SER: Species Determination- Crossover Electrophoresis

Principle

Crossover electrophoresis is used to determine the species origin of body fluids and tissues.

In this variation of the precipitin method, antigen and precipitating antiserums are brought together electrophoretically.

- The antigens in the fluid or tissue extracts are serum albumin and α and β globulins which, under the test conditions, move toward the anode.
- The antibodies in the antiserums are γ globulins and are moved by electroendosmosis toward the cathode.
- Reacting antibody and antigen form a precipitate that can be visualized with a protein stain.

Safety

Electrophoretic procedures have electrical hazards associated with them. Therefore, appropriate caution should be exercised when implementing this procedure.

Equipment and supplies

This procedure uses the following laboratory equipment and supplies:

- electrophoresis tank
- electrophoresis power supply
- analytical balance
- pH meter
- oven
- Bunsen burner, heating plate, or microwave
- glass or plastic support, approximately 7.5 cm x 5 cm
- pipette
- pipette tips
- microcentrifuge tubes
- gel bond
- filter paper
- weight (optional)
- stereomicroscope

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Reagents

This procedure uses the following reagents:

- 20% w/v Sodium Hydroxide
  – Dissolve 20 grams of sodium hydroxide in deionized water to bring to a volume of 100 mL.
- 1 M NaCl
  – Dissolve 58.4 grams of NaCl in deionized water to bring to a volume of 1 liter.
- 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

Buffer

This procedure uses the following buffer:

For 1 liter, mix:

- 21.8 g glycine
- 4.5 g Trisma base

Dissolve the chemicals in deionized water and bring to final volume to 1 liter. Check the pH and adjust to 8.4 using 20% w/v Sodium Hydroxide (see *Reagents*, above).

Store buffer solution in refrigerator at 5°C ± 5°C.

If problems are noted while performing this procedure (for example, no precipitin bands are visible between the human standard and the anti-human serum), discard the buffer.

NOTE: after every five uses, discard the buffer, rinse the tank, and refill the tank with buffer.

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Support medium

Using heat, dissolve 0.10 grams of agarose (SERI type E25 or agarose of similar EEO) in 10 mL of buffer.

Standards and controls

The following standards and controls must be used in each test:

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

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 *Crossover Electrophoresis 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.

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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.

Procedure

Use the following procedure to perform crossover electrophoresis:

Step	Action
1	Pour buffer into tank. Allow buffer to reach room temperature.
2	Pour the support medium on the hydrophilic side of the gel bond, using a glass or plastic plate for support.
3	Punch wells.
4	Load 5 µl of antiserum into the anodic wells and 5 µl of sample, known standard, and blank into the cathodic wells.
5	Place the gel in the tank. Fill the tank with enough buffer to submerge the current lines on the gel and run the gel for a minimum of 30 minutes at 120 volts.
6	Remove gel from tank. Wash gel overnight in 1 M NaCl.
7	Rinse in deionized water, cover with filter paper (and a weight, if desired) to remove water, and 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	Photograph the stained gel and record the results on the *Crossover Electrophoresis Worksheet*. Import image into worksheet. Seal the original gel in clear packaging and store it with the evidence.

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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.
- The absence of a precipitin band indicates a negative (-) reaction.
- Cross-reactions with closely related species may occur. See product insert, located in the *Biology Quality Control Log Book,* for further information.
- If bands are unclear or not in the proper position between wells, the test is inconclusive. The sample should be reanalyzed.

References

The following reference was used in the development of this procedure.

Methods Manual, Serological Research Institute.