Model Answers

M.Sc (III[rd] Semester) Examination, 2013

Forensic Science

Paper: III

(Forensic Biology and Serology)

AS-2336

I. Multiple Choice Questions

(i). While conducting Luminol test, luminol reacts with hydrogen salt and forms:
(a) Di-anion (b) Cation (c) Anion (d) All of the above
Ans: (a). Di-anion

(ii) Electrophoresis is mainly used for:
(a) Differentiate the biological sample
(b) To perform the human specific presumptive tests
(c) DNA isolation from biological material
(d) Separates the molecules
Ans: (d). Separate the molecules

(iii). Restriction enzymes are used in one of these techniques:
(a). Sequencing (b). Genotyping (c). RFLP (d). Polymerization
Ans: (c). RFLP

(iv). Which of the following statement is false
(a). Enzymes are differentiated by electrophoresis method
(b). While DNA sequencing both forward and reverse primers are used
(c). Amplification is done through PCR
(d). ABO blood grouping is mainly used for differentiating individuals
Ans: (b). While DNA sequencing both forward and reverse primers are used

(v). The fluorescence examination of the seminal stains indicates
(a). Pink color (b). White color (c). Blue color (d). Red color
Ans: (b). Blue color

(vi). The presumptive test for semen is
(a). Acid phosphatase test
(b). Sodium alpha naphthyl test
(c). Napthanil diazo test
(d). Barbiturate test
Ans: (a). Acid phosphatase test

(vii). For examination of diatoms sample should collect from
(a). Bone marrow (b). Blood (c). Tissue (d). Epithelial cells
Ans: (a). Bone marrow

(viii). A study of relationships between organisms and their environment
(a). Ethnology (b). Ecology (c). Monospecific (d). Monoecious
Ans: (b). Ecology

(ix). In wild life Forensics, identification of animals done by
(a). Grouping (b). Feathers (c). Twigs (d). Pug marks
Ans: (d). Pug marks

(x). Illegal way of trafficking animals
A. Kidnapping B. Poaching C. Harboring D. Smuggling
Ans: (b). Poaching

II. Long Answer Questions

2. Describe the identification methods of blood? Forensic significance of biological materials.
Ans:
The Forensics of Blood
After a homicide or an assault has been committed, police investigators usually find blood at the scene of the crime, giving them clues as to what happened. The blood's texture and shape and how it is distributed around the victim often help investigators determine when the crime was committed, whether the crime was preceded by a fight between individuals, and which weapon was used—say, a knife, a gun, or an object used to hit a person.

Physical Examination:
In natural light examination of exhibits for brown, reddish brown stains, powder or crystals of reddish brown colour, 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.

Luminol Tests:
The luminol is first activated with an oxidant, usually a solution of hydrogen peroxide and a hydroxide salt in water. When luminol reacts with the hydroxide salt, a di-anion is formed. The oxygen produced from the hydrogen peroxide then reacts with the luminol di-anion. Luminol is sensitive to the presence of extremely small amounts of blood.

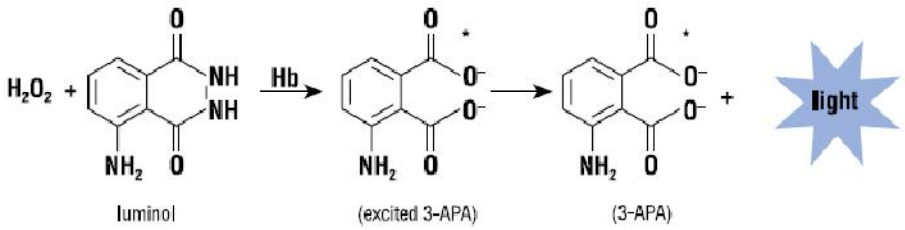


Fig 1. A chemical reaction between hydrogen peroxide (H_2O_2) and luminol (molecule pictured to the left) in the presence of hemoglobin (Hb) produces 3-aminophthalic acid (3-APA) and blue light.

Presumptive Test:

These suspected bloodstains; contaminated materials should be tested for positive for blood.

Tetramethyl Benzidine (TMB) Test:

NOTE: TMB is carcinogenic. Use of gloves is required.

Reagent Preparation:

Acetate Buffer

Sodium acetate	5.0g
Glacial Acetic Acid	43.0 ml
Deionized Water	50.0 ml

Working Solution

TMB	0.4g
Acetate Buffer	20.0 ml

Mix, filter and store in brown coloured bottle in refrigerator.

Procedure:

1. Place a cutting or swabbing of the stain on filter paper or spot test paper.
2. A drop of TMB Solution is placed on the stain, followed by a drop of 3% Hydrogen Peroxide.
3. An immediate blue-green colour is a positive test for peroxidase activity, indicative of hemoglobin. This is not a confirmatory test for blood.

OR

Phenolphthalein Test (Kastle-Meyer Test):

Reagent Preparation:

Stock Solution:

Phenolphthalein	2.0 g
Potassium Hydroxide	20.0g
Distilled Water	100 ml
Zinc Dust	20.0 g

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

Working Solution:

Solution # 1:	Ethanol	10 ml
Solution # 2:	Phenolphthalein Stock	2 ml
	Distilled Water	10 ml

	Ethanol	2 ml
Solution # 3:	3% Hydrogen Peroxide	10 ml

Procedure:
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 working phenolphthalein solution are added to the stain.
4. After waiting to insure that no colour develops at this stage, two or three drops of 3% Hydrogen Peroxide are added.
5. An intense pink colour is a positive test for peroxidase activity, indicative of hemoglobin. This is not a confirmatory test for blood.

Confirmatory Test:
Stains positive for the presumptive test should be further examined by the following tests:

Takayama Test:
Reagent Preparation:

Standard Glucose Solution (100g/100ml)	3 ml
10% Sodium hydroxide	3 ml
Pyridine	3 ml
Distilled Water	7 ml

Note: Reagents should be made fresh daily.

Procedure:
1. Place material to be tested on a microscopic slide and cover with a cover slip.
2. Add a drop of Takayama Reagent and allow to flow under the cover slip.
3. Warm slide gently on a hot plate at 65oC for 10-20 seconds
4. Allow to cool and observe under microscope at 100X.
5. The appearance of pink needle shaped crystals of pyridine hemochromogen (Pyridine ferroprotoprophyrin) is positive reaction for heme.

OR

Teichmann's Test:

Reagent Preparation:

Potassium Chloride or	0.1 g
Potassium Bromide	0.1 g
Potassium Iodine	0.1 g
Glacial Acetic Acid	100 ml

Mix and store in stoppered bottle.
Procedure:
1. Place material to be tested on a microscopic slide and cover with a cover slip.
2. Let the Reagent flow under the cover slip.
3. Warm slide gently on a hot plate at 65oC for 10-20 seconds.

4. Allow to cool and observe under microscope at 100X.
5. The appearance of brown rhombohedron shaped crystals of ferroprotoprophyrin chloride is a positive reaction for heme.

Forensic significance of biological materials:

Biological fluids such as blood, semen, and saliva are frequently encountered as physical evidence in many types of criminal investigations such as homicides, sexual assaults, assaults, and robberies. Since blood, semen, and saliva originate as liquids, they quickly coat or penetrate surfaces they are deposited on, and can be difficult to remove when dried. Because no two humans are genetically the same (except for identical twins) these body fluids are unique to the person they originated from. By performing DNA analysis of these fluids or stains, a genetic marker profile can be obtained which can then be compared to DNA profiles obtained from reference standards or from other items of evidence.

3. Describe about the DNA markers and their uses in Forensic cases.

Ans: DNA (deoxyribonucleic acid) is a molecule that is shaped like a double helix and made up of pairs of nucleotides, abbreviated as "A", "C", "G" and "T" DNA is packaged into chromosomes which are located within the nucleus of all cells. Every cell in the body contains all of the chromosomes that collectively make up the genome of that organism.

RFLP (Restriction Fragment length Polymerization):

RFLP is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of a particular restriction endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species (and even strains) from one another.

Applications of RFLP:

RFLPs can be used in many different settings to accomplish different objectives.

- RFLPs can be used in paternity cases or criminal cases to determine the source of a DNA sample.
- RFLPs can be used determine the disease status of an individual.
- RFLPs can be used to measure recombination rates.

RAPD (Random Amplification of Polymorphic DNA):

RAPD is a type of PCR reaction, but the segments of DNA that are amplified are random. The discovery that PCR with random primers can be used to amplify a set of randomly distributed

loci in any genome facilitated the development of genetic markers for a variety of purposes. The main reason for the success of RAPD analysis is the gain of a large number of genetic markers that require small amounts of DNA.

SNP (**Single nucleotide polymorphism)** or where alleles differ from each other by the sequence of only a single nucleotide base pair. SNP genetic tests focus on detecting precise single nucleotide base pair differences among the three billion nucleotide base pairs that make up the bovine genome.

Microsatellites: are stretches of DNA that consist of tandem repeats of a simple sequence of nucleotides (e.g. "AC" repeated 15 times in succession). The tandem repeats tend to vary in number such that it is unlikely two individuals will have the same number of repeats. To date, the molecular markers used to determine parentage have primarily utilized microsatellite markers.

4. (a). Describe the identification method of Urine and its Forensic significance.
(b). Explain about the Acid Phosphate Test.

Ans: 4 (a) Identification method of Urine
Physical Examination:
A suspected urine stain may fluoresce pale yellow or pale blue when viewed under long and short wave UV light. Safety eyeglasses, which absorb ultraviolet radiation, must be worn when viewing material for fluorescence.

Odour Test:
The characteristic odor of urine may be detected by placing a small sample of the stain in a test tube and heating it gently over flame. Avoid scorching the test material.

Urea Nitrate Crystal Test
An aqueous extract of stain is made and a thin film made on a microscopic slide.
Add one drop of conc. Nitric acid and cover. In the presence of urea, hexagonal stacked crystals of Urea nitrate are formed.

Creatinine Test
To a drop of stain extract on filter paper, add one drop of picric acid followed by one drop of 5% Sodium hydroxide. Brown/orange colour shows presence of creatinine.

Ans 4 (b). Explain about the Acid Phosphate Test.

Reagent Preparations:
Buffer

Glacial Acetic acid	1ml
Sodium acetate anhydrous	2gm
Distilled water	100ml

Step 1 Reagent

Buffer	50ml
Sodium alpha-naphthyl Phosphate, 0.25% (w/v)	126mg

Step 2 Reagent

Buffer	50ml
Naphthanil diazo blue B, 0.5% (w/v)	250mg

Step 1 Reagent and Step 2 Reagent can be made up in bulk and aliquoted into test tubes and frozen. When needed, one tube of each reagent can be thawed under warm running water for use.

Procedure:

1. Place a small piece (2 x 2 mm) of suspected seminal stain material on Whatman filter paper or other suitable test paper. Use proper standards and controls including positive, negative and unstained controls; see below.

2. Add 1-2 drops of Step 1 Reagent and allow to react for 30 seconds. (No colour should develop at this stage).

3. Add 1drop of Step 2 Reagent. Record the result after 10 seconds.

4. A positive reaction is recorded upon rapid development of a purple colour, which is indicative of semen. This is not a confirmatory test for semen.

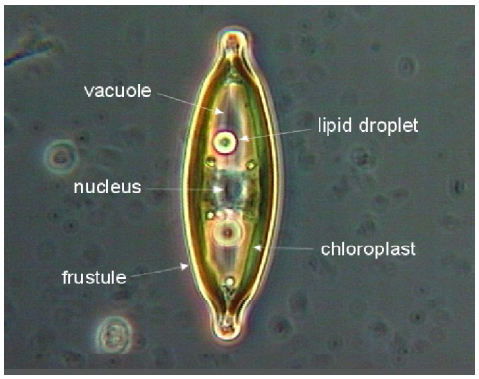
5. What are Diatoms. Explain the identification methods of Diatoms and its specificity.

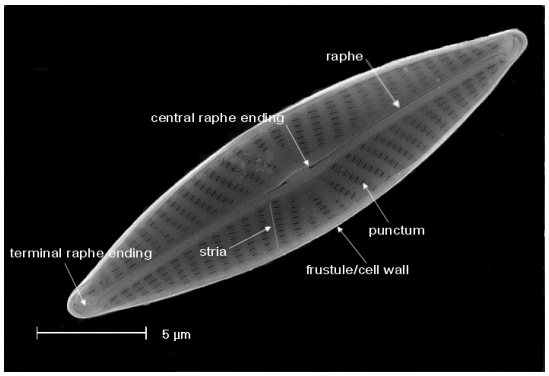
Ans:

Diatoms are a major group of eukaryotic algae, and are one of the most common types of phytoplankton. Most diatoms are unicellular, although some form chains or simple colonies. A characteristic feature of diatom cells is that they are encased within a unique cell wall made of silicate.

Role of diatoms

In cases, where the cause of death cannot be ascertained by conventional post-mortem examination in those cases presence of diatoms in lungs and other body tissues plays an important role in determining whether the death is due to drowning or not.





Investigation of Drowning

- One of the primary goals of forensic work is to determine the post-mortem interval (PMI), or time since death.
- • Diatoms are ubiquitous in both lotic (flowing water) and lentic (standing water) systems
- • Algal populations, though variable, persist throughout the year
- • Most diatoms can be identified to species with only a light microscope

Examining Diatoms:

The diatom test for drowning relies not only upon the identification of diatoms in the bone marrow, but also upon the identification of the same species of diatoms as found in a sample of water obtained from the place of recovery of the body.

Diatoms as Biological Evidence

The post-mortem examination of drowning, is one of the most difficult problems in forensic.
The post-mortem analysis of drowning is a classical problem in the pathology of sudden violent death.

Extraction of diatoms from water samples using acid digestion methods:

- Collected water samples from various water bodies like lakes, ponds, wells and drains.
- Water samples were treated with conc. HCI acid and supernatant was discarded.
- Then addition of conc. H2SO4 turned it blackish (conc. H2SO4 charred present organic material).
- The supernatant was cooled and added with solid NaNO3.
- Suspension was reheated until its color turned brown and finally cleared.
- Distilled water was used for the washing of the resultant suspension of silica diatom cells and residue was re-suspended in acetone.

OR

Extraction of diatoms from tissue samples using acid digestion method:

- Dissolve sternum bone marrow in a Kjeldahl flask containing 50 cc of nitric acid.
- After half an hour the yellow fluid turned transparent.

- This solution was allowed to cool and then centrifuged.
- The residue was put on the slide and examined under a microscope.

OR

Soluene-350 Method:

- Soluene-350 method for the extraction of diatoms from fresh water and seawater samples.
- At the time of collection water samples were fixed with formalin.
- 30 ml. of both fresh and seawater samples were washed thrice with distilled water and at every wash, samples were centrifuged at 3000 rpm for 5 min and the supernatant was discarded every time.
- Pellet was suspended in 8 volumes of Soluene-350.
- The solutions were then incubated at 50 °C for 2 hours and subsequently at room temperature overnight.
- Following centrifugations at 3000 rpm for 60 minute, samples were analyzed using the light microscope.
- Treatment was excessively destructive for seawater diatoms, probably because their frustules were less silicized and have less resistance as compare to the fresh water diatoms.

6. Define about mitochondrial DNA. What is the forensic significance of mtDNA.
Ans:
mtDNA (mitochondrial DNA):

Human mitochondrial DNA (mtDNA) is a circular double-stranded molecule, 16,569 base pairs (bp) in length that codes for 13 subunits of the oxidative phosphorylation system, 2 ribosomal RNAs (rRNAs), and 22 transfer RNAs (tRNAs). It is present in hundreds to thousands of copies in each cell, not within the nucleus, but within the cell's energy-generating organelles, the mitochondria. MtDNA consists predominantly of coding DNA, with the exception of a ~1100-bp long fragment that has mainly regulatory functions and is therefore termed the control region.

Mitochondrial DNA (mtDNA), an extra-nuclear genome, has certain features that make it desirable for forensics, namely, high copy number, lack of recombination, and matrilineal inheritance. mtDNA typing has become routine in forensic biology and is used to analyze old bones, teeth, hair shafts, and other biological samples where nuclear DNA content is low.

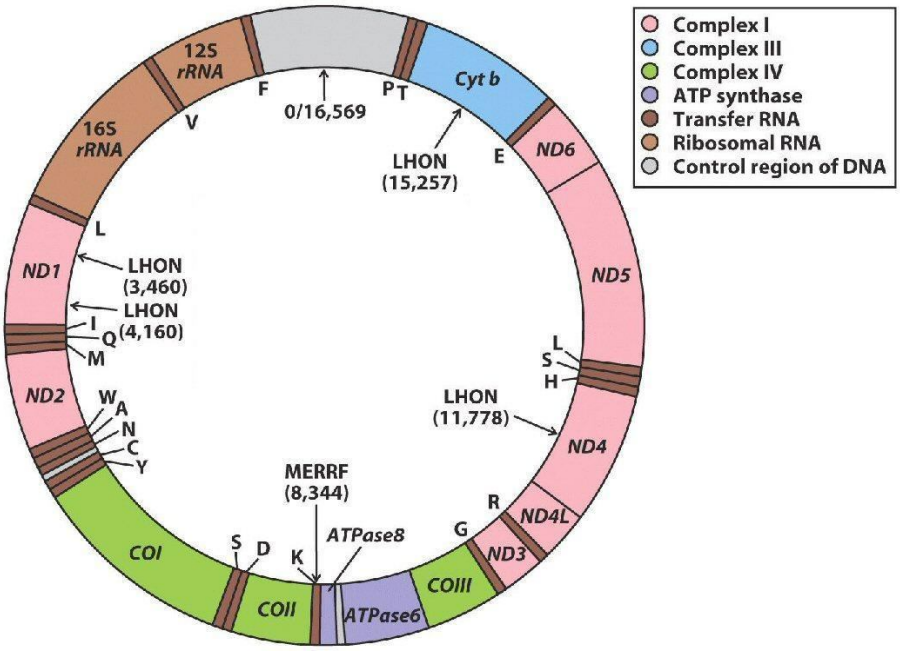


Fig: Structure of mtDNA (mitochondrial DNA)

Properties of Human Mitochondrial DNA:

High Copy Number:

MtDNA is present in high copy number in human cells. The average somatic cell has just two copies of any given nuclear gene or DNA segment, but hundreds to thousands of copies of mtDNA

Maternal Inheritance: The uniparental mode of inheritance is one of the great advantages of mtDNA, as it enables researchers to trace related lineages back through time, highlighting the maternal ancestry of a population, without the confounding effects of bi-parental inheritance and recombination inherent in nuclear DNA.

Mutation Rate: The mutation rate of mtDNA is several orders of magnitude higher than that of nuclear genes, with an estimated rate of 0.017×10^{-6} substitutions per site per year for the whole genome excluding the control region

The strengths of mitochondrial DNA (mtDNA):

1) Successful recovery from highly degraded sources where nuclear DNA typing may fail.

2) Application to cases where the only reference samples available are from matrilineal relatives.

3) Application to samples where nuclear DNA is virtually absent.

Forensic Significance of mtDNA:

- mtDNA enables identification of otherwise unidentifiable victims, if maternal relatives are alive for comparison. This is becoming increasingly relevant for victims of war or terror.
- mtDNA is increasingly used in so-called personalized genetic histories. This is the use of genetic testing to investigate individual genealogies, including tracing the origins of immigrant/slave ancestors.
- mtDNA typing analytical methods have been used worldwide to resolve identity issues in violent crimes, lesser crimes, acts of terrorism, missing persons cases, and mass disasters.
- Because of the high copy number of mtDNA molecules in a cell (16), typing mtDNA is particularly advantageous, compared with nuclear DNA, for certain kinds of forensic analyses.
- In cases where the amount of extracted DNA is very small or degraded, it is more likely that a DNA typing result can be obtained by typing mtDNA than by typing polymorphic markers found in nuclear DNA.

7. What are the different types of timber varieties encountered in forensic cases.

Ans:

Identification of Timber:

Timber has a variety of form which enables it to serve a variety of uses and contributes to its pleasing aesthetic appearance. However, the variability also means that there is lack of uniformity, which is a disadvantage when the timber is used for engineering applications. Timber identification aims at naming the species of tree from which the unknown sample has been cut, thus providing a means of assessing much useful information about the timber which would otherwise be unavailable.

There are two basic types of wood:

1) **Softwoods:** are from trees of Sub-phylum **Gymnospermae** – Order Coniferales - the conifers. The trees do not have true flowers, seeds are naked (not enclosed in any way) and borne on a scale of a cone. Leaves are usually needle-like and evergreen.

2) **Hardwoods:** are from trees of Sub-phylum **Angiospermae** – Class Dicotyledae - the flowering trees – seeds are produced inside an ovary or fruit. Leaves are typically broad and flat.

Viewing the microscopic characters of the wood can be carried out using the following tools:

- A magnifying glass which will give 4 to 20 times magnification - usually sufficient for hardwoods but not for softwoods
- A light microscope gives 5 to 400 times magnification and is the prime tool for wood identification
- Scanning electron microscopy (SEM) gives up to 100,000 times magnification, 3D views, no colour.

- **Important identifying features for softwoods:**
- In woods from regions with strong seasonal differences, there are noticeable seasonal changes between the tracheids that form the latewood and the earlywood whereas in woods tropical regions, tracheid size is basically the same throughout the growth ring.
- Presence of resin canals - present in Pinus, Larix (larch); Picea (spruce) and Pseudotsuga (douglas fir)
- Bordered pit arrangement (uniseriate is common; multiseriate staggered in Araucariaceae; multiseriate opposite in Sequoia)
- **Important identifying features for hardwoods:**
- In woods from regions with strong seasonal differences, there are obvious seasonal changes in vessel (pore) size.
- In woods from tropical regions, vessel (pore) size is often the same throughout the growth ring
- Vessels (pores) can be solitary or can be clustered into "radial multiples".
- Pore groups can be distributed evenly or arranged in radial, diagonal lines, or tangential lines.

8. (a). Explain about the examination of semen and its Forensic significance.
(b). Explain about the Forensic significance of Fiber evidence.

Ans: 8 (a)
Examination of Semen & Seminal Stains
Physical Examination:

Colour: Thick, yellowish white, glairy, opalescent, secretion having a characteristic odor known as seminal odor.

Confirmatory Test: Microscopic Examination

Upon obtaining a positive preliminary test for acid phosphates, the suspected stain can be extracted as follows:

1. Cut a small portion (1 cm2 maximum) of the stain and place in a test tube.

2. Add a few drops of acidulated water to cover the stain.

3. Agitate the stain on a vortex, or in an ultrasonic cleaner bath or manually, by flicking the tube. This will aid in freeing the spermatozoa from the dried stain.

4. When the solution is cloudy, withdraw the liquid with a pipette and place into a disposable 400ul plastic centrifuge tube and centrifuge in a microfuge for 30 seconds.

5. Carefully withdraw the supernatant, which contains soluble group-specific substances, enzymes and other solutes from seminal plasma.
6. Collect the button of cellular and other insoluble components from the tube and place on a clean-labeled microscope slide.
7. Fix in dilute H2SO4 acid and dry. It is now ready for staining.

Cross-Over Electrophoresis

Seminal Material can be identified by demonstrating the presence of P30, a semen specific protein. One method of doing this is Cross-Over Electrophoresis.

An extract of the suspected stain is placed in the cathode well of a gel plate and anti P30 is placed in the anode well. Electrophoresis is commenced for 20 minutes at 200 volts, forcing two components together. When the antigen (P30) meets the antibody (anti-P30), a precipitin band is formed. The presence of a precipitin band within an extract of an unknown stain proves that the stain contains seminal material.

Ans: 8 (b)
Forensic significance of Fiber evidence.

Textile fibers can be exchanged between individuals, between individuals and objects and between objects. When fibers are associated with a specific source, such as fabric from the victim, suspect or scene, a value is placed on that association. A fiber is transferred and detected is also dependent on the nature and duration of the contact between the suspect and the victim and/or scene and the persistence of the fibers after they have been transferred.

Fiber examinations involve a comparison of samples from known and questioned sources to determine whether they are consistent with having originated from the same source. Laboratory analysts examine various physical, chemical, and microscopic properties of fibers when performing a comparison between evidence fibers and a potential source. Conversely, individual characteristics allow the association between two or more items with each other to the exclusion of all other items. For fiber examiners, this most often occurs when pieces of fabric or cordage are physically matched.