

DNA Analyst Training Laboratory Training Manual

Protocol 2.16
Bloodstain Indication: Ouchterlony Test



This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the Illinois State Police. It has been included for training and example purposes only.

PRESIDENT'S
DNA
INITIATIVE



INTRODUCTION

Double immunodiffusion is a method which allows the immunological identification of a protein. The identification is made by simultaneously comparing the reaction of the unknown protein with the reaction of a known protein against a known antiserum.

SAFETY CONSIDERATIONS

Sodium Azide - Highly Toxic!
Danger! Reproductive Hazard!

PREPARATIONS

1/2% Agar Gel	
Agar	0.5 g.
Sodium chloride	0.85 g.
Sodium azide	0.01 g. (optional preservative)
Distilled water	100 ml.

1. Boil with constant stirring until agar is completely dissolved.
2. Pipette about 3 ml. of agar solution in 30 2-inch disposable plastic petri dishes and allow to cool on a level surface. Place the cover on each petri dish and store upside down in the refrigerator to prevent the gels from dehydrating.

INSTRUMENTATION

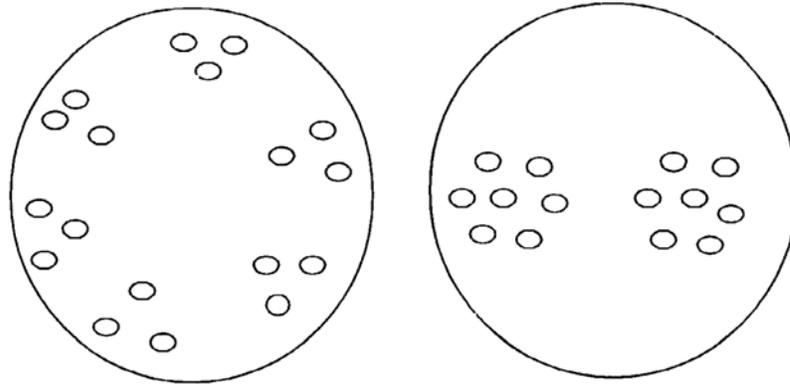
No Instrumentation Required.

MINIMUM STANDARDS & CONTROLS

A known sample for which the antiserum is directed against and a negative control.

PROCEDURE OR ANALYSIS

1. Using a template drawn on a plastic petri dish cover, punch the desired pattern of wells into the gel with a vacuum pipette. The pattern may be a series of 3 wells forming an equilateral triangle or 7 wells forming a hexagon with a central well. See below:



Note: When screening an unknown bloodstain to determine animal species origin, it is economical to use the hexagonal shape. Place an extract of the stain in the central well and antisera for 6 different species around it. Any antisera that forms a precipitin band must be checked by the conventional method described below. This method is only a screening technique to eliminate a majority of the possible species.

2. When using the customary 3-well pattern, position the wells in a triangle with apex above. (See diagram below.)
 - a. In the apex well, place antiserum.
 - b. In the right base well, place the known standard antigen extract or diluted serum.
 - c. In the left base well, place the questioned stain extract.

○ Anti-Human Serum

○ Extract of Questioned Bloodstain

○ Extract of Known Human Bloodstain

3. Set up a test triangle with the known sample in both left and right wells. This is a positive control.

4. Set up a triangle with a sample blank on one side, the known sample on the other. This serves as a negative control.
5. Cover the petri dish and leave undisturbed overnight. Refrigeration is not normally required.
6. Read the plate the following morning with the aid of a lamp. Precipitin bands which form a continuous arc of convergence (identity band) between the antiserum well and the 2 extract wells are considered positive results.

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