Examination of Blood and Blood Stains

Cases: - Murder

Rape

Assault

Accident

Evidence: - fresh/ slash/ dried

Spattering on wall

Pull of blood

Clothes

Bed sheets

Floor

Gun

Weapon

Bloody finger print

Rope

Wash down blood stains

Bathroom

Nail beads

Bite mark

Washbasin

Examination:

1) **Physical Examination:-**

   In natural light examination of exhibits for brown, reddish brown stains, powder or crystals of reddish brown color these areas should be demarcated. In case of absence of clear and visible stains, washed stains should be examined under 230-269 nm frequency UV light.

2) **Presumptive Test:-**

   These suspected blood stains contaminated materials should be tested for positive for blood.

a) **Tetra methyl Benzidine (TMB) Test:**

Place a cutting or swabbing of the stain on filter paper or spot test paper. A drop of TMB solution is placed on the stain, followed by a drop of 3% hydrogen peroxide. An immediate blue-green color is a positive test for peroxidase activity, indicative of hemoglobin.

b) **Phenolphthalein Test (Kastle-meyer test):**

Place a cutting or swabbing of the stain on filter paper or spot test paper.

Two or three drops of ethanol are placed on the stain. Two drops of working phenolphthalein solution are added to the stain. After waiting to insure that no color develops at this stage, two or three drops of 3% hydrogen peroxide are added. An intense pink color is a positive test.

3) **Confirmatory Test:**

a) **Takayama Test:**

Place material to be tested on a microscopic slide and cover with a cover slip. Add a drop of takayama reagent and allow to flow under the cover slip. Warm slide gently on a hot plate at 65 degree Celsius For 10-20 seconds. Allow to cool and observe under microscope at 100x. The appearance of pink needle shaped crystals of pyridine hemochromogen (pyridine ferroprotoprophyrin) is a positive reaction of heme.

b) **Teichmann's Test:**

Place material to be tested on a microscopic slide and cover with a cover slip. Let the reagent flow under the cover slip. Warm slide gently on a hot plate at 65 degree Celsius For 10-20 seconds. Allow to cool and observe under microscope at 100x. The appearance of brown rhombohedron shaped crystals of ferroprotoprophyrin chloride is a positive reaction of heme.

c) **Spectrophotometric Estimation:**

To a 1 cm long stained thread, add 10 ml of 0.2% sodium lauryl sulphate. Incubate at 37 degree Celsius for 15-20 minutes. Add 10 ml of 0.2% mercaptoethanol in 1 % NH_3 solution and mix. Transfer liquid to a microscopilary cuvette. On a spectrophotometer monitor the reaction at 560 nm against a reaction blank until absorption reaches maximum. When the reaction is complete, after 5-10 min scan the sample between 600 and 500 nm. Two peaks, which are clearly defined at 558 nm and 529 nm, indicate the presence of hemoglobin derivatives.