificity. The host animal that produced the antiserum should also be run against the antiserum to evaluate non-specific cross-reactions.
  - If an antiserum reacts with non-related species or the host animal, the antiserum should not be used.

Quality control records

The quality control tests for the normal serum and antiserum are recorded as follows:

- the dried gel is photographed and the image is imported into the *Ouchterlony Worksheet*
- the test is recorded along with the original gel, sealed in clear packaging, in the *Biology Quality Control Log Book* located in the Biology Laboratory

Controls

The following controls must be used in each set of tests:

- known blood or serum (human or other species, depending upon the species being tested for)
  - If using known human blood, dilute with deionized water to a red-brown color.
- extractant blank (deionized water or 5% Ammonium Hydroxide)

Sample preparation

Extract cutting, approximately 1 mm x 1 mm, of stained material in 50 µl deionized water, or place the cutting directly into the gel. Extracts containing blood should be a red-brown color.

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Procedure-single antiserum

Use the following procedure to test several stains against a single antiserum:

Step	Action
1	Use a cutter and template kit to punch wells in the gel. Use an aspirator or pipette to remove plugs from the gel.
2	Fill the center well with 8 µl of antiserum of interest.
3	Fill the outer wells with 8 µl of the controls, blank, and samples to be analyzed.
4	Allow the gel to diffuse for at least eight hours at room temperature.
5	Wash the gel in the 1 M NaCl for at least six hours.
6	Rinse in deionized water.
7	Place the gel on the hydrophilic side of a piece of gel bond using a glass or plastic plate for support. Cover with filter paper and a weight to remove water. Then, dry in oven at approximately 60°C.
8	Place gel flat in a container and stain with *Stain Solution* for 2-5 minutes.
9	Destain the dried gel in *Destain Solution* until the background is clear and the precipitin bands are visible.
10	Record the results on the *Ouchterlony Worksheet.* Seal the gel in clear packaging and store it with the evidence.

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Procedure- screening sample against multiple antiserums

Use the following procedure to screen a single sample against multiple antiserums:

Step	Action
1	Use a cutter and template kit to punch wells in the gel. Use an aspirator or pipette to remove plugs from the gel.
2	Fill the center well on the gel with 8 µl of sample.
3	On each gel, fill the outer wells with 8 µl of assorted animal antiserums.
4	Allow the gel to diffuse for at least eight hours.
5	Wash, rinse, dry, and stain the gel as described in Steps 5-9 and record as in Step 10, above.
6	If a positive result is obtained, rerun the sample with the appropriate controls using *Procedure- single antiserum*, above.

Interpretation

The following information must be considered when interpreting the results of this analysis.

- With a stereomicroscope, examine the area between the opposing wells. The presence of a stained precipitin band between two opposing wells is a positive (+) reaction.
  - Multiple precipitin bands may occur if the amounts of antigen and antibody are not balanced. Multiple bands do not negate a positive finding.
  - For the test in which several stains are tested against one antiserum, fusion of the bands between adjacent wells means that the materials are antigenically identical.
    - The presence of spurs indicates non-identity or an inconclusive result.
- The absence of a precipitin band indicates a negative (-) reaction.
- Cross-reactions with closely related species may occur.
- If bands are unclear or not in the proper position between wells, the test is inconclusive. The sample should be reanalyzed.

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References

The following references were used in the development of this procedure:

DOJ Sacramento Regional Laboratory *Technical Procedures Manual* 4/94

Lee, H., "Identification and Grouping of Bloodstains," *Forensic Science Handbook*, Ed. R. Saferstein, New Jersey: Prentice Hall, 1982, pages 272-275.