A HIGH THROUGHPUT PROTOCOL FOR SPERM DNA FRAGMENTATION ANALYSIS USING THE SDF-INDEXTM ALGORITHM.

Elizabeth C. Cox, Ricardo P. Bertolla

C.S. Mott Center for Human Growth and Development, Department of Obstetrics and Gynecology, Wayne State University

WAYNE STATE
School of Medicine

SDF-Index™

Introduction

Sperm DNA fragmentation (SDF) is an important biomarker of male fertility that has been associated with reduced natural conception, poorer assisted reproductive outcomes, and recurrent pregnancy loss. Acridine orange flow cytometry provides a rapid, high-throughput method for assessing SDF using the DNA fragmentation index (DFI) and high DNA stainability (HDS).

Conventional protocols recommend acquisition shortly after staining because of concerns regarding fluorescence instability over time. However, this can drastically limit laboratory throughput due to interrupted workflow. This study evaluates whether delayed acquisition following acridine orange staining can maintain analytical performance while increasing testing flexibility using the proprietary SDF-Index™ algorithm.

Objective

To determine whether delayed post-staining acquisition can support higher-throughput sperm DNA fragmentation testing by maintaining accurate DFI and HDS measurements using the SDF-Index™ algorithm.

Design

Prospective experimental study with time-series analysis.

Materials and Methods:

Semen specimens obtained from a biorepository at the C.S. Mott Center for Human Growth and Development at Wayne State University were diluted to 1–2 x 10^6 sperm/mL , exposed to acid-detergent denaturation for 30 seconds, stained with acridine orange, and maintained at 4–8°C before flow cytometric acquisition. Samples were analyzed at the standard 2.5-minute time point and serially through 7 days using the proprietary SDF-Index™ algorithm to quantify DNA fragmentation index (DFI) and high DNA stainability (HDS). DFI was categorized as low (<15%), average (15–<25%), high (25–40%), or very high (>40%), while HDS was classified as normal (<25%) or elevated (≥25%) **(Figure 1).** Analytical stability was evaluated using changes in percentage points based on the origin, Lin concordance correlation coefficient (CCC), tolerance retention, and paired statistical testing with Holm adjustment. All specimens were obtained under an Institutional Review Board-approved waiver.

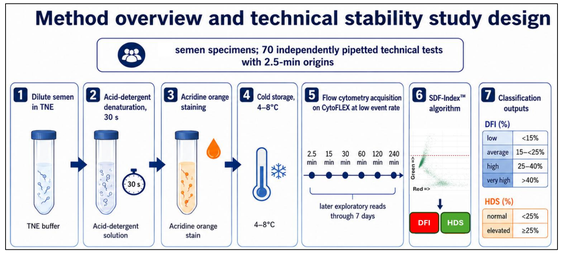

Figure 1. Experimental workflow for delayed-acquisition sperm DNA fragmentation analysis.

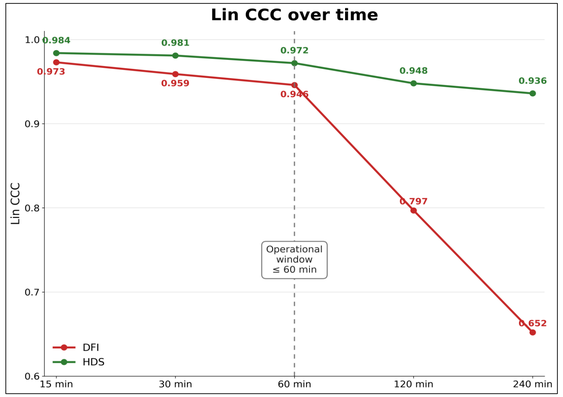

Figure 2. Concordance of DFI and HDS measurements with baseline over time.

Results:

Seventy analyses from 16 semen specimens were included in the study. Baseline DFI was 19.0 ± 12.8%, with 39 low, 14 average, 11 high, and 6 very high classifications. Baseline HDS was 9.5 ± 5.5%, and all specimens demonstrated normal HDS values. DFI remained quantitatively stable through 60 minutes after staining, with mean non-significant changes of +0.11, +0.28, and +1.04 percentage points at 15, 30, and 60 minutes, respectively. Concordance with baseline remained high throughout (CCC = 0.946–0.973), and 81–91% of measurements remained within ±5 percentage points of their original value **(Figure 2).** Although DFI category discordance increased modestly over time, most classification changes occurred in samples with baseline values within 5 percentage points of established category thresholds **(Figure 3).** At 120 minutes, DFI demonstrated significantly greater deviation from baseline (+3.40 percentage points), reduced concordance (CCC = 0.797), and increased category discordance (30.0%). HDS measurements remained stable through 240 minutes.

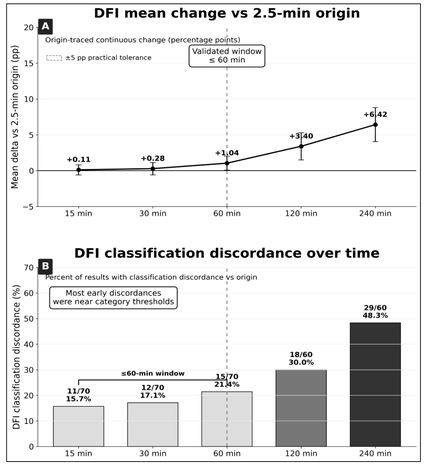

Figure 3. Stability of DFI measurements and classification over delayed acquisition times.

Conclusions:

DNA fragmentation measurements generated by acridine orange flow cytometry and analyzed using the SDF-Index™ algorithm **(Figure 4)** remained analytically stable for up to 60 minutes after staining when samples were maintained under refrigerated conditions. Delayed acquisition within this interval may provide greater laboratory flexibility and improve testing throughput without compromising DFI assessment. Although HDS measurements also remained stable, additional evaluation of specimens with elevated HDS is needed to fully establish delayed acquisition performance for chromatin immaturity assessment.

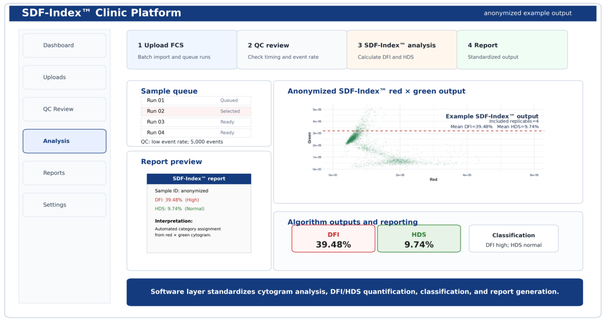

Figure 4. Representative SDF-Index™ analysis of DFI and HDS.

Support: None