School of Medical and Allied Sciences

Course Code: BMLT3002 Course Name: Pathology

Semen examination

Classification

Semen

examination

Physical examination
Or
Macroscopic examination

Gross examination

- 1. Color,
- 2. Odor,
- 3. volume,
- 4. viscosity,
- 5. reaction and
- 6. liquefaction

Microscopic examination

- 1. Motility
- 2. Morphology
- 3. Viability
- 4. Count

Chemical examination

- 1. Fructose test
- 2. Acid
- phosphatase test

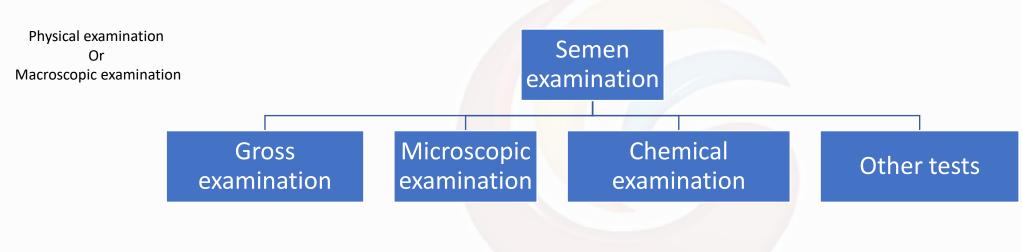
Other tests

- I. Immunological assays
- 2. Microbiological assays
- 3. Sperm function tests
- 4. Semen cryopreservation

Physical examination

- 1. Colour. Normally it is whitish, grey-white or slightly yellowish.
- 2. Volume. Normally, volume of semen is between 2.5 and 5 ml. The volume is slightly more in patients of infertility. The volume does not vary with the period of abstinence.
- 3. Viscosity. When ejaculated, semen is fairly viscid and it falls drop by drop.
- 4. Reaction. Normally, it is slightly alkaline with pH between 7 and 8.
- 5. Liquefaction. Liquefaction occurs because of presence of fibrinolysin. Normally liquefaction occurs at room temperature

within 10-30 minutes (average 20 minutes).



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2. Microscopic Examination

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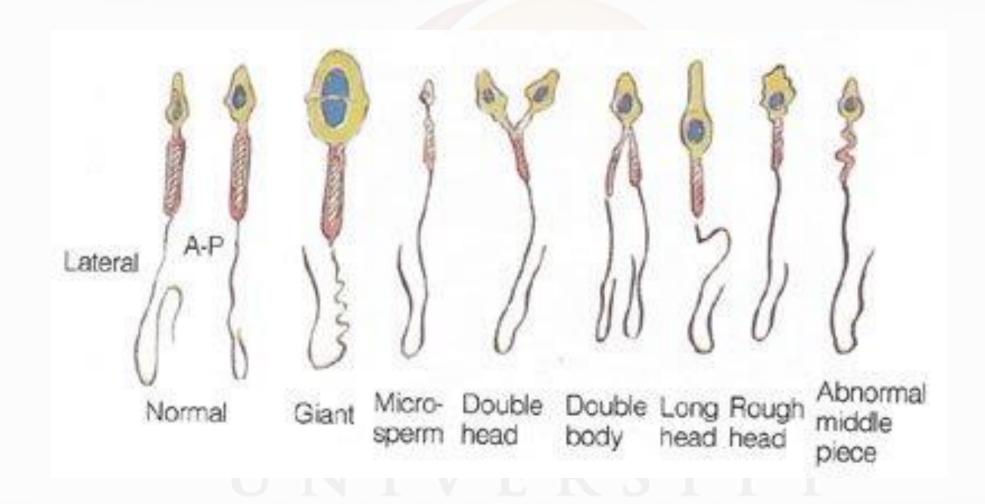


a. Motility test

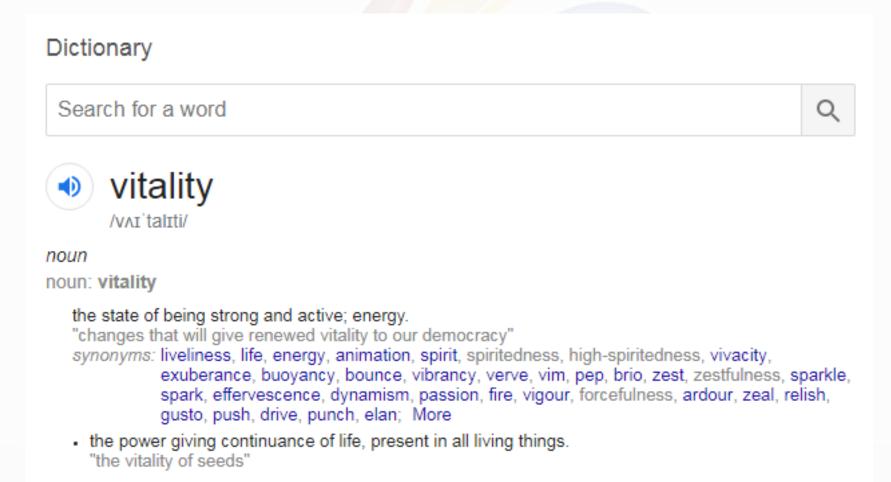
- 1. Place a drop of liquefied semen on a clean glass slide.
- 2. Put a coverslip over it and examine it under the microscope, first under low power and then under high power.
- 3. Normally within 2 hours of ejaculation, more than 60% of spermatozoa are vigorously motile, and in 6-8 hours 25-40% are still motile.
- 4. If motility is less than 50%, a stain for viability such as eosin Y with nigrosin as counterstain can be done. Heads of non-motile sperms show red dye.

b. MORPHOLOGY

- 1. Prepare a thin smear from liquefied semen on a glass slide.
- 2. Stain it with any of the Romanowsky stains, Pap or H & Estain.
- Observe at least 200 spermatozoa for any abnormality in their morphology.
- 4. Normally 80% of spermatozoa are normal.
- The abnormal forms of spermatozoa are with double head, swollen and pointed head, double tail and rudimentery forms.
- 6. Also look for the presence of RBCs or WBCs, if any.
- Computer-assisted morphologic screening is particularly useful in samples with very low numbers of normal sperm count which may otherwise remain undetected.

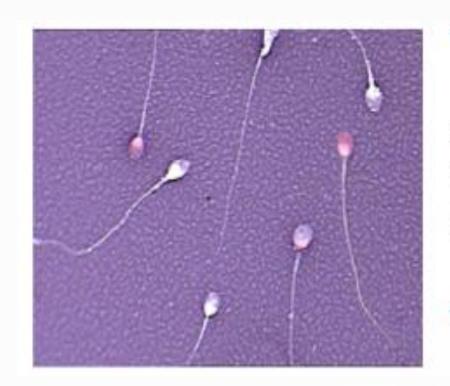


c. Sperm Vitality (viability)test



Sperm Viability

- Sperm Viability
- Decreased sperm viability may be suspected when a specimen has a normal sperm concentration with markedly decreased motility.
- Viability is evaluated by mixing the specimen with an eosin-nigrosin stain, preparing a smear, and counting the live sperms.



Eosin—nigrosin staining method:

The dead spermatozoa are stained red or dark pink, while the live spermatozoa white or light pink.

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3. Chemical Examination

- 1. Fructose test
- 2. Acid phosphatase test

FRUCTOSE TEST

- This test determines androgen deficiency or ejaculatory obstruction to semen; the level of seminal fructose is low in both these conditions.
- Normal seminal fructose level is
- 150-600 mg/dl.
- Fructose is measured qualitatively by Resorcinol test.
- Procedure
- 12 Take 5 ml of dilute HCl in a test tube.
- 2 Add 1 ml of semen.
- 2 Add 5 mg of resorcinol.
- 2 Boil.
- Interpretation. Appearance of red colour indicates
- presence of fructose which can be measured by spectrophotometer.

Seliwanoff's test



 Aldoses and Ketoses are detected by Seliwanoff's Test

ACID PHOSPHATASE TEST

- This test is used for seminal stain and on vaginal aspirate in medicolegal cases.
- Normally semen has 2500 KA units/ml of acid phosphatase.

Acid Phosphatase Test

- Acid Phosphatase enzyme that is secreted by the prostate gland into seminal fluid.
- This enzyme will react with an acidic solution of sodium alpha naphtylphosphate and Fast Blue B dye.
- 4-methyl umbelliferyl phosphate (MUP) will fluoresce under UV light when in contact with the enzyme.

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Reference:

 https://www.who.int/docs/default-source/srhrdocuments/infertility/examination-and-processing-of-human-semen-5ed-eng.pdf?sfvrsn=5227886e_2

• https://www.amboss.com/us/knowledge/Semen analysis