Presumptive Tests - Blood

What is a presumptive test?
- It is an indication of probability
- Helps establish what type of further testing should be employed

Presumptive Test - Blood
- Positive Reaction with presumptive tests DO NOT indicate that substance is definitely what you’re testing for
- Is the stain really blood?
  - Visual Exam

Presumptive Test - Blood
- Properties of a good presumptive test:
  - Sensitive and specific to heme
  - Relatively stable and non-toxic
  - Reliable and economically feasible to use
  - Non-destructive to evidence and crime scene
  - Meets the Frye standard for general acceptance

Presumptive Test - Blood
- How do presumptive tests work?
  - They function as a result of chemical, catalytical, or enzymatic reactions with blood enzymes or the hemoglobin found in red blood cells
  - Produce a visible color reaction
  - Produce a “light” reaction

Presumptive Test - Blood
- Sensitivity is the amount by which blood may be diluted and still produce a reaction to a preliminary blood test
- Specificity is the likelihood with which a preliminary blood test will give a true reaction ONLY in the presence of hemoglobin

Presumptive Test - Blood
- Phenolphthalein Test
  - Also known as Kastle-Meyer Test
  - Its sensitivity is **1:10,000**
  - It is specific due to chemical activity of hemoglobin in animal blood
  - Consists of 3 steps:
    - alcohol
    - phenolphthalein
    - hydrogen peroxide
  - The alcohol’s function is to increase the sensitivity of the test
    - By breaking down the globin and exposing the heme, it “cleans up” the area in and around the bloodstain to better expose the hemoglobin
  - The phenol’s function is to produce a color change (from clear to pink) when oxidized
  - The hydrogen peroxide’s function is to do the oxidizing
    - The oxygen radical is freed and binds itself to the phenol: this is our pink color change

- TMB (tetramethylbenzidine)
  - Produces a **green** color when heme reacts with sodium perborate
  - Sensitivity is comparable to KM
  - Most benzidine derivates were believed to be carcinogenic
Luminol (3-aminophthalhydrazide)
- Produces a luminescent reaction in the presence of heme
- Sodium perborate is the oxidant which reacts with luminol
- Its **Sensitivity is up to 1:1,000,000**
  - Excellent for visualizing blood that has been cleaned up
- 3 ingredients:
  - Sodium carbonate: adjusts pH to make solution basic
  - Luminol: acts as your luminescing indicator!
  - Sodium perborate: acts as the oxidant
- This test is applied directly to suspected evidence, not filter paper or swabs
- Best to perform this test only after other tests have been utilized
- Reacts with certain other chemical oxidants, metal salts, and plant peroxidases

**Advantages:**
- Highly sensitive
- Helps visualize large areas of cleaned blood

**Disadvantages:**
- Reaction can only be observed in darkness
- Exams can be tricky in confined spaces
- Reagent is basic (slippery feeling)

Presumptive Test - Blood
- Will they interfere with DNA analysis?
  - DNA analysis targets nuclear material in WBCs
- Protein stains won’t affect presumptive blood tests

**Other Bodily Fluids: Semen & Saliva**

Semen
- **Obvious Forensic Importance**
  - Fluid commonly demonstrative of sexual assault
- How do we visually examine for semen?
  - Check items/areas where you would expect
  - Look for whitish crusty stain (yellows with age)
  - Don’t be fooled; detergents and blood will give same reaction with UV light

Semen – Presumptive Test
- **Acid Phosphatase**
  - Enzyme secreted by prostate into seminal fluid
  - Not unique to seminal fluid
  - Can be found in breast milk and contraceptive creams, among other items

Saliva
- **Associated with violent crimes**
- Dried stains nearly invisible to naked eye
- Check items you would expect

Saliva – Presumptive Test
- **Radial Diffusion/Starch Iodine**
  - Labs may perform test to determine levels of alpha-amylase
- Interpretation of test:
  - Finding amylase DOES NOT PROVE saliva is present
  - Amylase is found in many other body fluids
- **Other Presumptive Tests**
  - Press Test
Tube Test using *Phadebas* Reagents

**Confirmatory Tests: Bodily Fluids**

**Blood – Confirmatory Tests**
- Crystal Tests
  - Useful because blood has its own crystalline structure
  - Teichmann:
    - Blood is heated in presence of acid and chloride
    - Developed in 1853
  - Takayama:
    - Chemicals added to blood sample
    - Developed in 1912

**Species Origin Determination**
- Ring Precipitin:
  - Antisera added to extract of suspect bloodstain
  - Precipitate forms at juncture
  - If extract of bloodstain is not human, no reaction occurs
- Ouchterlony:
  - Gel Diffusion
  - Antibodies and Antigens move toward one another
  - Produces line of precipitation

**Semen – Confirmatory Tests**
- p30 (PSA):
  - Prostate Specific Antigen
  - Unique to seminal fluid
  - Enzyme responsible for liquified semen after clotting
- Microscopic ID:
  - Use “Christmas Tree” Stains to visualize
  - Positively ID sperm when heads are separated from tails