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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.
Clinical Trial Registration Number: ClinicalTrials.gov identifier: NCT02475395. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
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