BIOLOGICAL EVIDENCE

Semen, Saliva, Urine and Feces

Semen

- Avg. ejaculate is 3.5mL
  - Each mL can contain between 10-50 million sperm cells
- Medical Conditions:
  - *Oligospermia* – low sperm count
  - *Aspermia* – no sperm
  - *Vasectomy* –surgical procedure that leaves the male incapable of producing sperm

Semen: Presumptive Tests

1. Alternate Light Source
   - Semen fluoresces under ultraviolet light
   - Blue-yellow fluorescence

- False positive: many other substances will fluoresce under the same light

Semen: Presumptive Tests

2. Acid Phosphatase Test (AP)

- Male prostate gland produces the enzyme *acid phosphatase* and secretes it into seminal fluid
- 2 reagents used:
  - Alpha-naphthyl acid phosphate
  - Brentamine Blue
- If acid phosphatase is present, it will turn dark purple/blue

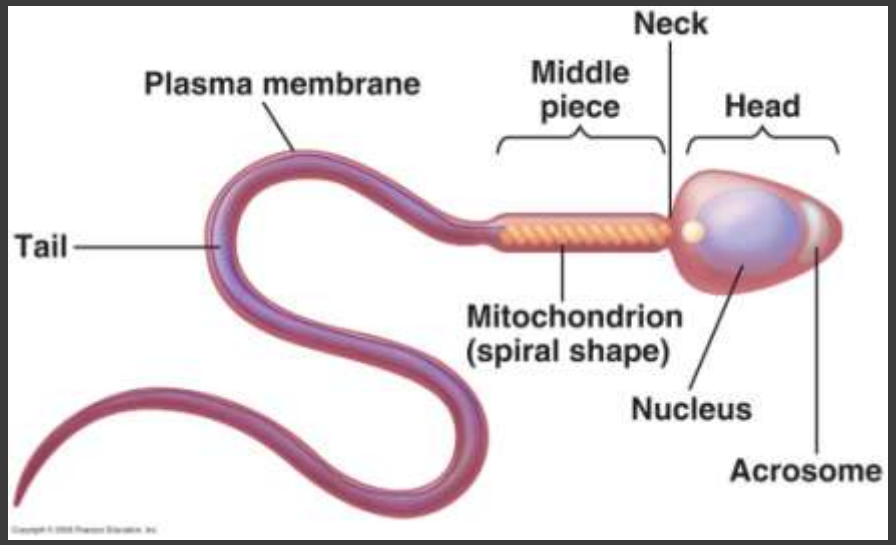
- False positives: other body fluids like vaginal secretions contain this enzyme

Semen: Presumptive Tests

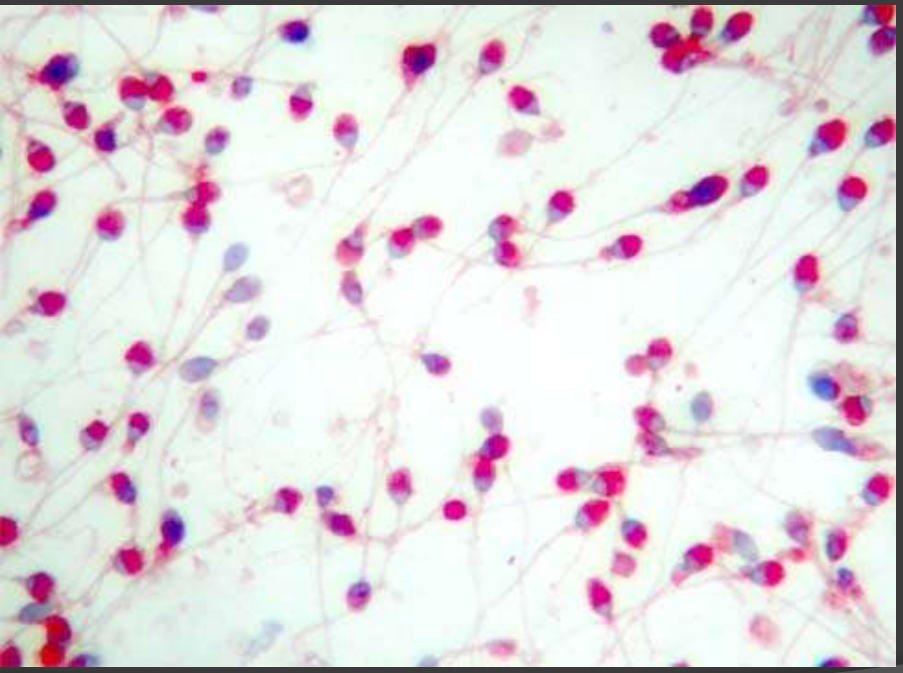
3. Prostate Specific Antigen (PSA) or P30
   - PSA or P30 is produced by the prostate gland
   - ABAcard test like Hematrace
- False positives: P30 can be found in feces, sweat, and female urine and breast milk

Semen: Confirmatory Tests

1. Visual confirmation of sperm under the microscope
   - Christmas Tree Stain
     - Picroindigocarmine stains the neck and tail green
     - Nuclear Fast Red stains the head and acrosomal cap a reddish/pink color

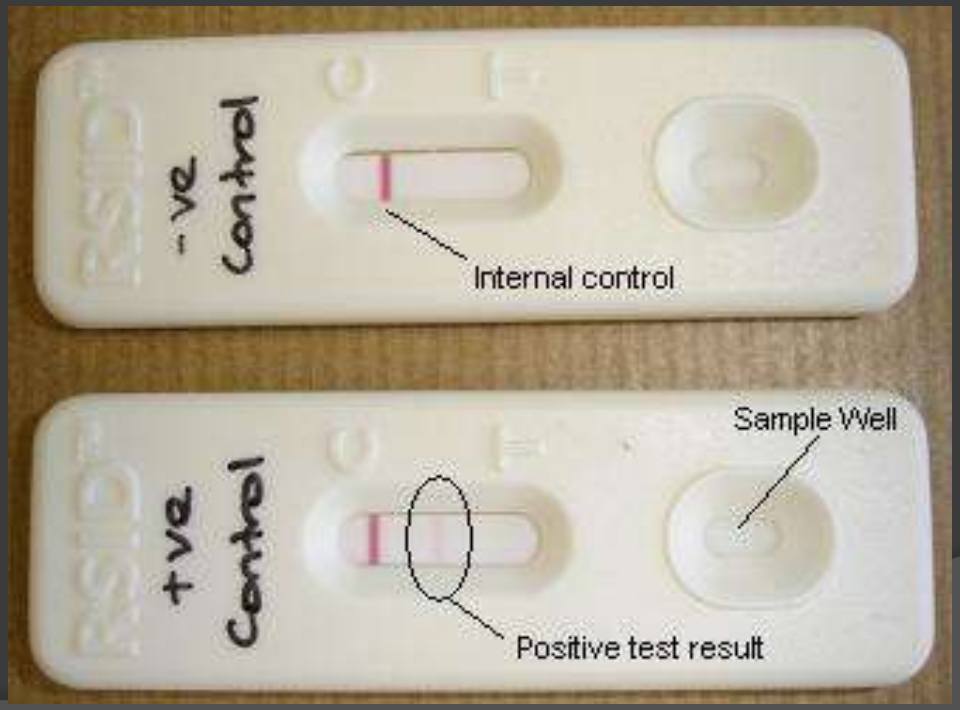

Neck
Plasma membrane
Middle piece
Head
Tail
Mitochondrion (spiral shape)
Nucleus
Acrosome


Sperm cell with intact tail



Semen: Confirmatory Tests

2. RSID- Semen (Rapid Stain Identification)
   - Test for presence of semenogelin
   - No cross reactivity with other body fluids or animals



Saliva

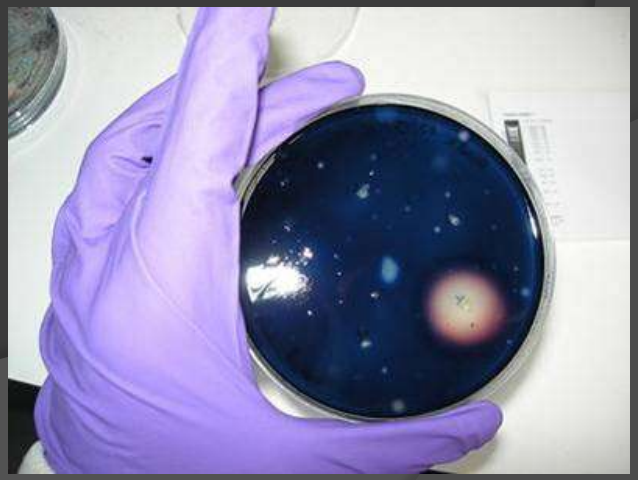
- Human salivary glands produce 1.0-1.5 liters per day
  - Mostly water
  - *Amylase* – an enzyme that digests starch in the mouth and intestine
    - breaks down starch into simple sugars
- Two types of amylase
  - β – amylase
  - α - amylase

Saliva

- β – amylase (beta amylase)
  - plant and bacterial sources
- α – amylase (alpha amylase)
  - human sources
  - found in saliva **and** pancreas

Saliva: Presumptive Tests

1. Starch-iodine assays
   - E.g. Amylase overlay assay and amylase radial diffusion
   - Tests for presence of starch
   - If starch is present - will change to dark blue-black in the presence of iodine



Saliva: Presumptive Tests *Starch-Iodine Assays*

- False positive reactions
  - any substance with amylase activity
    - e.g. bacteria, plants, vomit
- Not species-specific
  - reacts with saliva from any animal that produces it
    - cats and dogs DO NOT produce amylase

Saliva: Presumptive Tests

2. Phadebas method
   - Add saliva stain to water
   - Add tablet that consists of insoluble starch bound to a blue dye
   - If amylase is present, it will break down the starch and release the blue dye

Saliva: Confirmatory Tests

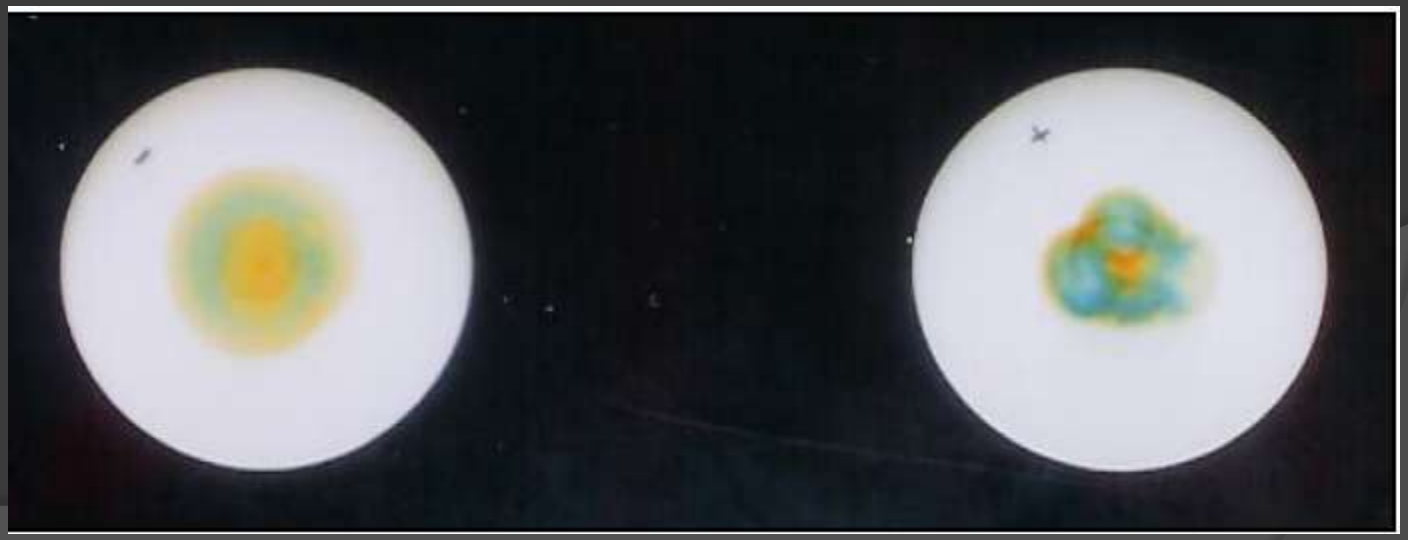
- RSID- Saliva
  - Distinguishes between salivary and pancreatic amylase
  - Very sensitive
    - Your hands would probably test positive due to mouth-hand contact
  - False positive – will react with amylase from gorillas and rats

Urine

- Not tested for often in forensics
- All animals must get rid of excess nitrogen from the breakdown of proteins and amino acids
  - Aquatic animals get rid of nitrogen as ammonia
  - Birds and terrestrial animals use uric acid
  - Mammals use **urea** and expel it in urine

Urine: Presumptive Tests

1. Urease Test
   - Uses Whatman Filter Paper
   - If urea is present, then urease will catalyze the reaction of urea to ammonia
   - The paper will turn blue



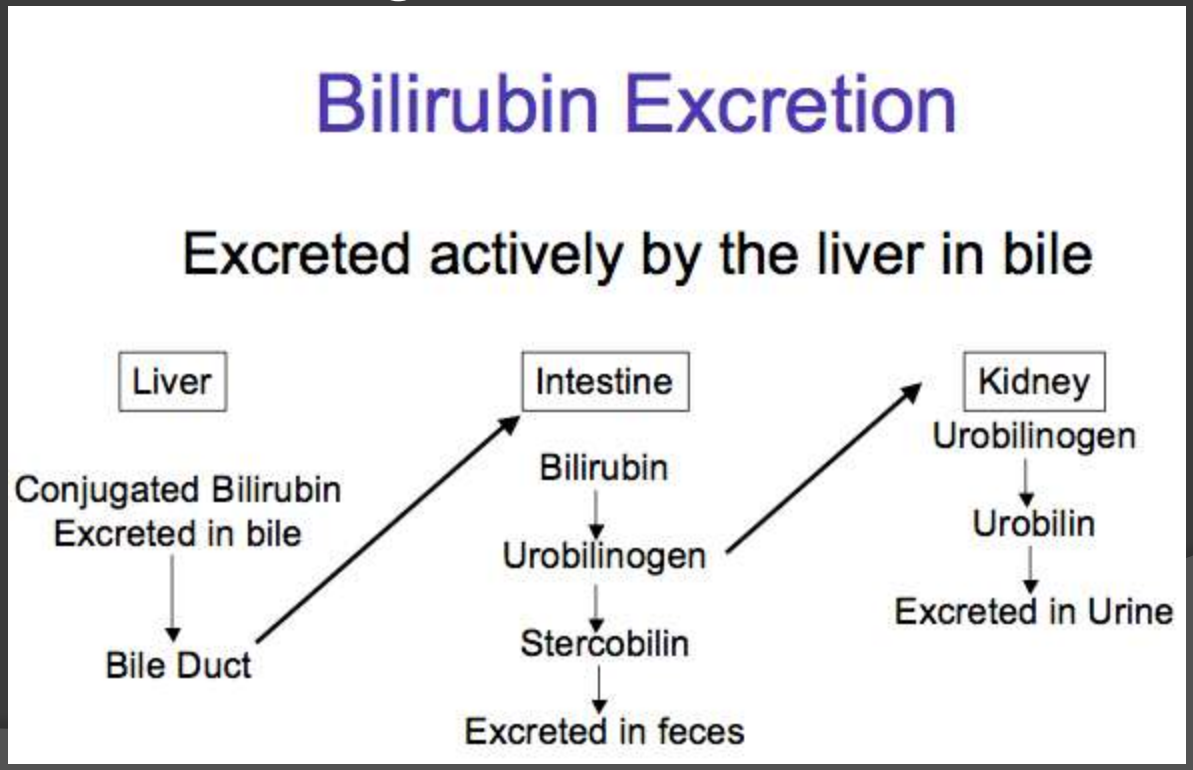
Urine: Presumptive Test

2. RSID-Urine
   - tests for a glycoprotein abundant in urine
   - NOT HUMAN SPECIFIC

- No confirmatory tests for urine!

Feces

- Waste product called bilirubin is excreted in feces…gives it the brown color
- Bacteria in the gut also break down bilirubin into urobilinogen



Feces: Presumptive Test

- Tests for the presence of urobilinogen
- If present, will fluoresce under UV light
  - Fluoresces green

Feces: Confirmatory Tests

- Microscopic Test
  - Look for the presence of animal and plant cells due to digestion