Electronic Supplementary Information

Paper-based sperm DNA integrity analysis

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Contents

Materials and Methods

Device fabrication and operation. The paper-based sperm DNA analysis device was designed in power point and printed on nitrocellulose paper (mean pore diameter of 0.45 μm, Bio-Rad Laboratories Ltd., Canada) using a solid wax printer (ColorQube 8570N, Xerox, Canada). Pattered nitrocellulose paper then heated at 125 ºC for 5 min to let the wax to diffuse through the thickness of the paper and from the hydrophobic boundary. To add ICP functionality to the paper, 0.5 μL of cation-selective nanoporous Nafion (20% wt. in lower aliphatic alcohols and water, Sigma-Aldrich, US) was pipetted at the beginning of the sample channel, following by hydration of the membrane in DI water for 30 min. Devices were air-dried at room temperature and stored in Petri dishes upon use. To operate with the device, 3 μL of the sample was pipetted into the sample channel, following by saturation of the device with DI water. ICP was induced by applying a voltage of 150 V/cm along the sample channel for 15 min. Following this step, an upright fluorescence microscope (Axiophot, Carl Zeiss AG, Germany) was used to capture images in green (dsDNA) and red (ssDNA) fluorescence. Captured images were processed in ImageJ and a written script in Matlab was used to for data quantification.

Semen sample preparation. Fresh human semen from healthy donors and patients (n=7) were obtained by masturbation after 2-4 days of sexual abstain at Royal Victoria Hospital in Montreal, Canada. All donors signed and informed consent, and the information remained confidential within the institution. After liquefaction (30 min incubation at 37 ºC), computer-assisted sperm analysis

(CASA; Penetrating Innovations, Ingersoll, Canada) equipped with an Olympus BH2 Microscope and a Sperm Vision HR software (Version 1.0.5, 2008) was used to obtain standard semen parameters in accordance to WHO guidelines.[1] The semen sample was diluted to concentration of 20 million sperm per mL (M/mL) using TNE buffer (0.01 M Tris-HCl, 0.15 M NaCl, 1 mM EDTA, pH 7.4). Following this steps, sperm cell were lysed by treating 10 µL of the diluted sample with 40 µL of 50 mM dithiothreitol (DTT), 50 µL of distilled water, and 300 µL of 8 M GuHCl in separate steps (the solution was vortex mixed for 30 s between each step and for 15 s after the last step).[2] Lysed sperm DNA was fluorescently labeled by adding 50 µL of acridine orange (AO) staining solution (0.1 M citric acid, 0.2 M Na2PO4, 1 mM EDTA, 0.15 M NaCl, 6 µg AO/mL staining buffer, pH 6.0) to 50 µL of lysed sperm DNA, followed by storage on ice for 3 min. Stained sample was diluted 500-fold in DI water and used instantly.

Sperm chromatin structure assay. Flow cytometry-based SCSA was used to clinically test for sperm DNA integrity.[3,4] Semen samples were diluted to a concentrate of 10 M/mL with TNE buffer. After dilution, 200 µL of the sample was treated with 400 µl acid detergent solution (0.08 N HCl, 0.15 M NaCl, 0.1% Triton-X 100, pH 1.2) for 30 s. Following this step, the cell suspension was stored on ice and treated with 1.2 ml of AO staining solution for 3 min. after staining, the sample was run through a MACSQuant Analyzer (Miltenyi Biotec, Bergisch Gladbach, Germany) equipped with 488 nm air-cooled laser. About 5,000 to 10,000 sperm were analysed for each sample at a flow rate of 100-200 events/sec. A reference sample was used to set the red and green photomultiplier tube (PMT) voltage gains to yield the same mean fluorescence levels (~ 120/1000 channels for red and 400/1000 channels for green). WinList software (Verity Software House Inc., Topsham, ME) was used to analyse the data which generated cytogram (the red vs green fluorescence) and histogram (total cells vs %DFI), as well as %DFI and %HDS.

Table S1. Comparison of processing time for conventional SCSA and paper-based sperm DNA integrity test.

Task	**Processing time (min)**	
	Conventional SCSA	Paper-based sperm DNA integrity test
Liquefaction	30	30
Semen Analysis	10	10
Sample preparation	6	8
Flow cytometry (including prep time)	22	N/A
ICP concentration (including prep time)	N/A	17
Data Analysis	5	5
Total †	**73**	**70**

† In terms of test time, the method is comparable to SCSA (Table S2). This comparison misses the more important point that clinics do not, in general, run SCSA from fresh samples, as would be possible with the paper-based method. Rather, samples are frozen and tested in batches, typically once or twice per week, to (i) reduce reagent-use and technician time in clinics with SCSA capabilities and (ii) allow for shipping of samples in clinics without an in-house SCSA.

Table S2. Estimated capital and operating costs for conventional SCSA and paper-based sperm DNA integrity test.

Conventional SCSA			**Paper-based sperm DNA integrity test**		
Equipment	Capital cost (USD)	Operating cost (USD/100 test)	**Equipment**	Capital cost (USD)	Operating cost (USD/100 test)
Consumables	N/A	500	Paper device	N/A	40
Reagents	N/A	2,500	Reagents	N/A	10
MACSQuant Analyzer 10 *	165,000	N/A	Voltage source*	500	N/A
			Basic fluorescence microscope*	2,500	N/A
Total	**165,000**	**3,000**	**Total**	**3,000**	**50**

*As quoted by suppliers

References

1 *WHO laboratory manual for the Examination and processing of human semen*, WHO Press, Geneva, 5th edn., 2010.

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