Novel centrifugal technology for measuring sperm concentration in the home

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Objective: To evaluate the analytical performance and usability of the Trak Male Fertility Testing System, a semiquantitative (categorical) device recently US Food and Drug Administration (FDA)-cleared for measuring sperm concentration in the home by untrained users.
Design: A three-site clinical trial comparing self-reported lay user results versus reference results obtained by computer-aided semen analysis (CASA).
Setting: Simulated home use environments at fertility centers and urologist offices.
Patient(s): A total of 239 untrained users.
Intervention(s): None.
Main Outcome Measure(s): Sperm concentration results reported from self-testing lay users and laboratory reference method by CASA were evaluated semiquantitatively against the device's clinical cutoffs of 15 M/mL (current World Health Organization cutoff) and 55 M/mL (associated with faster time to pregnancy). Additional reported metrics include assay linearity, precision, limit of detection, and ease-of-use ratings from lay users.
Result(s): Lay users achieved an accuracy (versus the reference) of 93.3% (95% confidence interval [CI] 84.1%–97.4%) for results categorized as $\leq$15 M/mL, 82.4% (95% CI 73.3%–88.9%) for results categorized as 15–55 M/mL, and 95.5% (95% CI 88.9%–98.2%) for results categorized as >55 M/mL. When measured quantitatively, Trak results had a strong linear correlation with CASA measurements ($r = 0.99$). The precision and limit of detection studies show that the device has adequate reproducibility and detection range for home use. Subjects generally rated the device as easy to use.
Conclusion(s): The Trak System is an accurate tool for semiquantitatively measuring sperm concentration in the home. The system may enable screening and longitudinal assessment of sperm concentration at home.
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Key Words: Sperm count, male fertility, home test, cytometry, semen analysis

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For at least the past seven decades, the cornerstone of clinical male fertility evaluation has been semen analysis (1). Male factor infertility is a relatively common condition, contributing to 30%–50% of infertility cases (2, 3). However, conventional semen analysis suffers from several drawbacks, including restriction to specialized physician offices or centralized laboratories, and interlaboratory variability due to a diversity of standard practices (4). Furthermore, many men find the process of producing a semen sample in a clinical setting to be embarrassing, disconcerting, and inconvenient (5). Population data indicate that just 9.4% of men aged 25–44 years used infertility services compared with 13% of similarly aged women (6). This gender discrepancy is emphasized by Centers for Disease Control and Prevention survey data suggesting that up to 27% of infertile

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couples forgo a male evaluation entirely during an infertility evaluation (7).

The gap in evaluation of the male partner has spurred development of more convenient, home-use semen tests. Most prior products provide binary (yes or no) results for sperm concentration based on the World Health Organization-recommended cutoff of 20 M/mL and more recently 15 M/mL (1, 8–12). However, there is not broad agreement on a single threshold value for male subfertility for any semen analysis parameter (1, 11, 13–18), nor is there a sharp transition in the probability of unassisted pregnancy across these cutoff thresholds (19). For example, a 2002 study showed that time to natural pregnancy improves linearly with increasing sperm concentration up to approximately 55 M/mL (17). A 2001 analysis from the Reproductive Medicine Network recommended two cutoffs dividing results into three categories: fertile, indeterminate, and subfertile (13). Therefore, although binary tests provide a convenient home screening option, they have limited clinical utility for assessing one's fertility status, do not help identify longitudinal trends in sperm concentration, may provide undue reassurance for a positive (yes) result near the cutoff, and have not seen substantial adoption by reproductive medicine practitioners.

Here we report the development and clinical validation of a home-use device called the Trak Male Fertility Testing System. The centrifugal microfluidic device, which recently received 510(k) clearance from the US Food and Drug Administration (K153683), provides linear sperm concentration measurement with three category (two cutoff) semiquantitative results: low ($\leq$15 M/mL), moderate (15–55 M/mL), and optimal (>55 M/mL). This categorical approach combines the World Health Organization threshold with an evidenced-based reference value for faster time to pregnancy (1, 17).

MATERIALS AND METHODS

System Components and Operation

The Trak Male Fertility Testing System (Sandstone Diagnostics, Inc.), shown in Figure 1A, comprises a battery-powered reusable instrument (Engine), single-use test cartridges (Props), and various consumables. The Props (Fig. 1B) use centrifugal force to process a defined volume of semen and visually display sperm concentration. To operate the system, a user collects a semen sample in an enzyme-coated collection cup that promotes liquefaction (viscosity reduction). The user transfers approximately 0.25 mL of semen to the Prop inlet chamber, attaches the Prop to the Engine, and closes the lid to initiate the spin sequence (~6.5 minutes at 7,000 rpm). As the Prop spins, a precise volume of semen is metered by centrifugal action from the sample inlet into the metering chamber, and sperm are compacted into a narrow channel at the end of the Prop. When the spin sequence is complete, the sperm cells form a visible, measurable white column that is proportional to the concentration of sperm in the sample and visually interpreted by the user (Fig. 1C).

The system also includes an external control comprising a bead suspension formulated to simulate Trak results at a calibrated sperm concentration. Users may run the control on a Prop to verify proper operation of the system components, as well as to practice the assay protocol before testing a semen sample.

Assay Principle

Assessing sperm concentration by compacted cell volume has been previously explored by loading semen into the glass tubes and high speed centrifuges typically used with hematocrit (20). However, the pellet heights produced using conventional hematocrit accessories have poor correlation with sperm concentration (21, 22). The Trak System differs in several important aspects from previous "spermatocrit" incarnations. First, the Trak Prop is manufactured with a preloaded liquid density medium that prevents low density contaminants from interfering with test results. Semen typically contains cell debris, immature sperm cells, and other contaminant particulates that can be filtered by a density gradient (23–27). Second, the internal geometry of the disposable Prop forces sperm cells from a much larger volume of semen (170 vs. 50 μL) into the cell collection channel, producing a larger and more distinct pellet, especially at low sperm concentrations. Third, the Prop cell collection channel cross-section increases away from the tip, accommodating differences in packing density on the physiological range of human sperm concentrations and thereby increasing dynamic range.

Calibration

The spatial position of the reference marks on the Trak Prop were calibrated by running a series of defined semen samples derived by pooling individual samples and diluting to the desired concentration with cell-free seminal plasma. Trak results were imaged within 3 minutes of assay completion in the presence of a calibrated ruler using an Mu300 camera (AmScope), and analyzed using ImageJ software version 1.48 (National Institutes of Health). For analytical performance studies, Trak results were converted from pellet length to concentration using the calibration data.

Consumer Clinical Study Design

Device performance and usability were evaluated in a consumer clinical study carried out at three sites in the United States. Subjects were provided with the Trak System and the instructions for use, and were given no further assistance. While waiting during the semen liquefaction step, lay subjects completed a control test, proceeding to test their sample only after obtaining a positive control result. A 500-μL aliquot of the sample was transferred to a reference technician for computer-aided semen analysis (CASA) measurement. The remaining semen was transferred to a trained technician for testing by Trak. The subject and trained Trak operator were

FIGURE 1

The Trak Male Fertility Testing System. (**A**) Photo of the system contents, including the reusable Engine, fluid transfer device, instructions, liquefaction cups, seal-before-spin stickers, the Prop in a foil pouch, and several unpouched Props. Photo shows a Prop placed on the Engine with a seal-before-spin sticker in place. (**B**) Photo of the Prop, with and without label. (**C**) Example test results of semen samples with low (≤15 M/mL), moderate (15–55 M/mL), and optimal (>55 M/mL) sperm concentrations.

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blinded to the reference result, and the reference technician was blinded to all Trak results by assigning unique identifier numbers to each subject and collecting results with paper case report forms completed by each independent operator or technician.

Lay users reported each result as ≤15, 15–55, or >55 M/mL according to the instructions for use. Likewise, the trained technicians reported an interpretation of each subject's result to evaluate lay user interpretation. Trak results (from the lay subject and trained technician) were also photographed within 2 minutes of assay completion for verification. After the test, lay subjects were instructed to complete a survey about their results interpretation and the device ease-of-use using a 5-point Likert scale (1 = very easy, 5 = difficult).

Ethical Approval, Exclusion Criteria, and Recruitment

Approval was granted by Salus Institutional Review Board (Austin, TX) for analytical performance validation studies, and by Aspire Institutional Review Board (Santee, CA) (#SD001) for clinical consumer studies at all sites. Semen samples from consenting donors were used for all analytical assay development and validation studies. Donors were instructed to collect samples after a 2- to 7-day abstinence, and deliver samples to the analysis site within 1 hour of collection. In the clinical consumer studies, site technicians measured the subject's semen volume after collection and subjects who could not produce at least 1.8 mL of semen (minimum required ejaculate volume to complete all tests for the

clinical study) were excluded from the study. Clinical subjects were recruited from site patient pools and online study listings.

Statistical Analyses

Deming regression analysis was used to evaluate the linear assessment study and clinical results. Regression analysis was carried out using R version 3.2 (The R Foundation for Statistical Computing). Where relevant, the Pearson correlation coefficient, *r*, was also calculated. Semiquantitative conditional probabilities were calculated by dividing the number of correct calls by the total number of reference method results for each category ($\leq$15, 15–55, or >55 M/mL). Wilson scores were used to calculate 95% confidence intervals (CIs).

Precision

To evaluate the assay precision and reproducibility, quantified semen was pooled and diluted to concentrations of 13, 15, 17, 18, 47, 55, and 63 M/mL, and tested by Trak at five separate time periods during 24 hours. For each concentration tested, three Prop lots were tested by three operators using three separate Engines during each period. A total of 60 replicates were tested at each concentration in total, and components of variation were determined using a three-factor nested analysis of variance (ANOVA) model implemented in R version 3.2.

Linearity

To evaluate the assay's linear response, quantified semen was diluted to concentrations of 5, 10, 20, 25, 35, 45, and 65 M/mL. In addition, a pooled sample of cell-free seminal plasma was included as a negative control. Trak assays were performed on each sample in replicates of four and the resulting pellet lengths were measured by photographic analysis.

Limit of Blank and Limit of Detection

Semen samples from vasectomized donors were confirmed azoospermic by microscopic inspection, and were tested in 120 replicates on two lots of the Trak device to establish the maximum assay response from cell-free semen (limit of blank) in accordance with guidance from the Clinical and Laboratory Standards Institute, *CLSI-EP17-A2* (28). Four low concentration samples between 0.8 and 3.5 M/mL were tested in 30 replicates each to establish the limit of detection by Equation (1)

$$\mathrm{LoD} = \mathrm{LoB} + 1.645\sigma_s, \quad (1)$$

where LoB is limit of blank, σ_s is the pooled standard deviation for low concentration samples, and LoD is the limit of detection.

Laboratory Reference Method

To provide a consistent reference method across clinical sites and avoid potential intersite and intertechnician bias or variability associated with manual semen analysis, sperm concentrations at all sites were measured with a validated cell-counting procedure using Hamilton-Thorne CEROS CASA instruments running version 14.13 software for all studies. The instrument settings were defined according to the manufacturer's recommendations. Four separate 6-μL portions of each sample were loaded onto 20-μm chamber slides (Leja) and analyzed at 37°C. To minimize error in the reference method, a larger number of replicates and total number of cells were analyzed than in standard practice. Each sample was measured a minimum of four times, with each measurement consisting of 1,000 total cells counted, or 20 microscopic fields, whichever occurred first. Measurements were repeated if the concentration range of the four measurements exceeded 20% of the mean. To accommodate the concentration limits of the instrument, samples with sperm concentrations exceeding 50 M/mL were diluted 1:1 with D-phosphate-buffered saline (PBS; Sigma) using a minimum of 50 μL of semen and diluent. As a control, each operator evaluated two sets of defined concentration beads (Hamilton-Thorne) by CASA each day of analysis. In repeated comparisons, with multiple operators the CASA reference procedure was found to produce equivalent results to the World Health Organization-recommended method for manual analysis of semen, with improved intermeasurement and interoperator precision (1). Seminal debris was largely excluded by the image analysis algorithm, and the induced error was well below the interoperator variation for the manual method (data not shown).

RESULTS

Study Population and Participants

In all, 239 men were recruited including 166 (69.5%) healthy volunteers, 46 (19.7%) partners in a couple having difficulty conceiving, 12 (5.0%) diagnosed with male factor infertility, 13 (5.4%) patients after vasectomy, and 1 (0.4%) patient after vasectomy reversal. Subjects were 33.9 years old on average (range, 20.0–49.0 years), with 51.9% white and 33.1% with some college education (Supplemental Table 1, available online).

Clinical Performance (Method Comparison)

Table 1 shows the 3 × 3 contingency table comparing Trak results from the subject/tester to the CASA reference method according to the semiquantitative categorization ($\leq$15, 15–55, or >55 M/mL) across all sites. The results indicate accuracy of 93.3% (95% CI 84.1%–97.4%) for results categorized as $\leq$15 M/mL, 82.4% (95% CI 73.3%–88.9%) for results categorized as 15–55 M/mL, and 95.5% (95% CI 88.9%–98.2%) for results categorized as >55 M/mL.

Of the n = 24 lay user versus CASA discrepant results, n = 6 (25%) were within 5 M/mL of the 15-M/mL threshold with CASA results often reading >15 M/mL and the apparently false Trak readings below the 15-M/mL threshold, indicating that slight variations in CASA quantitative results around the 15 M/mL cutoff represents one source of error. For example, the CASA method is known to occasionally read debris as sperm cells, thereby yielding a falsely high sperm concentration result.

TABLE 1

A 3 × 3 contingency table for agreement between lay subject Trak results versus computer-aided sperm analysis (CASA) at all study sites (N = 239).

	Reference value		
Subject interpretation	**≤15 M/mL**	**15–55 M/mL**	**>55 M/mL**
≤15 M/mL	56	8	1
15–55 M/mL	4	75	3
>55 M/mL	0	8	84
Accuracy (95% CI)	93.3% (84.1%–97.4%)	82.4% (73.3%–88.9%)	95.5% (88.9%–98.2%)

Note: Summary accuracy of lay subject Trak result versus reference is shown below each respective category (95% confidence intervals [CI] are calculated by Wilson score).

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Consumer Usability

Of the 239 subjects who met study inclusion criteria, 100% were able to successfully run a control test and obtain a positive result. A summary of the responses from the questionnaire presented to subjects enrolled in the clinical study can be found in Table 2. Of the subjects, 98.7% believed that they performed the steps correctly and obtained a valid result.

Precision

Trak generated stable results across different operators, disposable lots, and devices with the component of coefficient of variation (CV) due to these combined factors calculated as <1.5% for all tested concentrations (Supplemental Table 2, available online). Likewise, the within-run component of CV was <7% for all tested concentrations indicating adequate reproducibility between replicate tests. For comparison, the CV of sperm concentration by CASA has been reported to be approximately 10% (29). Trak results are considered valid for 2 hours after sample collection. Average Trak results were initially within 5% of reference values for all concentrations (Supplemental Table 3, available online), and as expected showed a declining value at longer time intervals after collection (Supplemental Fig. 1, available online). This effect may be due to changes in the properties of semen caused by enzymatic action and progressive changes in sperm cell integrity.

TABLE 2

Results of consumer use surveys.

Survey question: how difficult was…	**Survey result: easy or very easy (out of 239)**	**%**
Collecting a sample	223	94.1
Following the test instructions	235	98.3
Running the control test	235	98.4
Waiting 30 min for liquefaction & opening Prop pouch	227	95.0
Transferring sample to Prop	233	97.5
Attaching Prop to Engine	228	95.4
Understanding when spin sequence began & ended	235	98.3
Interpreting the result	223	93.3

Note: Survey questions are condensed for brevity. See Supplemental Information for the original survey questions.

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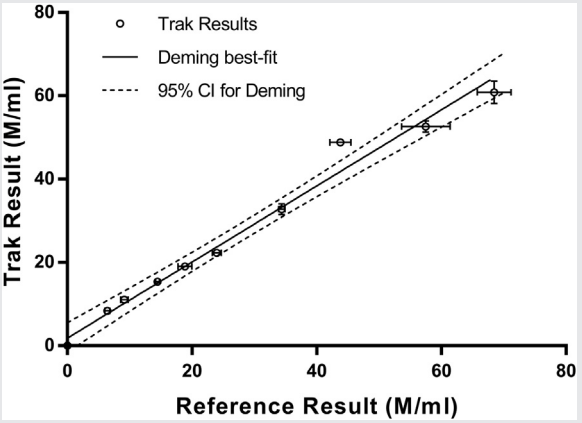
Linearity

Linearity assessment with pooled and diluted semen samples revealed a strong linear correlation with reference results between 0 and 67.7 M/mL ($r = 0.99$), a range that includes the 15-M/mL and the 55-M/mL thresholds (Fig. 2) A best-fit line was found by Deming regression to have a slope of 0.91 (95% CI 0.81–1.0) and a y-intercept of 1.9 M/mL (95% CI -1.6–5.5). The 95% CIs for slope and intercept contain 1 and 0 M/mL, respectively.

Limit of Blank and Limit of Detection

The data demonstrated that vasectomized samples were consistently assigned correctly into the ≤15-M/mL category and produced results close to zero and not visible without magnification. The average Trak result for vasectomized samples was 0.30 M/mL. Trak results from this group were typically not visible without magnification. Trak could reliably detect sperm concentrations as low as 1.19 M/mL, the calculated limit of detection. Both the limit of blank and limit of detection were well below the 15-M/mL lower clinical threshold.

FIGURE 2



Linear assessment. A Deming regression best-fit line is also plotted for reference (slope = 0.91, intercept = 2.0 M/mL), along with 95% confidence interval (CI) estimates for the Deming best-fit line. $r = 0.99$.

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DISCUSSION

The clinical and performance studies reported in the present study demonstrate that the Trak System provides lay users with an accurate and consistent measurement of sperm concentration. The primary sources of error for clinical subjects were: [1] interpretation of apparently blank channels (no pellet) resulting from zero or very low sperm concentrations, and [2] misrepresentation of results close to, but above the 15-M/mL threshold. Results falling into category [1] were interpreted correctly by the trained technician, whereas results falling into category [2] generally resulted in pellets that were just below the reference mark on the disposable cartridge. Due to the device's linear visual readout, results misinterpreted as slightly above or below a cutoff may be noted as a borderline result and treated accordingly.

Several important limitations of this study warrant mention. Many of the coauthors have a financial interest in the company. Although strict blinding of the data and analysis should limit bias in data collection, it remains possible. Whereas validated in the literature, the sperm concentration cutpoints chosen may not reflect infertility or fertility in all populations. Given the variation in semen quality based on geography and race/ethnicity, semen quality and normative values may vary (30).

Trak measures only one semen parameter—sperm concentration, which is one of several semen parameters that impact male fertility. Others, including sperm motility, morphology, DNA integrity, semen volume, pH, liquefaction, may affect fertility outcomes independent of sperm concentration results. Due to the widespread use of total motile count by andrologists and reproductive endocrinologists for male fertility evaluation, the inability to measure sperm motility is a particularly notable limitation of the technology. It is possible to have moderate or optimal sperm concentration and low sperm motility or morphology. Furthermore, the test provides markings for three interpretive categories and may miss finer variations in sperm concentration. For these reasons, Trak should not be considered as a replacement for a comprehensive semen analysis.

Nonetheless, Trak's linear test results may enable longitudinal measurements to support medical interventions aimed at improving sperm concentration. For example, patients undergoing common medical treatments or procedures aimed at improving sperm production, including varicocelectomy, vasectomy reversal, and hormone therapy (HT), may benefit from a more accessible testing option (31–36).

It is expected that by allowing in-home evaluation and retesting of sperm concentration, the Trak System will lower the barrier for more comprehensive testing. The system may help men identify suboptimal sperm concentration levels early, and thus may ultimately help more men receive medical evaluation and treatment for infertility. The system may further enable new strategies for reproductive health management by allowing lay users to longitudinally measure sperm concentration at home alongside medical consultation and treatment. Last, although Trak cannot measure total motile sperm count, the result correlates linearly with sperm concentration, which remains a parameter under active study (18, 37–44). A standardized method for home testing may enable more detailed population studies of sperm concentration.

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SUPPLEMENTAL TABLE 1

Summary of demographics of the consumer clinical study (n = 239 men).

Parameter	Result
Age (y), continuous: mean (SD) (min–max)	33.9 (7.6) (20.0–49.0)
Race, n (%)	
White	124 (51.9)
Native Hawaiian/Pacific Islander	2 (0.8)
Black or African American	43 (18.0)
American Indian or Alaska Native	1 (0.4)
Asian	28 (11.7)
Two or more races	10 (4.2)
Other race	31 (13.0)
Education, n (%)	
Grade school	0 (0.0)
High school or equivalent	18 (7.5)
Some college	79 (33.1)
AA degree	37 (15.5)
4-y (Bachelor's) degree	72 (30.1)
Postgraduate	33 (13.8)

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SUPPLEMENTAL TABLE 2

Summary of precision study results.

			Within-run		Between run		Between period		Between operator[a]		Total SD and %CV	
ID	N	Mean	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
1	60	13.0	0.47	3.6%	0.36	2.8%	0.43	3.3%	0.00	0.0	0.73	5.6%
2	60	14.1	0.44	3.1%	0.27	1.9%	0.67	4.4%	0.00	0.0	0.80	5.7%
3	60	15.9	0.37	2.3%	0.31	2.0%	0.47	3.1%	0.00	0.0	0.68	4.3%
4	60	16.1	1.03	6.4%	0.00	0.0	1.47	9.1%	0.00	0.0	1.80	11.2%
5	60	47.4	2.42	5.1%	0.00	0.0	0.00	0.0	0.00	0.0	2.42	5.1%
6	60	55.2	2.66	4.8%	0.00	0.0	0.00	0.0	0.76	1.4%	2.77	5.0%
7	60	61.7	2.44	3.9%	0.47	0.8%	1.55	2.5%	0.00	0.0	2.92	4.7%

Note: Standard deviation (SD) for each factor is reported in mole per milliliter. The elevated component of percent coefficient of variation (%CV) for "Between period" for sample identification number (ID) 1–4 is caused by degradation of the semen samples versus time.

[a] Operator factor is convolved with Lot and Engine.

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SUPPLEMENTAL TABLE 3

Precision results from time period 1 (< 2 hours after initiation).

ID	Mean Trak result ± SD (M/mL)	Mean CASA result ± SD (M/mL)	Difference
1	13.6 ± 0.4	13.3 ± 0.9	2.3%
2	14.8 ± 0.8	15.4 ± 0.9	−4.0%
3	16.7 ± 0.4	16.7 ± 0.1	0.0
4	17.4 ± 0.7	18.2 ± 0.8	−4.7%
5	47.7 ± 2.2	46.3 ± 2.6	3.0%
6	55.9 ± 2.0	56.7 ± 3.2	−1.4%
7	60.5 ± 3.3	61.9 ± 3.8	−2.4

Note: The mean and standard deviation (SD) for n = 12 replicates for Trak results and n = 4 replicates for reference computer-aided sperm analysis (CASA) results are included for each concentration. ID = identification number.

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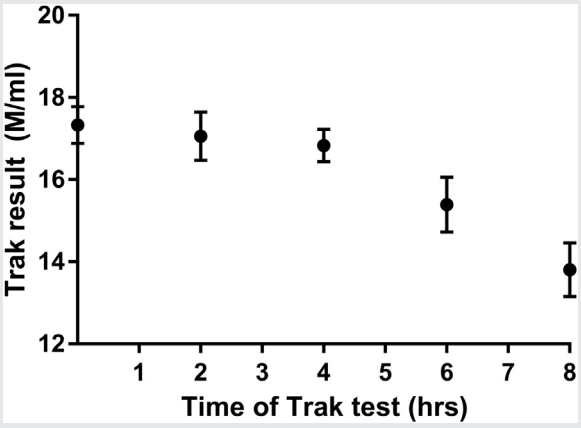
SUPPLEMENTAL FIGURE 1



Decrease in Trak result with time. Aliquots from each sample were taken and tested on Trak at 2-hour increments. The graph shows the mean Trak result for sample 4 during the course of the precision study. It is known that Trak results for a given sample decrease over time due to degradation of the semen sample, especially for lower concentration samples. The instructions for use indicate that the test is to be run within 2 hours after sample collection to ensure valid results. Error bars show the 95% confidence intervals for the average result during that time period.

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