

---

<b>Protocol Section:</b>	Post-Thaw Sperm Evaluation	<b>Policy No:</b>	P-CMMR4-PTE-00
<b>Protocol Subject:</b>	Progressive Motility, Viability, Sperm Concentration	<b>Effective Date:</b>	
		<b>Date Reviewed:</b>	08 March 2004
		<b>Date Revised:</b>	

---

## 1. Determination of sperm concentration

Sperm concentration is shown in the proportion of spermatozoa cell (WHO Laboratory manual for the examination of human semen and sperm cervical mucus interaction, Cambridge University Press; 1999).

### 1.1 Material

- 1.1.1 Makler counting chamber
- 1.1.2 Sperm immobilization solution
- 1.1.3 Eppendorf tube (1.5mL)
- 1.1.4 Micropipette

### 1.2 Method

- 1.2.1 Dilute semen 1:1 with sperm immobilization solution (100 mL distilled water + 1 mL neutral buffered formalin %10 + 5 grams sodium bicarbonate) for fixation of sperm cells in a eppendorf tube
- 1.2.2 Place 20  $\mu$ L of diluted semen sample on sperm examination area
- 1.2.3 Put special cover slide on the examination area
- 1.2.4 Examine under 10 x magnification
- 1.2.5 Count sperm cells within ten boxes and record that number
- 1.2.6 Count sperm cell concentration on the following formulation

$$\text{Sperm concentration} = \text{Sperm number} \times 10^6 \times 2 \text{ (dilution factor)}$$

## 2. Determination of sperm motility

In order to achieve fertilization, a sperm must not only be able to move but be capable of movement that results in forward progression (WHO Laboratory manual for the examination of human semen and sperm cervical mucus interaction, Cambridge University Press ;1999)

### 2.1 Material

- 2.1.1 Makler counting chamber
- 2.1.2 HTF or PBS with BSA (S-3311 Sigma)
- 2.1.3 Eppendorf tube (1.5mL)
- 2.1.4 Micropipette

**Protocol Section:** Post-Thaw Sperm Evaluation  
**Protocol Subject:** Progressive Motility, Viability, Sperm Concentration

**Policy No:** P-CMMR4-PTE-00  
**Effective Date:**  
**Date Reviewed:** 08 March 2004  
**Date Revised:**

---

## 2.2 Method

- 2.2.1 Dilute it 1:4 or 1:8 with HTF or PBS prior to examination fresh or post-thaw semen in a eppendorf tube
- 2.2.2 Place 20 µL of diluted semen sample on sperm examination area
- 2.2.3 Put special cover slide on the examination area
- 2.2.4 Examine under 10 x magnification
- 2.2.5 Count only the motile sperm, in forward progression, within ten boxes and record that number
- 2.2.6 Recount the same boxes, this time only counting the non-motile sperm
- 2.2.7 Count motility on the following formulation

$$\% \text{ Motility} = \frac{\text{motile sperm}}{\text{motile} + \text{non-motile sperm}} \times 100$$

## 3. Determination of sperm viability

Sperm vitality is reflected in the proportion of spermatozoa that are "alive". The eosin-nigrosin staining technique is based on the principle that dead cells will take up the eosin and as a result stain red. The nigrosin provides a dark background, which makes it easier to assess the slides (WHO Laboratory manual for the examination of human semen and sperm cervical mucus interaction, Cambridge University Press ;1999)

### 3.1 Material

- 3.1.1 Stain A- 20 ml of 1% Eosin Y in water
- 3.1.2 Stain B- 30 ml of 10% Nigrosin in water
- 3.1.3 Test tube
- 3.1.4 Frosted slide

### 3.2 Method

- 3.2.1 Mix 50 µL of semen with 2 drops of stain A in a sterile test-tube
- 3.2.2 After 30 seconds, add three drops of stain B and mix thoroughly
- 3.2.3 Within 30 seconds of adding stain B, place a drop of semen-stain mixture on a microscope slide and make a thin smear using a cover glass
- 3.2.4 Allow to air-dry and examine under oil immersion (1000 x magnification) under bright field microscope.  
Colorless spermatozoa is given live and red stained spermatozoa is given dead sperm rate
- 3.2.5 Count 100 cells
- 3.2.6 Store reagents between 4 °C and 25 °C.

**Issued by Lab Manager:** \_\_\_\_\_ **Date:** \_\_\_\_\_

**Approved by Facility Management:** \_\_\_\_\_ **Date:** \_\_\_\_\_