Validation of LensHooke® X1 PRO and Computer-Assisted Semen Analyzer Compared with Laboratory-Based Manual Semen Analysis

Ashok Agarwal1, Manesh Kumar Panner Selvam1, Rafael F. Ambar1,2,3
1American Center for Reproductive Medicine, Cleveland Clinic, Cleveland, OH, USA, 2Urology Department of Centro Universitario em Saúde do ABC/Andrology Group at Ideia Fertil Institute of Human Reproduction, Santo André, 3Hope Clinic—Human Reproduction, São Paulo, Brazil

Purpose: To compare two automated semen quality analysis systems (LensHooke® X1 PRO [X1 PRO] and IVOS CASA) for accuracy, precision and agreement with laboratory-based manual semen analysis (MSA).

Materials and Methods: Semen samples (n=31) were obtained from normozoospermic healthy male volunteers and infertile men with a minimum abstinence period between 2–3 days. After complete liquefaction, 101 seminal aliquots were prepared and tested according to WHO 5th Edition (2010) guidelines. The results obtained by X1 PRO and IVOS CASA were compared with that of MSA. Additionally, 10 samples were used to evaluate the intra- and inter-rater agreement for X1 PRO and MSA.

Results: The semen parameters (sperm concentration, total, and progressive motility) showed strong correlation and agreement for both automated semen analyzers and MSA (Spearman’s rank correlation ≥0.92, p<0.0001). X1 PRO and IVOS CASA were able to differentiate samples with abnormal concentration with a positive predictive value (PPV) of 100%. Furthermore, the PPV for X1 PRO (86.5%) was higher than that for IVOS CASA (71.7%) in differentiating samples with abnormal motility. The X1 PRO device showed a high PPV (97.7%) in identifying normal sperm forms compared to MSA. Semen parameters evaluated showed a high inter-rater (kappa >0.91) and intra-rater (kappa >0.92) agreement for X1 PRO compared with MSA.

Conclusions: Both automated semen analyzers demonstrated a high level of concordance and their performance was comparable with MSA analysis. Furthermore, high-levels of inter-and intra-rater reliability for semen analysis indicate that the new X1 PRO can be used in a clinical laboratory to offer accurate and quick test results.

Keywords: Computer assisted semen analyzer; LensHooke® X1 PRO; IVOS CASA; Semen analysis

INTRODUCTION

Infertility affects approximately 190 million people globally, where male factor infertility is implicated in approximately 50% of the total infertility cases [1-3]. Manual semen analysis (MSA) is an important part of laboratory evaluation of male infertility [4]. It can assess the macroscopic (pH, volume and appearance) and microscopic (sperm concentration, total motility, normal sperm morphology, vitality) parameters of the
semen sample [5]. MSA does not assess the fertility potential of men, but it differentiates men with normal and abnormal semen parameters based on the World Health Organization (WHO) guidelines (5th edition, 2010) [5]. The evaluation of microscopic parameters is particularly time-consuming and requires extensive training of the operator [6]. High subjectivity makes it challenging to rely on the results of MSA as it is prone to inter- and intra-observer variations. To overcome these shortcomings, computer-assisted semen analyzers (CASA) have been introduced to replace MSA [7,8].

Due to these limitations in MSA, many CASA systems were introduced about four decades ago. In general, CASA systems utilize a microscope to capture and generate successive images of spermatozoa with multiple static view fields. These images are then analyzed by customized commercially-available software. The major advantage of CASA systems is to provide sperm kinematic data with high precision, especially when MSA is unavailable or unreliable. In addition, when individual tracking data are needed, CASA systems can overcome the burden of measuring sperm tracks. A further important advantage of CASA is that the test samples can be analyzed in a shorter period of time as compared to MSA. The IVOS CASA system, from Hamilton Thorne (Beverly, MA, USA), is a popular semi-automated semen analyzer that is used in many andrology laboratories [8,9]. The latest generation of IVOS includes integrated phase contrast optics, improved image resolution and tail detection for better spermatozoon discrimination [8].

Despite the recent advances in automation technology, CASA systems still require manual intervention to rectify errors and provide reliable results [9]. Even though semi-automated semen analyzers have been used in andrology laboratories for the past three decades, most of them continue to be large, complicated and expensive instruments. Recently a new semen quality analyzer (LensHooke® X1 PRO [X1 PRO], BonyRayBio, Taichung, Taiwan) was introduced to offer a quick and reliable analysis of semen parameters in the form of a compact and portable device. It is based on artificial intelligence optical microscopic (AIOM) technology [10]. The LensHooke® X1 PRO semen quality analyzer was designed for in vitro diagnostic use and analyzes sperm concentration, total, progressive, and non-progressive motility as well as normal sperm morphology. It also measures sperm kinematics such as straight-line (rectilinear) velocity, average path velocity, straightness and amplitude of lateral head displacement. Diagnostic capability of the X1 PRO device with CS1 test cassette has been reported with a high level of correlation and agreement with the MSA [10]. However, the performance of the X1 PRO against other CASA systems (HT IVOS) and its inter-and intra-rater agreement for semen analysis remains to be investigated. We therefore conducted this study to compare two automated semen quality analysis systems (LensHooke® X1 PRO and IVOS CASA) for accuracy, precision and agreement with laboratory-based MSA.

MATERIALS AND METHODS

1. Study design and participants

Semen samples (n=31) were obtained from 8 healthy male volunteers and 5 patients presenting for male infertility after an abstinence period of 2 to 3 days. While the 8 healthy semen donors produced a total of 26 ejaculates, which were split into 88 aliquots, the 5 infertile patients produced 5 ejaculates that were split into 13 aliquots (Supplement Fig. 1). A total of 101 aliquots were prepared from the native semen samples either by dilution or concentration using seminal plasma of the respective donors (Supplement Table 1). Furthermore, 10 semen samples were used to evaluate the intra- and inter-observer agreement for the X1 PRO and MSA.

2. Semen analysis

The semen samples were collected after 2 to 3 days of sexual abstinence by masturbation into a sterile container and placed in an incubator at 37°C for 30 minutes to liquefy. Semen analysis was performed according to the WHO 5th edition guidelines (WHO, 2010). Semen parameters such as pH, concentration, total, and progressive motility, and morphology were determined by standard MSA and by two automated semen quality analyzers (IVOS CASA and X1 PRO). MSA was carried out using a disposable Leja counting chamber (Spectrum Technologies, Healdsburg, CA, USA) to evaluate sperm concentration, as well as total and progressive motility [11]. Automated semen analysis was performed by the LensHooke® X1 PRO semen analyzer and the IVOS CASA system with HTM-IVOS software (version 12.4), as per the manufacturer’s protocols. Sperm morphology was evaluated by WHO 5th edition criteria us-
ing Diff-Quik staining kit (RAL Diagnostics, Martillac, France) according to the standard protocol [12].

For the LensHooke® X1 PRO analyzer, 40 μL of semen sample was loaded into disposable CS1 semen test cassettes (Bonraybio) following complete liquefaction. The test cassette was inserted into the LensHooke® X1 PRO and the built-in AIOM system automatically generated results in five minutes (Supplement Fig. 2). Using another aliquot of the same sample, semen analysis was also carried out using the Hamilton Thorne IVOS CASA. A Leja counting chamber was loaded with 6 μL of semen sample and analyzed as per established protocol [13].

3. Determination of intra- and inter-observer variation

Semen parameters including sperm concentration, as well as total and progressive motility were evaluated for intra- and inter-observer agreement. Intra-observer variation was determined on semen samples (n=10) in triplicates by MSA and LensHooke® X1 PRO. Furthermore, inter-observer variation was also determined on the 10 semen samples. Each sample was split into three aliquots and analyzed independently by three different operators by both MSA and LensHooke® X1 PRO.

4. Statistical analysis

Statistical analysis was performed using MedCalc Software (V. 19.4.1; MedCalc Software, Ostend, Belgium). After testing for normal distribution by means of the Kolmogorov–Smirnov test, semen parameters evaluated using MSA and automated semen analyzers were compared using the Wilcoxon test. Spearman’s rank correlation (rho) was employed to evaluate correlations for the various parameters between MSA and automated semen analyzers (X1 PRO and IVOS CASA). Concordance correlation was used to determine the accuracy and precision.

The different methods (MSA, X1 PRO, and IVOS CASA) were compared by using Bland–Altman plots as well as the Passing–Bablok regression analysis. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the automated semen analyzers to identify samples with abnormal concentration (sperm concentration <15×10⁶/mL) and abnormal motility (total motility <40%) were calculated using the results of the MSA as reference. A p-value of <0.05 was considered as statistically significant.

| Table 1. Summary results of sperm parameters (concentration, total motility, progressive motility, and morphology) evaluated by MSA, X1 PRO, and IVOS CASA |
|---------------------------------------------------------------|
| Semen parameter (n=101)                                      | MSA                          | X1 PRO                         | IVOS CASA                        |
| Mean±SD Median Range                                         | Mean±SD Median Range         | Mean±SD Median Range           |
| Concentration (×10⁶/mL)                                       | 59.14±38.62 54.0 0–180       | 58.70±38.82 49.3 0–184         | 61.6±38.54 56.5 0–171            |
| Total motility (%)                                            | 48.14±23.00 49.0 0–88        | 48.96±26.91 53.0 0–98          | 42.32±23.36 41.0 0–89            |
| Progressive motility (%)                                     | 42.49±22.61 43.0 0–91        | 48.62±23.35 40.0 0–91          | 42.17±23.39 41.0 0–89            |
| Normal sperm morphology (%)                                   | 8.25±2.67 8.0 0–14           | 8.25±2.67 8.0 0–14             | 8.25±2.67 7.0 0–17               |
| P-value                                                      | MSA vs X1 PRO                 | MSA vs IVOS CASA               | X1 PRO vs IVOS CASA              |
| Concentration (×10⁶/mL)                                       | 0.3133 <0.0001                | <0.0001                        | <0.0001                          |
| Total motility (%)                                            | 0.4222 <0.0001                | 0.3861 <0.0007                 | 0.0701 <0.0001                   |
| Progressive motility (%)                                     | 0.0016 <0.0001                | 0.0007 <0.0001                 | <0.0001                          |
| Normal sperm morphology (%)                                   | 0.0016 <0.0001                | 0.0007 <0.0001                 | <0.0001                          |

A p-value was calculated by Wilcoxon test. MSA: manual semen analysis, SD: standard deviation.
5. Ethics statement
This cross-sectional study was approved by the Institutional Review Board (IRB) of Cleveland Clinic (IRB # 18-771). Written informed consent was acquired from all study participants.

RESULTS

1. Summary statistics of semen parameters
The mean age of the study participants was 30.6±7.4 years, while the mean semen volume was 2.7±1.2 mL. In the healthy donors, the mean age and volume of semen sample was 30.1±9.1 years and 2.8±1.1 mL, respectively. Whereas, the mean age and semen volume of infertile men were 31.4±6.3 years and 2.1±1.6 mL, respectively. There was no significant difference in the mean age (p=0.8452) and semen volume (p=0.2349) between the healthy donors and infertile men (Supplement Table 2).

Semen analysis results obtained by MSA, X1 PRO, and IVOS CASA are depicted in Table 1. While there was no difference in the average sperm concentration and total motility between the X1 PRO and MSA, the average sperm progressive motility obtained by the X1 PRO was lower than that by MSA (p=0.0016). Sperm concentration measured by the IVOS CASA (61.6±38.5×10⁶/mL) was significantly (p<0.0001) higher than that using MSA (59.1±38.6×10⁶/mL). In contrast, no difference was observed between MSA and IVOS CASA for the measurement of progressive motility (p=0.3861). Whereas, results of sperm morphology evaluated by X1 PRO were significantly (p<0.0001) lower than manual results (Table 1).

When sperm concentration, total, and progressive motility evaluated using MSA, X1 PRO, and IVOS CASA were compared, the Passing–Bablok regression analysis did not show any significant (p>0.05) deviation.
Table 2 provides Spearman’s rank correlation for sperm concentration, total, and progressive motility, obtained by automated analyzers (X1 PRO and IVOS CASA) and MSA. Concordance correlation coefficient was >0.90 (p<0.0001) with bias correction factors (Cc) for accuracy >0.99 for X1 PRO compared with MSA (Table 3).

2. Specificity and sensitivity

Comparison of the results obtained by X1 PRO and IVOS CASA with those of MSA for sperm concentration, total, and progressive motility are shown in Table 4. When MSA was used as a reference, the X1 PRO device showed a sensitivity and specificity of 100% for the identification of samples with abnormal concentration, with PPV and NPV of 100%. The X1 PRO device had also a very high sensitivity (94.1%) and specificity (92.5%) for the identification of samples with abnormal motility (at cut-off value of 40%) (Table 4). Except for progressive motility, sensitivity, specificity, PPV, and NPV values were generally higher for the X1 PRO than those for IVOS CASA. In addition, the X1 PRO showed a comparable diagnostic ability with the IVOS CASA for sperm progressive motility at a cut-off value of 32%. The X1 PRO demonstrated a high PPV (97.7%) and a low NPV (9.1%) for sperm morphology when compared to manual results.

3. Intra-and inter-operator agreement

The average intra-operator coefficients of variation for sperm concentration, total, and progressive motility were <11% in both X1 PRO and MSA (Table 5). Intra-class correlation coefficients were >0.92 for sperm concentration, total, and progressive motility as obtained from linearity (Fig. 1-3).
Table 2. Spearman's coefficient of rank correlation for sperm concentration, TM, and PR evaluated using MSA, X1 PRO, and HT IVOS CASA

| Comparison            | n  | Concentration (ρ) | TM (ρ) | PR (ρ) |
|-----------------------|----|-------------------|--------|--------|
| X1 PRO vs. MSA        | 101| 0.96 (p<0.0001)   | 0.94 (p<0.0001) | 0.92 (p<0.0001) |
| HT IVOS CASA vs. MSA  | 100| 0.98 (p<0.0001)   | 0.92 (p<0.0001) | 0.92 (p<0.0001) |
| X1 PRO vs. HT IVOS CASA | 100| 0.97 (p<0.0001)   | 0.87 (p<0.0001) | 0.87 (p<0.0001) |

TM: total motility, PR: progressive motility, MSA: manual semen analysis.

Table 3. Concordance correlation coefficient, Pearson (precision) and accuracy (bias correction factor) of the X1 PRO and HT IVOS CASA devices in evaluating sperm concentration, TM, and PR compared to MSA

| Device                  | Concentration correlation coefficient | Pearson (p) | Bias correction factor (Cj) |
|-------------------------|--------------------------------------|--------------|----------------------------|
|                         | Concentration | TM | PR | Concentration | TM | PR | Concentration | TM | PR |
| X1 PRO (n=101)          | 0.96          | 0.93 | 0.91 | 0.96          | 0.94 | 0.92 | 0.99          | 0.99 | 0.99 |
| HT IVOS CASA (n=100)    | 0.97          | 0.89 | 0.92 | 0.97          | 0.92 | 0.93 | 0.99          | 0.97 | 0.99 |

PR: progressive motility, TM: total motility, MSA: manual semen analysis.
by both the X1 PRO and MSA. Similarly, inter-operator agreement (kappa) was >0.91 for sperm concentration and total motility by using the X1 PRO and MSA, respectively. Furthermore, the inter-operator agreement for progressive motility was higher for X1 PRO ($\kappa = 0.93$) than that in MSA ($\kappa = 0.88$) (Table 6).

**DISCUSSION**

Semen analysis is considered as the cornerstone of evaluation for male infertility, even though the results of MSA are controversial due to its subjective nature and high intra-individual variability. In andrology and in vitro fertilization (IVF) clinics, CASA is being used [8] to overcome the reported problems and the repeated criticisms of the manual analysis [14]. One of the primary benefits of CASA is improved accuracy and precision of semen analysis and efficiency of processing semen samples [15,16]. There are several types of CASA devices using different technologies, based on phase-contrast microscopy, electro-optics, or integrated visual optical system. In many diagnostic laboratories, the Hamilton Thorne IVOS CASA, a large unit with an incorporated microscope and camera [8,17], is a popular device and has been clinically validated in several studies [13,18,19]. It runs an image processing system to capture the graphics of the sample and conduct the analysis, and has been proven to be effective in clinical use to reduce operator subjectivity and uncertainty in results [20]. However, the IVOS CASA requires a considerable amount of laboratory bench space and a trained operator to perform the quality control and testing of semen samples. In addition, the maintenance of this CASA system requires experienced and trained professionals. In the current study, we have validated the performance of the novel, automated, AIOM-technology-based semen analyzer X1 PRO compared with the IVOS CASA, and standard MSA.

In general, sperm concentration was shown to highly correlate between both approaches (IVOS CASA and MSA) and the X1 PRO. In the current study, there was no significant difference in the average sperm concentration measured using X1 PRO and MSA. Similarly, the sperm concentration results for IVOS CASA and X1 PRO were comparable. Previous studies have reported that the ability of the computer-based analyzers to evaluate samples that had very low or high sperm concentrations is limited [21-24]. Generally, these analyzers tend to slightly overestimate sperm concentration [25]. However, our results clearly suggest that the X1 PRO can be effectively used as a substitute for MSA. In fact, we have noticed that the X1 PRO device has a sensitivity and specificity of 100% for the identi-
fication of samples with abnormal sperm concentration compared to MSA. This is likely due to the fact that the X1 PRO device has a lower detection limit of $0.1 \times 10^6$ sperm/mL and upper detection limit of $300 \times 10^6$ sperm/mL for sperm concentration. Such a wide detectable range for sperm concentration is not available in any other type of current CASA devices.

In the present study, there was no significant difference in the average total motility measured using the X1 PRO and MSA. Similarly, the total motility results for the IVOS CASA and the X1 PRO were comparable. Strong correlations were previously observed for sperm motility using different CASA systems [15,21,26,27]. This can be credited to the ease of marking and tracking sperm to count and follow its movement using computer algorithms. However, CASA systems can have difficulties in distinguishing between immotile sperm, non-sperm cells, and debris [9]. This lack of distinction causes inaccurate evaluation of sperm motility as well as counting of spermatozoa, which also affects the evaluation of sperm concentration. One of the limitations includes the discrepancies in evaluating the progressive motility of the sperm by using X1 PRO and MSA. Progressive motility evaluated by the X1 PRO was significantly lower compared to the standard MSA. However, our results demonstrate that the X1 PRO device has a very high sensitivity and specificity to detect samples with abnormal motility (at a cut-off value of 40%). The PPV for samples with abnormal motility measured by the X1 PRO was higher than that of the IVOS CASA. The X1 PRO also demonstrated a higher level of specificity and a similar level of precision in identifying samples with abnormal motility than that by the IVOS CASA. Furthermore, the diagnostic ability of X1 PRO was comparable with the IVOS CASA to evaluate progressive motility with a cut-off value of 32%.

Intra- and inter-observer agreement are important parameters to be analyzed when validating a device in a laboratory setup. These values indicate the ease of use of equipment for the reproducibility and repeatability of the results. In the current study, our results suggest that the repeatability and intra-operator agreement of the X1 PRO device was comparable with MSA for sperm concentration, total motility, and progressive motility. Similarly, a high inter-observer agreement indicates that sperm progressive motility measured by the X1 PRO was comparable to MSA, which will reduce inter-laboratory variability. In addition, the X1 PRO requires less bench space and takes less than 5 minutes to generate complete semen analysis results [10]. Moreover, the touch-screen and easy-to-use operational interface make it more user-friendly, when compared to other CASA devices.

Another important feature of X1 PRO is the use of AI technology to recognize the spermatozoa, analyze their every movement, and define if they have normal or abnormal morphology. The algorithm uses WHO 5th edition criteria and machine learning technology to train the system for its high accuracy and reproducibility. Furthermore, the portability of the X1 PRO makes it possible to be used in an IVF clinic or even in a medical office, especially in cases of patients who need fast semen analysis results, such as in cases of fertility preservation, post-vasectomy reversals or before varicocele surgery. It can also be carried with clinicians to multiple outreach clinics and operating rooms, if necessary. One limitation of the X1 PRO is
that the sperm morphology values did not correlate with manual results. Even though the device showed a high PPV in detecting normal sperm forms, the low NPV seen in our study is due to the very low number of samples with abnormal sperm forms (<4%) included in the analysis. Due to its high PPV, the andrology and IVF laboratories can use the morphology results generated by X1 PRO in all cases when normal sperm forms are ≥4%. A manual evaluation should be done in patients with abnormal morphology (<4%) (Fig. 4).

CONCLUSIONS

Both automated semen analyzers (X1 PRO and IVOS CASA) showed high levels of predictive power for oligo- and asthenozoospermic samples, and their performances were comparable with laboratory-based MSA. Furthermore, high levels of inter-and intra-rater agreement indicate that the X1 PRO provides reliable semen analysis results. The X1 PRO’s combination of speed, ease of use, accuracy, portability, and ability to detect a wide range of sperm concentration makes it a good choice of device for use in many settings, from small medical offices to large IVF centers. Lastly, the high PPV of Lenshooke® X1 PRO to correctly identify normal sperm forms makes it a very attractive device for diagnostic use.

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Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: AA. Data curation: MKPS, RFA. Formal analysis MKPS, RFA. Funding acquisition: AA. Investigation: AA. Methodology: MKPS, RFA. Software: MKPS. Validation: MKPS, RFA. Writing – original draft: MKPS, RFA, AA. Writing – review & editing: MKPS, RFA, AA.

Supplementary Materials

Supplementary materials can be found via https://doi.org/10.5534/wjmh.200185.

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