SCOPESCREEN

6/28/17 Revised 01/11/2020 (Formerly known as BIOSCREEN)

LEUCOSCREEN™

Cytochemical Stain For Detecting Granulocytes in Semen

(about 25 to 300 determinations)

Principle:

This staining method detects the presence of the enzyme peroxidase in cells.

A semen sample is mixed with a substrate specific for the enzyme peroxidase. If peroxidase is present, it will reduce the substrate, hydrogen peroxide. At the same time, diaminobenzidine (DAB) will be oxidized to form an insoluble brown product:

$$DAB + H_2O_2 \xrightarrow{\text{peroxidase}} \text{oxidized DAB (brown pigment)}$$

Using a formula, it is possible to calculate the number of peroxidase-positive cells in each semen sample by knowing the concentration of spermatozoa.

Reagents:

Buffer: 7 ml buffer, pH 7.4. Ready to use.
DAB: 1 ml diaminobenzidine solution. Ready to use.
Warning: Diaminobenzidine is a possible carcinogen.
Peroxide: 1 ml hydrogen peroxide solution. Ready to use.
Fixative: 12 ml dilute ethanol. Ready to use.
Peroxidase: 0.5 ml peroxidase suspension. Ready to use.

Materials Required But Not Provided:

1. Deionized water.
2. Sperm counting chamber.
3. Glass slides and coverslips.
4. Pipettors and tips.
5. Test tubes and rack.
6. Positive and negative controls (see Controls).
7. Bright-field microscope with 100X to 400X total magnification.

Warning and Precaution:

All semen samples should be considered potentially infectious. Handle all specimens as if capable of transmitting HIV or hepatitis. Specimens should be disposed of in accordance with OSHA guidelines.

Storage and Stability:

Store the reagents at 2°C to 8°C.They can be used until the expiration date on each label.The expiration date is 18 months from the date of manufacture.

Specimen Collection:

Semen should be collected in a clean cup. The semen sample can be stored at room temperature until using.

Preparation:

1. Bring all reagents to room temperature.
2. Prepare fresh substrate by adding the following to a test tube:
   1 ml water
   250 µl **Buffer**
   40 µl **DAB**
   exactly 1 drop **Peroxide**
3. Mix gently. Discard after use.

Procedure:

1. Allow semen sample to liquefy.
2. Count spermatozoa.
3. Pipette 20 µl semen into a test tube.
4. Pipette 20 µl **Peroxidase**, a positive control, into a second test tube.
5. Pipette 20 µl water, a negative control, into a third test tube.
6. Add exactly 1 drop **Fixative** to each test tube.
7. Pipette 60 µl fresh substrate into each test tube and mix briefly.
8. Observe the test tubes with **Peroxidase** and water and note any color change. The test tube with **Peroxidase** should turn dark brown. This indicates that the fresh substrate is working properly. Proceed with the next step if the fresh substrate is working properly.
9. Prepare specimen for viewing using Method I or Method II.
   Method I: Pipette 10 µl to 20 µl onto a glass slide and place a coverslip on top of the liquid. Examine at a total magnification of 400X using a microscope. Count brown cells and sperm within the same viewing area.
   Method II: Pipette about 5 µl onto a Makler Chamber or a disposable counting chamber. Examine at a total magnification of 100X using a microscope. Count brown cells within the entire grid area.

Calculation Of Granulocytes In Semen, Method I:

$$\text{Granulocytes/ml} = \text{Sperm Count} \times \frac{\text{Number of brown cells}}{\text{Number of sperm}}$$

Example: The following data were obtained for a tested semen specimen placed on a glass slide with a coverslip and viewed at 400X:
Sperm Count = 73 X 10^6 cells/ml
Number of brown cells = 4
Number of sperm = 66
Applying the formula: 73 x 10^6 X 4/66 = ~4 x 10^6 granulocytes/ml

Calculation Of Granulocytes In Semen, Method II:

Granulocytes/ml = Number of brown cells X 5 X counting chamber factor where 5 is the dilution factor because semen was diluted 20µl in a total volume of 100 µl.

Example: The following data were obtained for a tested semen specimen viewed at 100X in a Cell-VU:
Number of brown cells in 100 squares = 16
Cell-VU chamber factor = 10^5/2 cells/ml
Applying the formula: 16 x 5 X 10^5/2 = 4 x 10^6 granulocytes/ml

Selected References:

Vujisić S, Lepej SŹ, *et al*. Antisperm antibodies in semen, sera and follicular fluids of infertile patients: relation to reproductive outcome after in vitro fertilization. Am J Reprod Immunol 2005:54:13-20.

World Health Organization. *Laboratory manual for the examination of human semen and sperm-cervical mucus interaction*. 4th ed. New York: Cambridge University Press, 1999.

SCOPESCREEN
Scopescreen Inc. www.scopescreen.com +1.212.260.6692