Toluidine Blue Image Cytometry Assay for Sperm Chromatin Integrity:

- Simple, unexpensive and reliable test for infertility diagnostics
- Provides complementary data for standard semen analysis
- Sensitive for both DNA strand breaks and chromatin packaging density
- Sensitivity for infertility diagnostics reaches 92%

Contact information:

Andrology laboratory
Riga Stradins University
Dzirciema 16
Riga LV 1007, Latvia
phone: +371 29471361
fax: +371 67331739
e-mail: Juris.Erenpreiss@rsu.lv

ERAF
EIROPAS REĢIONĀLĀS ATTĪSTĪBAS FONDS

EIROPAS SAVIENĪBA

References:

1. Erenpreisa J, Erenpreiss J, Freivalds T, et al. Toluidine blue test for sperm DNA integrity and elaboration of image cytometry algorithm. Cytometry. 2003;52A(1):19-27.
2. Erenpreiss J, Jepson K, Giwercman A, et al. Toluidine blue cytometry test for sperm DNA conformation: comparison with the flow cytometric sperm chromatin structure and TUNEL assays. Hum. Reprod. 2004;19(10):2277-2282.
3. Tsarev I, Bungum M, Giwercman A, et al. Evaluation of male fertility potential by Toluidine Blue test for sperm chromatin structure assessment. Hum. Reprod. 2009;24(7):1569-1574.

RĪGA STRADIŅŠ UNIVERSITY

Andrology Laboratory Riga Stradins University

Toluidine Blue Image Cytometry Assay for Sperm Chromatin Integrity:

a novel and efficient assay for male infertility diagnostics

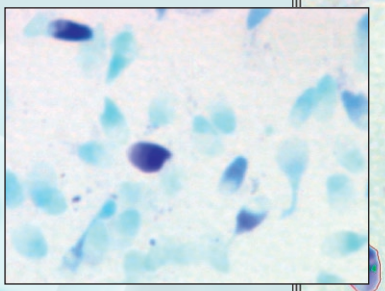
Riga,
Latvia 2011

RSU

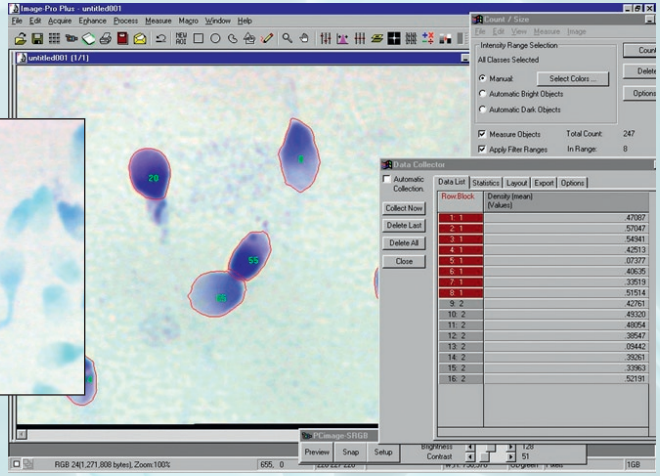
Semen analysis is routinely used to evaluate the male partner in infertile couples. However, it has been demonstrated that due to a considerable overlap in the values of sperm concentration, motility and morphology between fertile and infertile men, prognostic power of semen analysis for infertitlity diagnosis is limited.

It has been sugested that assessment of sperm DNA damage yields significant diagnostic and prognostic information complementary to that obtained from standard sperm parameters. Sperm DNA integrity is an important, independent parameter of sperm quality that has been associated with male fertility potential both in vivo and in vitro. A widely used tests for chromatin/DNA integrity are the sperm chromatin structure assay (SCSA) and the terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling (TUNEL) assay. However, both these tests require expensive eguipment and highly trained staff.

We have developed an alternative simple and unexpensive method for sperm chromatin structure assessment: the Toluidine Blue (TB) test. The test measures proportion of sperm cells with abnormal chromatin integrity, which is dependent on both the protein state and DNA integrity. TB is a nuclear dye used for external metachromatic and orthochromatic staining of chromatin depending on chromatin packaging state. Cells with disturbed chromatin packaging are detected by assesing optical density during image cytometry analysis. TB test is perfomed on slides using light microscope, no flow cytometer or other specific equipment required. Slides are permanent and easy to handle and store.



Toluidine Blue
staining of sperm smear.
Note dark cells with disturbed
chromatin structure



Toluidine Blue Image Cytometry. Cell optical density are measured.

We have demonstrated that the results of the TB test correlate well with the results of the SCSA and TUNEL assays. The proportion of sperm cells with abnormal chromatin conformation, detected by the TB test (dark cells with high optical density as measured by image cytometry) correlated significantly with the proportion of spermatozoa containing denaturable DNA detected as SCSA percentage DFI (r = 0.84, P = 0.001, Figure 1) and with the fraction of spermatozoa with fragmented DNA in the flow cytometry TUNEL test (r = 0.80, P = 0.001, Figure 2).

We also have evaluated the clinical applicability of the TB test in assessing male fertility potential using well-defined groups of fertile and infertile men. There was a significant difference in the percentage of TB dark cells between fertile and infertile men.

Thresholds for the TB test between fertile and infertile men were set by ROC curve analysis. Sensitivity and specificity for the thresholds were calculated. A threshold for TB dark cells by ROC curve analysis was set at 45% (Fig. 3); if percentage of sperms cells with abnormal chromatin structure exceeds 45% – a man with 92% probability is infertile, corresponding specificity is 42%. Odds ratio for infertility was 7.5 (95% CI: 2.7 –20.8) when the 45% TBDC threshold was used.

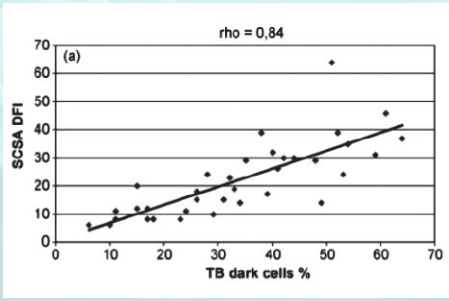


Fig. 1. Correlation between sperm cells with disturbed chromatin structure as measured by TB and SCSA tests.

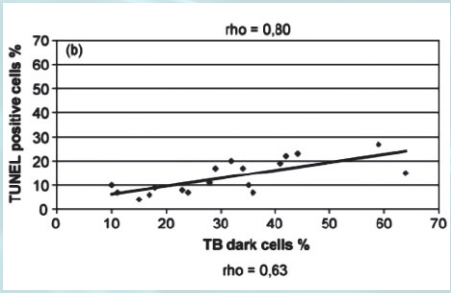


Fig. 2. Correlation between sperm cells with disturbed chromatin structure as measured by TB and TUNEL tests.

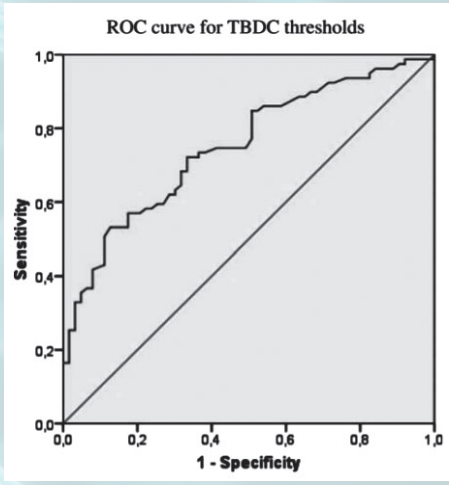


Fig. 3. ROC curve for TB dark