SEMEN DILUTING FLUID



PRINCIPLE

Semenal fluids mainly contain mucous and sperm cells. Whereas, the sperm cells are de-mucused by the action of bicarbonates and fixed subsequently by formalin to enumerate them on a Neubauer counting chamber.

REAGENTS COMPOSITION

Sodium Bicarbonate 50 G/L Formalin 3 mL/L

Working Reagent Preparation

Reagent is ready to use. May be filtered through watmann No 1 if precipitates or dust particles visible under microscope.

STORAGE AND STABILITY

The reagent is stable till the expiry marked on label if stored tight capped at room temperature away from bright light.

Materials required but not supplied.

- 1. Microscope with 45 x objectives
- 2. Neubauer counting chamber with cover glass.
- 3. $20 \,\mu\text{L}$ pipette with tips.
- 4. 2 mL pipette with tips.
- 5. Test tubes (75 x 12 mm)
- 6. Petri dish with moist filter paper.

Sample collection and handling

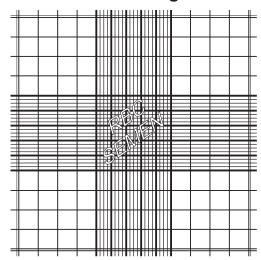
Collect seminal fluid in a clean container free from dust and dirts.

Samples can be stored in 2-8°C for a week if the test need to perform at a later time.

PROCEDURE

- 1. Label test tubes with Sample ID
- 2. Pipette 2 mL Semen diluting fluid.
- 3. Pipette 20 µL well mixed liquified whole semen sample.
- 4. Mix well and Charge in a Neubauer counting chamber.
- 6. Keep the charged chamber for 5 minutes under a petridish with moist filter paper.

Neubauer counting chamber



- 7. Count sperm cells from 400 smallest RBC column under 45 x microscope
- 8. Calculate total sperm count as per result calculation column.

RESULT CALCULATION

Total Sperm cell count / mL = $\frac{\text{T C}}{20}$ x $\frac{2020}{20}$ x $\frac{10 \times 1000}{10^6}$

Total Sperm cell count in million / mL = TC x 1.01

Where TC = Total Sperm Cell Counted in 1 mm²

 $\frac{2020}{20}$ = Dilution of sample

10 = Depth of counting chamber.

 $\frac{1000}{10^6}$ = Converted in to millions/ mL

EXPECTED VALUES

Total Sperm cell count 20 to 120 millions / mL

Each laboratory should establish its own normal values.

BIBLIOGRAPHY

- 1. Rider RF., Semin. Hematology 11.423
- John D Bauer., Numerical Evaluation of formed elements of blood., Gradwohl's clinical laboratory methods and diagnosis vol I page 797/9

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