

DNA Analyst Training Laboratory Training Manual

Protocol 2.09
Saliva Stain Indication:
Radial Gel Diffusion 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

This test is developed as a preliminary screening test to aid in the identification of saliva stains.

SAFETY CONSIDERATIONS

NaH₂PO₄ - Sodium Phosphate Monobasic - Caution! Irritant!

Na₂HPO₄ - Sodium Phosphate Dibasic - Caution! Irritant!

KI - Potassium Iodide - Caution! Irritant!

Iodine - Caution! Irritant!

Incompatibilities - Acetylene & Ammonia

PREPARATIONS

Reagent Preparation:

1. Phosphate buffer, pH 6.9 prepared as follows:

NaH₂PO₄, anhydrous 2.7 g. (.01m)

Na₂HPO₄, anhydrous 3.9 g. (.01m)

NaCl 0.2 g. (.002m)

Distilled water 500 ml.

2. Gel test plates (2% agarose, 0.1% soluble starch)

Buffer, pH 6.9 10.0 ml.

Agarose 0.2 g.

Soluble starch 0.01 g.

Heat to boiling and continue stirring constantly until all the agarose is dissolved. Divide gel solution and pour into 3-2" disposable plastic petri dishes. Allow to polymerize completely. Store gels inverted (to retard dehydration) at 4°C.

3. Iodine development solution

KI 1.65 g.

I₂ 2.54 g.

Distilled H₂O 30 ml.

Dissolve by stirring for 5 minutes at 65°C in a fume hood. Store saturated I₂ solution in dark stoppered bottle.

Working solution in 1/50 dilution with distilled water.

Sample Preparation:

Extract a small piece of stained material with 50 µl of distilled H₂O.

INSTRUMENTATION

No Instrumentation Required.

MINIMUM STANDARDS & CONTROLS

1. Positive Controls: known dilution of fresh liquid saliva (1/500 in H₂O).
2. Negative control: distilled H₂O.

PROCEDURE OR ANALYSIS

1. Punch holes in gel plate with a vacuum pipette, leaving 1.5 cm. between the sample wells.
2. Place samples to be tested in the sample wells using a precision pipettor. Each well holds approximately 4 µl of liquid.
3. Cover the petri dish and place in an incubator at 37°C. for 6 hours or overnight.
4. Stain the plate by pouring a 1:50 dilution of saturated iodine solution onto the surface. Rinse with H₂O.
5. Clear circles around the wells indicate areas of amylase activity. The diameter of the clear circle is proportional to the square root of the concentration of amylase. Record the diameter and results in notes.
6. A positive test is one in which the ring size is equal or greater in size than the positive control.
7. An inconclusive result is one in which the ring size is less than the positive control but greater than the negative control.
8. A negative result is an absence of any clear ring.

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