

# Urinalysis and Body Fluids CS

## Unit 5

### Seminal Fluid

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## Seminal Fluids - objectives

1. Discuss the major components of seminal fluid with regard to source, function, normal and abnormal appearance.
2. List three (3) reasons for semen analysis.
3. Outline instructions to give to a patient for the correct method for collecting a semen specimen for laboratory analysis.
4. List two (2) methods for identifying a questionable fluid as semen.
5. State the significance of finding increased acid phosphatase in a suspicious fluid.
6. Calculate a sperm count when provided with the number of sperm counted, the dilution factor and the area of the counting chamber used.
7. List the normal values for: semen volume, viscosity, pH, sperm count, motility and morphology.

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## Seminal Fluids

### • Composition of Semen

- Spermatozoa
- Fluids to provide nutritional support and media

	% of total	Description / Purpose
Spermatozoa	2-5%	Formed in testes, stored in epididymis and vasa differentia
Seminal Fluid	60-75%	Alkaline fluid.; primarily responsible for nutritional support through: amino acids, enzymes, fructose. Also to suppress possible immune response by female
Prostate Fluid	25-30%	Acid phosphatase, citric acid, proteolytic enzymes and zinc
Bulbourethral glands	1 - 5%	Galactose, mucous

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## Seminal Fluids

- **Anatomy, composition and formation.**
  - **Testes** - source of sperm (2-5%)
  - **Seminal vesicles** - provides fructose & nutrients and is primary provider of fluid (@ 60-75%)
  - **Prostate gland** - Provides enzyme, acid phosphatase, citric acid, zinc, and proteolytic enzymes (for coagulation and liquification). 2<sup>nd</sup> source of fluid(25-30%)
  - **Bulbourethral glands** - @ 5%. Thick alkaline mucous-like fluid that neutralizes acids.



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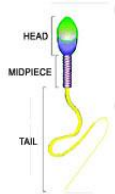
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## Seminal Fluids

- Spermatozoa - produced in the testes, mature in the epididymis.



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## Seminal Fluids

- **Reasons for Testing**
  - **Infertility issues** - more often a problem with the woman, but easy to rule-out the male.
    - With assisted reproductive technology, greater emphasis placed on sperm quality and quantity.
  - **Post- vasectomy** - frequent reason for testing
    - *Test at one month intervals until 2 consecutive months are negative for sperm.*

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## Seminal Fluids

- Reasons for Testing
  - Forensic analysis of fluid as being semen
    - *as in alleged rape.*
    - *Vaginal swab, washings, or scrapings microscopically evaluated for sperm*
    - *chemical test for enzyme: acid phosphatase*
      - *Contributed by prostate gland*
      - *Present even in the absence of sperm cells*
  - Sperm donors - artificial insemination programs

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## Seminal Fluids

- Specimen Collection
  - Sterile container
  - Direct deposit preferred
    - no lubricants, spermicides, condoms, etc.
  - Complete specimen
    - Majority of sperm are in first part of ejaculate
  - 3 day sexual abstinence required
    - But not more than 5 days.
  - ***Best if collected at laboratory site.***
    - If other, specimen must be kept warm and delivered to lab within 1 hour
  - Time of collection important.
    - *Must be recorded!*

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## Seminal Fluids

- Physical characteristics
  - Liquefaction - fresh specimen will clot, then liquefy within 30 - 60 minutes
  - Persistence of clot is abnormal
  - ***All further evaluation must wait until liquefaction is complete.***

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## Seminal Fluids

Semen: Appearance	
Opaque Gray, white, light yellow	Normal
Shades of yellow	Correlate with flavin concentration Could also indicate contamination with urine.
Deep yellow	Associated with certain drugs
Brown or red	May contain blood
Highly turbid	Usually contains leukocytes indicating infection or inflammation

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## Seminal Fluids

Semen: Appearance, cont.	
Volume	2.0 – 5.0 mL Measured in serological pipet Recorded to 1 decimal place
pH	7.2 – 8.0 Measured with pH paper Alkaline to off-set acid vaginal environment Acid may indicate increased prostatic fluids pH > 8 may indicate infection

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## Seminal Fluids

Semen: Appearance, cont.	
Viscosity	Pours in droplets (as shown in picture) Rating: 0 = water-like 4 = gel-like
	

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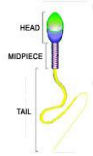
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## Semen: Microscopic Analysis

- Motility
- Concentration / cell count
- Morphology
- Agglutination
- Viability
- Penetration of cervical mucous



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## Semen: Microscopic Analysis

- Microscopic examination
  - Generally performed 30 - 60 min after collection
  - **Must be after liquidification has occurred**
    - Motility
      - Motility is a very necessary quality of sperm. Must propel through uterus & fallopian tubes which is quite a long distance.
      - Must be evaluated within the 1<sup>st</sup> hour following collection
      - Will decrease over time

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## Semen: Sperm Motility

- Analysis to begin within 1 hour of specimen collection
- Evaluation times may vary between labs, but usually at set intervals
- Consistency in technique and procedure important
- Using hemocytometer & coverslip, examine a drop of undiluted specimen using high dry objective.
- Brightfield microscopy with light level reduced
- Some labs use phase microscopy
- Alternate method: High-resolution video photography / CASA (computer assisted semen analysis)

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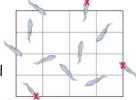
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## Semen: Sperm Motility

- Manual Subjective evaluation
  - Observe immediately following liquidification; and within 1<sup>st</sup> hour.
  - Place well mixed undiluted drop on **pre-warmed** hemacytometer slide
  - Observe under high-dry objective; with reduced light.
  - Rated from "0" to 4.0"
    - 4.0 - rapid and straight line movement
    - 3.0 - slower, and some lateral movement
    - 2.0 - slow forward progression, noticeable lateral
    - 1.0 - no forward progression
    - 0 - no movement
- Other types of rating scales may be used
- Normal (authors vary greatly) but > 50-60% show 2.0 or greater at 1 hour.




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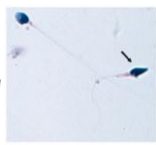
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## Semen: Microscopic Analysis

- Morphology
  - May be performed in cytology, pathology, or hematology
  - Oval/egg shaped head (3x5um)
    - While oval from the front, appears flattened when viewed from the side appears flattened
    - @  $\frac{1}{2}$  covered with an enzyme laden acrosomal cap, which contains
  - Middle piece
    - provides energy
  - Tail piece of @ 45 - 55 um long




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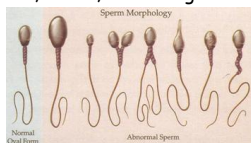
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## Semen: Microscopic Analysis

- Morphology
  - At least 200 cells evaluated on smear (Wright's, Giemsa or Papanicolaou) stained.
  - Usually evaluated by pathologist, or cytologist**
  - Looking for double heads, pin heads, giant heads, or amorphous heads, double, coiled, or missing tails, etc.
  - Many sources of good pictures available




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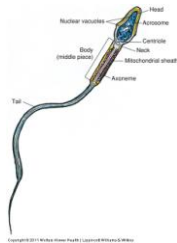
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## Semen: Microscopic Analysis

- Morphology
  - Normal = < 30% abnormal forms (NV varies considerably based on strictness of criteria.
  - WBC, RBC, bacteria presence should be noted & **may indicate infection**
  - Round cells (neutrophils and immature sperm) should be noted as well.




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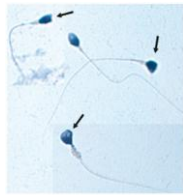
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## Semen: Abnormal forms

- 2 headed sperm
  - Sternheimer - Malbin stain
  - X 320
- Flat-headed sperm




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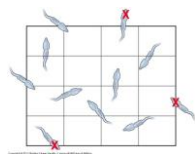
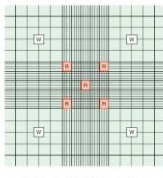
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## Semen: Microscopic Analysis

- Sperm count
  - NV= 20 - 160 million/mL
  - Make 1 to 20 dilution with sodium bicarbonate and formalin, count 5 small squares (within the center laræ saquare) of the Neubauer hemacytometer.




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## Semen: Microscopic Analysis

- Sperm cell count
- standard method to begin calculation of # cells (mature sperm) per microliter:

$$\frac{\text{ave. \# cells counted} \times \text{dilution}}{\text{\# squares counted} \times \text{volume of each square}}$$

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## Semen: Microscopic Analysis

- Microscopic examination
- Example:  $\frac{52 \text{ cells (mature sperm)} \times 20}{5 \text{ (squares)} \times 0.004}$ 
  - This provides results as \_\_\_ cells /  $\mu\text{L}$ ;  
Normal values are reported as \_\_\_ cells / mL.
- Must multiply X 1000 to convert  $\mu\text{L}$  to mL
  - =  $52.0 \times 10^6$  / mL

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## Semen: Microscopic Analysis

- Metric
  - Internationalized system using decimals
  - Common system of measuring units
    - Length (M), volume (L), mass (G), time (s), temperature ( $^{\circ}\text{C}$ )
    - Prefixes allow for mL,  $\mu\text{L}$ , etc.
- International System of Units (SI)
  - Modified / modern form of metric system
  - Has 7 base units (but, unlike the original metric system does not include volume)
  - Other units, such as volume are 'derived'
  - Basic unit for volume is  $\text{m}^3$
  - mL = cubic centimeter (cumm),  $\mu\text{L}$  = cubic millimeter ( $\text{mm}^3$ )

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## Semen: Sperm Agglutination

- Observed while performing motility evaluation.
- Few clumps are normal.
- Distinctly head-to-head or tail-to-tail clumping may indicate the presence of antisperm antibodies.
  - IgG
  - IgA

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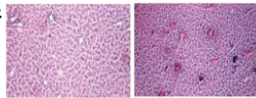
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## Semen: Sperm Viability

- Eosin - Nigrosin stain
  - supravital stain
  - Add to drop of fresh sample
  - Smear is made and allowed to dry
  - Evaluated on oil immersion (1000x)
  - Viable / live sperm do not take up the stain and remain colorless or blue-white
  - Non-viable / dead sperm stain orange-red
- Reported as % viable
- Normal >75%



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## Semen: Analysis

- Other tests
  - Sperm penetration
    - Evaluates ability of sperm to make progressive movement through the cervical mucous.
  - Microbial testing
    - Increased WBC (>1 million/ mL) suggestive of infection
    - Aerobic and anaerobic cultures

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## Semen: Chemical Analysis

- pH
  - Measure within 1 hour of collection
  - Normal 7.2-8.0
- Acid Phosphatase
  - Used to evaluate the secretory function of the prostate
  - Also used in forensic analysis - as prostatic fluid acid phosphatase is higher than other fluids. (>200 units)
- Fructose
  - Provides energy / nutrition to sperm
  - Indication of viability
  - Presence of fructose - screen using resorcinol test
- Hormones
  - Testosterone, LH, FSH

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## Post- Vasectomy Analysis

- Post-vasectomy semen analysis
  - Specimens tested at monthly intervals starting 2 months post-vas.
  - 2 consecutive months of negative microscopic for sperm
  - Wet prep with phase microscopy
  - Examination of centrifuged specimen as well

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## Semen: Forensic Analysis

- Examination of fluid as to being semen (forensic)
  - Acid phosphatase - highly sensitive, as no other body fluid contains as high level (2500 units compared to @ 5 units)
  - ABO, HLA typing
  - DNA analysis
  - UV light scan, semen fluoresces green/white

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## Semen: Analysis QC

- Quality control
  - Previously little or no QC materials available
  - Commercial products now becoming available
  - Proficiency testing now available
    - CAP
    - American Association of Bioanalysts (AAB)

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## Reference Listing

- > Please credit those whose work and pictures I have used throughout these presentations.
- > Lillian Mundt & Kristy Shanahan, Graff's Textbook of Urinalysis and Body Fluids, 2<sup>nd</sup> Ed.
- > Susan Strassinger & Marjorie Di Lorenzo, Urinalysis and Body Fluids, 5<sup>th</sup> Ed.
- > Wikipedia, the free encyclopedia
  - > [www.wikimedia.org](http://www.wikimedia.org)

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