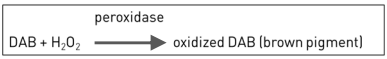
Leukocytes slides SCA Protocol

KIT FOR DETECTION OF PEROXIDASE POSITIVE CELLS IN SEMEN (CYTOCHEMICAL STAIN FOR DETECTING GRANULOCYTES) · *FOR RESEARCH USE ONLY*

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:



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. Pipettors and tips.
3. Test tubes and rack.

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 2ºC to 8ºC. 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.

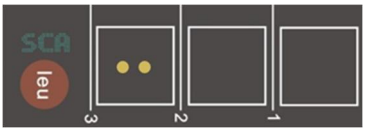
Staining Procedure:

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.

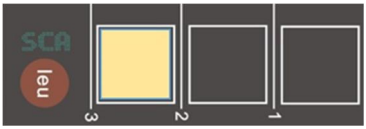
6. Add exactly 1 drop Fixative to each test tube.
7. Pipette 60 µl fresh substrate into each test tube and mix briefly.
8. Observe the test tubes with Peroxidase and water and note any color change. The test tube with Peroxidase should turn dark brown. This indicates that the fresh substrate is working properly. Proceed with the next step if the fresh substrate is working properly.

Analysis:

a. Put 2 equidistant drops of stained sample (2µl/drop) in the centre of one square of the slide:



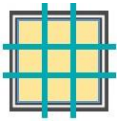
b. Take one coverslip (15 x 15 mm) and put it on the square carefully (shape of coverslip and printed square should be coincident). Sample should spread all over the cover surface:



c. Put the slide at the microscope and analyse it.

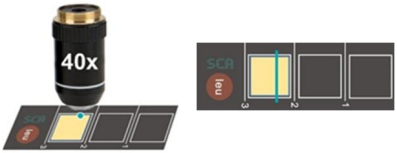
ANALYSIS with the SCA®:

- The full screening of the sample consists in doing 4 transects similar than this:



To do that,

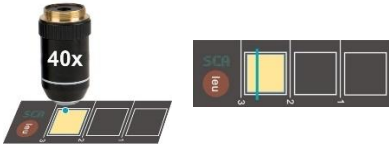
- Go to one horizontal edge of the coverslip and screen the sample following a vertical transect (only "Y" axis from the microscope stage should be moved).



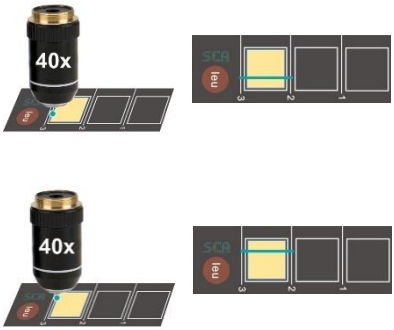
- Capture the round cells observed along the transect. The SCA will recognize the presence of peroxidase activity on them (when present).

Don't include in the analysis those cells partially out of the transect. In no case the objective should be moved out the transect to capture a cell outside the transect limits.

- Move the objective to the opposite edge and screen a second vertical transect.



- Move the objective to the vertical edge of the cover and repeat the process, but describing two horizontal transects (only the "X" axis from the stage should be moved for each transect).



ANALYSIS with the SCA SCOPE:

- Locate the slide into the SCA SCOPE and introduce into the system the reference and position (number of the square) of the sample to be analysed. Start the analysis.

Interpretation of results:

- ‹ 0.5 M Peroxidase positive cells/ml: Very low Risk of infection.
- 0.5 – 0.9 M Peroxidase positive cells/ml: Risk of infection.
- › 1M Peroxidase positive cells/ml: Infection.

Selected References:

Vujisic S, Lepej SZ, *et al*. Antisperm antibodies in semen, sera and fol¬licular 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.

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