

CONCEPTION TECHNOLOGIES

LEUCOSCREEN™

INTRODUCTION:

Most human ejaculates contain leucocytes (Wolff and Anderson, 1988; Aitken and West, 1990; Barratt et al, 1990), the predominant cell type being the neutrophil. Presence of large numbers of these cells (leucocytospermia) may indicate the existence of reproductive tract infection.

Furthermore, leucocytospermia may be associated with defects in the semen profile, including reductions in the volume of ejaculate, sperm concentration and sperm motility, as well as loss of sperm due to oxidative stress (Aitken et al, 1989; Aitken and West, 1990) and/or secretion of cytotoxic cytokines (Hill et al, 1987). It is difficult to define a threshold concentration of leucocytes beyond which fertility will be impaired. The impact of these cells depends upon the site at which the leucocytes enter the semen, the types of leucocytes involved, and their state of activation.

As a general rule, a normal ejaculate should not contain more than 5×10^6 round cells/mL, while the number of leucocytes should not exceed 1×10^6 /mL.

When the semen contains more than 1×10^6 /mL white blood cells, microbiological tests should be performed to investigate if there is an accessory gland infection.

METHOD (ENDTZ, 1972):

1. Preparation of Work Solution
Add 30 μ l of reagent 2 to 1 mL of reagent 1. This working solution remains stable for 1 day.
2. Mix one drop (10 μ l) of sperm with one drop (10 μ l) working solution, using the edge of a clean coverslip. Mix thoroughly for at least 1 minute.
3. Cover with the cover slip approximately two minutes after initial mixing, avoiding air bubbles. Formation of small air bubbles is normal and is due to the peroxidase reaction. The greater the number of peroxidase positive cells in the sample, the greater the number of bubbles which form.
4. Read the results after 2 minutes at a magnification of 400X.

INTERPRETATION:

Yellow to brown stained cells are peroxidase positive cells: neutrophilic polymorphous leucocytes.

*Pink stained cells: all other cells.

COMPONENTS:	REAGENT 1	20ML
	REAGENT 2	1 ML

Note: Formation of a sediment in Reagent 1 is normal and it may be removed with filter paper.

STORE REAGENTS AT ROOM TEMPERATURE

BIBLIOGRAPHY

1. Aitken, R.J., West, K.M. (1990) Analysis of the relationship between reactive oxygen species production and leucocyte infiltration in fractions of human semen separated on Percoll gradients. *International Journal of Andrology*, 13:433-51.
2. Aitken, R.J., Clarkson, J.S. Fishel, S. (1989) Generation of reactive oxygen species, lipid peroxidation and human sperm function. *Biology of Reproduction*, 41:183-7.
3. Endtz, A.W. (1972) Een methode om het vichtige uniresediment en het vochtige menselijke sperma rechtstreeks te kleuren. *Nederlands Tijdschrift voor Geneeskunde*. 116(17):681-5
4. Hill, J.A., Haimovici, F., Politch, J.A. Anderson, D.J.(1987) Effects of soluble products of activated lymphocytes and macrophages (lymphokines and monokines) on human sperm motion parameters. *Fertility and Sterility*, 47:460-5
5. WHO (1992) WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. Third Edition, Cambridge University Press, Cambridge, p.107.
6. Wolff, H., Anderson, D.J. (1988), Immunohistological characterization and quantification of leukocyte subpopulation in human semen. *Fertility and Sterility*, 53:528-36

CAUTION: Reagent 1 contains benzidine and cyanosine. **Very poisonous by inhalation, skin contact or swallowing.** Risk of irreparable damage. Remove contaminated clothing immediately. Wear protective clothing. In case of accident, seek immediate medical attention.

Reagent 2 contains Hydrogen Peroxide (H_2O_2), corrosive, causes burns. In case of skin contact, wash immediately with water and soap. Wear eye/face protective equipment.



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