

Biological Screening Workshop

Other Body Fluids and Tissues



Vaginal Secretions

- Vaginal secretions are a complex mixture of cells and secretions
- There is no confirmatory test to identify vaginal secretions
- Several screening tests based on microscopy have been proposed



Vaginal Secretions

- Vaginal epithelial cells are large, and many contain glycogen (a polysaccharide) which can be demonstrated by staining with iodine in the form of a solution or exposing to iodine vapor
- The presence of glycogenated cells is variable depending on the stage of the estrous cycle
- Glycogenated cells can be found in other body secretions (i.e. oral, anal)



Lugol's Staining





Fecal Material

- Feces are food residues passed after completion of travel through the digestive system
- Has a characteristic odor mainly due to skatole, an organic compound that occurs naturally in feces



Fecal Material

- Microscopy
 - Microscopy has been used to identify fecal material
 - Looking for undigested residues of food material
- Chemical Tests
 - Detection of urobilinogen, a bile pigment excreted in feces, which may be detected using its fluorescent reaction to Edelman's reagent





Urine

- No confirmatory tests for the presence of urine
- Urine stains fluorescent under ultraviolet light
 - This can be useful for locating stains prior to chemical testing
- Has a characteristic odor



Urine

- Contains a large amount of urea, a chemical byproduct of normal metabolic processes in the body
 - Identification of high levels of urea can serve as a screening test for urine in fluids or stains
 - Perspiration can give reactions similar to urine



Urea – Litmus Paper Test

- Litmus paper test for the detection of ammonia
- Relies on the indirect identification of urea by reacting a test sample with urease to generate ammonia from the urea
- Litmus paper is used to detect the ammonia

Urea +
$$H_2O \leftrightarrow CO_2 + 2NH_3$$



Urea – Litmus Paper Test

- A known urine sample and a blank are tested as a positive and negative control
- A substrate control may be tested, as needed



Urea – Nitrogen Tube Test

- Relies on the indirect identification of urea by reacting a test sample with urease to generate ammonia from the urea
- Ammonia is subsequently identified by the production of a deep blue-colored solution

Urea +
$$H_2O \leftrightarrow CO_2 + 2NH_3$$



Urea – Nitrogen Tube Test – How to Perform

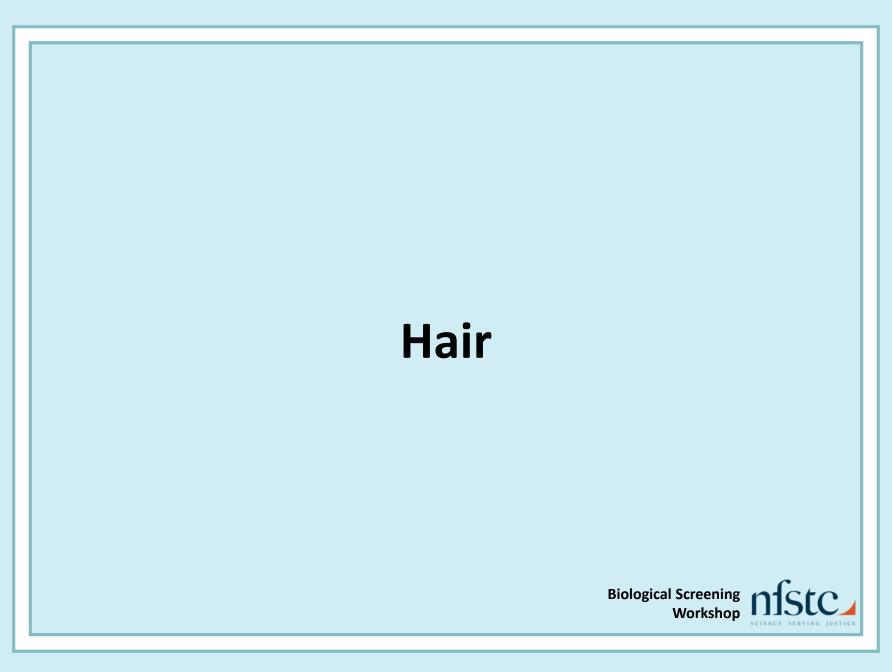
Sample	Expected Results
Negative Control	No color
Standard	Deep blue color
Positive Control	Deep blue color
Substrate Control	No color
Question Sample (without urease)	No color



Creatinine

- Jaffe Test
 - One of the oldest tests for the detection of creatinine-1886
 - Creatinine forms a red compound with picric acid
 (Jaffe test)





- Composed of cylindrical structures or shafts made up of tightly compacted cells that grow from follicles
- Diameter ranges from 15 to 120 μm
 - Depends on type of hair and body region
- Root material can be used for nuclear DNA testing
- Shaft material can be used for mtDNA testing



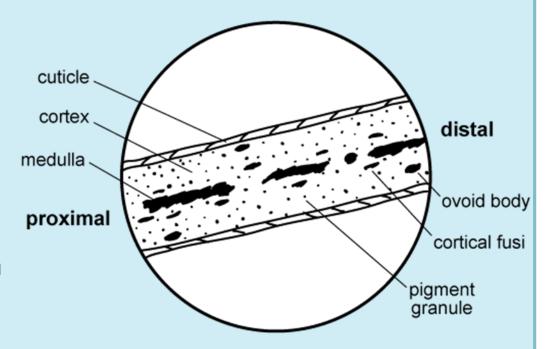
- Human hairs are distinguishable from hairs of other mammals
 - Human hairs are generally consistent in color and pigmentation throughout the length of the hair shaft
 - Animal hairs may exhibit radical color changes in a short distance, called banding



- The medulla, when present in human hairs, is amorphous in appearance, and the width is generally less than one-third the overall diameter of the hair shaft
- The medulla in animal hairs is normally continuous and structured and generally occupies an area of greater than one-third the overall diameter of the hair shaft



- Structure
- Three cell types
 - Outer cuticle
 - Central cortex
 - Central medulla



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- In some instances human hairs can be classified by racial origin such as:
 - Caucasian (European origin)
 - Negroid (African origin)
 - Mongoloid (Asian origin)
- In some instances the region of the body where a hair originated can be determined by its gross appearance and microscopic characteristics



- The length and color can be determined
- It can also be determined whether the hair was forcibly removed, damaged by burning or crushing, or artificially treated by dyeing or bleaching



- The growth phase of the hair is important in determining whether the root is suitable for nuclear DNA analysis testing
- Growth Cycles
 - Anagen phase
 - Catagen phase
 - Telogen phase



Hair – Anagen Phase

- Active hair growth
- Contains nucleated cells in the root and in the surrounding sheath material
- Generally suitable for nuclear DNA analysis



Hair – Anagen Hair



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Hair – Catagen Phase

- Transitional phase after active hair growth, cell division stops
- Characteristic club appearance of root
- May be suitable for nuclear DNA analysis



Hair – Catagen Hair



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Hair – Telogen Phase

- Follows transitional phase-growth ceases
- Shedding phase
- Telogen hairs without follicular tissue may not be amenable to nuclear DNA analysis because of the lack of nucleated cells
 - May contain sufficient mitochondrial DNA in their roots and hair shafts for analysis



Hair – Telogen Hair



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Hair - Basic Evaluation Steps

- 1. Determine if the sample is a hair.
- 2. Determine if the hair is of human origin.
- 3. Determine if the hair has root material that is suitable for nuclear DNA analysis (characteristic of a particular growth phase).
 - If not suitable for nuclear DNA analysis, determine if the hair is sufficient in size for mtDNA analysis (two to three centimeters)



- DNA analysis of hair is a destructive technique and results in the consumption of portions of the hair
 - Hair characteristics, such as color, length, shape, and texture should be noted in the case file for future reference prior to DNA analysis
 - Notes and digital images



Questions? Biological Screening Workshop