DNA Analyst Training
Laboratory Training Manual

Protocol 2.15
Bloodstain Indication: Kastle-Meyer 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 utilized as a preliminary screening test for blood.

SAFETY CONSIDERATIONS

Hydrogen Peroxide 30% - Danger! Corrosive!

Phenolphthalein - Caution! Irritant!

Potassium Hydroxide - Danger! Corrosive!

Zinc powder or dust in contact with water or damp air evolves hydrogen. The heat of reaction is sufficient that the hydrogen may ignite. Therefore, zinc should not be discarded in the wastebasket. The following procedure should be followed for less than 20 grams of zinc dust:

1. Follow standard laboratory chemical handling practices and work in the hood with the hood on, wearing safety glasses and rubber gloves. With the zinc in a large beaker, add small amounts of concentrated hydrochloric acid with a pipet. The solution will bubble and give off heat. Proceed slowly. Allow time for the bubbling and heat to dissipate before adding more acid. Continue slowly adding acid until no more bubbles are formed and no gray powder is visible (about 3 mls. HCl for 1 gram of zinc).

2. When all the zinc has dissolved (forming soluble zinc chloride), cautiously neutralize the acid solution by adding small amounts of sodium carbonate. Again, foaming will occur. Continue slowly adding sodium carbonate until no more bubbling occurs (about 2 g. sodium carbonate for 1 gram of zinc). At this point, all the zinc should now be in the form of zinc carbonate, a white precipitate.

3. The zinc carbonate may be filtered out of solution and disposed of in a trash can since zinc carbonate is nontoxic.

PREPARATIONS

Stock Solution:	Phenolphthalein	2 g.
	Potassium hydroxide	20 g.
	Distilled water	100 ml.
	Zinc dust	20 g.

Mix, add a few boiling chips and boil under reflux 2-3 hours or until the solution has lost all its pink color. Cool and decant into a bottle containing some zinc to keep it in the reduced form.

Working Solutions:

Solution #1:	Ethanol	10 ml.
Solution #2:	Phenolphthalin stock solution	2 ml.
	Distilled water	10 ml.
	Ethanol	2 ml.
Solution #3:	3% Hydrogen peroxide	10 ml.

(Dilute 30% Hydrogen peroxide to 3% using distilled water)

INSTRUMENTATION

No Instrumentation Required

MINIMUM STANDARDS & CONTROLS

A known bloodstain and negative control.

PROCEDURE OR ANALYSIS

1. A small cutting, swabbing, or extract of the suspected bloodstain is placed on filter paper or spot test paper.

2. Two or three drops of ethanol are placed on the stain.

3. Two drops of the working solution of phenolphthalin are added to the stain.

4. After waiting to insure that no color develops at this stage, two or three drops of 3% H_2O_2 are added.

5. An intense pink color is a positive test for peroxidase activity, indicative of hemoglobin. This is not a confirmatory test for blood.

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