The Effectiveness of Lenshooke™ Semen Quality Analyzer X1 Pro for Human Semen Analysis

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ABSTRACT

Background: Lenshooke™ Semen Quality Analyzer (SQA) X1 Pro is an automated semen analysis. The accuracy of Lenshooke™ SQA X1 Pro has never been analyzed with World Health Organization (WHO) standard method.

Aim: This study aims to examine whether the Lenshooke™ SQA X1 Pro method provides reliable results according to the WHO standard method.

Methods: This study was a laboratory analytic observational study using 60 patients in Andrology clinic of Dr. Soetomo Hospital. The concentration, progressive motility (PR), total motile sperm count (TMSC), and morphology results of the Lenshooke™ SQA X1 Pro and standard method were analyzed statistically using correlation, Bland Altman, and diagnostic test.

Results: Significant correlation between two methods were found in all parameters (concentration: r = 0,970; PR: r = 0,781; TMSC: r = 0,952; morphology: r = 0,568). The mean difference for concentration, PR, TMSC, and morphology between the two examination methods were 1,165 million/ml, 7,05%, 7,584 million/ejaculate, and 2,25%. However, it found that the correlation and agreement were weaker in sample with low number of spermatozoa per high power field. The results revealed a sensitivity of 100%, 81%, and 59% for oligozoospermia, astenozoospermia, and teratozoospermia, respectively. The specificities were shown to be 100%, 74%, and 100% for oligozoospermia, astenozoospermia, and teratozoospermia, respectively.

Conclusion: The Lenshooke™ SQA X1 Pro gives a reliable result for determining oligozoospermia and asthenozoospermia, but in the situation that the clinicians need the accurate data, standard method should be used.

Keywords: Concentration, Lenshooke™ Semen Quality Analyzer X1 Pro, Morphology, Progressive Motility, WHO Standard Method

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INTRODUCTION

Semen analysis is an important procedure for assessment of male reproductive function. It is mandatory to perform semen analysis for male fertility evaluation at least twice.[1,2,3] Various factors can cause semen to have a large variation on each examination even though the sample comes from the same individual.[4] However, semen analysis prone to have a high level of uncertainty which could be contributed by the subjectivity of the examiner.[5]

An automated semen analysis methods known as computer assisted sperm analysis (CASA) has been used for routine semen analysis in some center.[6,7] CASA systems have evolved to become powerful tools for the rapid and objective assessment of sperm concentration, motility and kinematics, as well as morphology.[8] Various attempts to make simpler equipment have been carried out, including using Lenshooke[TM] Semen Quality Analyzer (SQA) X1 Pro.[9] However, the accuracy of Lenshooke[TM] SQA X1 Pro has never been analyzed to standard methods from World Health Organization (WHO).

This study aims to examine whether the Lenshooke[TM] SQA X1 Pro method provides reliable results according to the WHO standard methods.

MATERIAL & METHOD

1. Study design

This study was a laboratory analytic observational study using 60 patients who attended to Andrology clinic of RSUD Dr. Soetomo Surabaya.

2. Patient's recruitment

Patients who came to Andrology Clinic of RSUD Dr. Soetomo Surabaya for fertility treatment during research period were explained and offered to be volunteer for the research until the number of sample were fullfiled. Patients were required to sign an informed consent letter after counseling and explaining the research.

3. Semen collection

Ejaculate samples were obtained from men who had fulfilled the inclusion and exclusion criteria. The inclusion criteria were sexual abstinence between 48 hours – 7 days and the semen volume should be more than 1 mL. We excluded hematospermia samples and no spermatozoa obrserved in wet preparation. Ejaculate samples were obtained by masturbation. Patients were not permitted to use condoms. Samples that had been collected in a container C-KUP[TM] CK that had been provided, then were placed in a heater or incubator at 37ºC until the liquefaction. The liquefaction were checked as described in WHO manual and confirmed by homogenized by flipping C-KUP[TM] CK 8-10 times and looking at the drops of semen falling from the V stick contained in the C-KUP[TM] CK. The volume were measured based on the volume scale found on the C-KUP[TM] CK.

4. Standar method
4.1 Initial microscopic investigation

After complete liquefaction, the wet preparations were made by mixing the semen sample well, removed 10 μl aliquot of semen immediately after mixing into a clean glass slide. The aliquot was covered with a coverslip 22 mm × 22 mm to provide a chamber approximately 20 μm deep Remixed the semen sample before removing replicate aliquots. Aggregation and agglutination were examined, only sample with no agglutination were enrolled in this study.

The wet preparations were examined to estimate the number of spermatozoa per high power field (HPF) and categorized the sample into three group (<16 spermatozoa/HPF, 16-100 spermatozoa/HPF, and >100 spermatozoa/HPF).

4.2 Motility examination

The motility examination performed in two wet preparation with phase-contrast optics at ×400 magnification. The examination were started when the sample were stop drifting. The observation area was at least 5 mm from the edge of the coverslip to prevent drying artefacts affecting motility. We evaluated at least 200 spermatozoa in a total of at least 5 fields in each replicate, categorized the motility into three grade, progressive (PR), non-progressive (NP), and immotile (IM). The average percentage and

difference between the two percentages for the most frequent motility in the replicate were counted in order to determine the acceptability of the difference based on WHO's manual for semen analysis 5th edition. If the difference between the percentages was acceptable, we reported the average percentage for each motility grade (PR, NP and IM). If the difference was too high, two new aliquots from the semen sample should be taken in order to make new preparations and assessment.

4.3 Concentration examination
4.3.1 Semen dilution

Based on the number of spermatozoa/HPF, dilution was made according to WHO's manual for semen analysis 5th edition. The semen sample mixed well. Appropriate volume of semen was aspirated immediately after mixing using a positive-displacement pipette. The semen off the outside of the pipette tip were removed by wiping with no touch of the opening of the tip. Dispense the semen into the fixative and rinse the pipette tip by aspirating and expressing the fixative. Mix the semen sample well again, and prepare the replicate dilution.

The dilution mixed well, removed immediately approximately 10 μl of fixed suspension. The pipette tip was touched carefully against the lower edge of one of the chambers. The plunger of the pipette was depressed slowly, allowing the chamber to fill by capillary action. Second dilution was treated by the same procedure and loaded into the second chamber of the haemocytometer. The haemocytometer were kept horizontally for at least 4 minutes at room temperature in a humid chamber.

4.3.2 Assessing sperm numbers in the counting chambers

Sperm number should be assessed in both chambers of the haemocytometer. The haemocytometer were examined using phase-contrast optics at ×400 magnification. At least 200 spermatozoa were counted in first chamber, the assessment should continue until all spermatozoa in the row of the 200th spermatozoa present were complete to be counted. The same number of rows would be used for counting number of spermatozoa in

other chamber of the haemocytometer. If the two values agree sufficiently, the aliquots taken can be considered representative of the sample. Determination of the acceptability of the difference were made according to WHO's manual for semen analysis 5th edition. The concentration calculation would be performed If the difference was acceptable.

4.3.3 Calculation of the concentration of spermatozoa in semen

The concentration was counted with formula below.

$$C = \frac{N}{n} \times \frac{1}{20} \times D$$

C = Concentration (10^6/ml)
N = Number of spermatozoa
N = Number of rows examined
D = Dilution factor.

4.4 Morphology assessment

Two or more smears were made from the fresh semen sample by mixing the semen sample well, removed 10 μl aliquot of semen immediately after mixing into a clean glass slide. "Feathering" method" were performed for making the semen smear. The semen smears were fixed with alcohol and stained with Safranin and crystal violet method. Tygerberg Strict Criteria were used for the evaluation of spermatozoa morphology. Morphological evaluation were performed on every assessable spermatozoon using brightfield optics at ×1000 magnification with oil immersion. At least 200 spermatozoa in each replicate, in order to achieve an acceptably low sampling error. The average percentage and difference between the two percentages for the normal morphology in the replicate were counted in order to determine the acceptability of the difference based on table 1. If the difference between the percentages was acceptable, we reported the average percentage for normal morphology. If the difference was too high, reassessment would be performed.

5. Lenshooke™ SQA X1 Pro method

During liquefaction, The LensHooke™ X1 SQA was turned on and several data

should be inputted manualy. After liquefaction, two drops of semen were dropped into LensHooke[TM] Semen Test Cassette. LensHooke[TM] Semen Test Cassette inserted appropriately into The LensHooke[TM] SQA X1Pro. The LensHooke[TM] X1 SQA would analyze the semen and show the results on the internal and external monitor.

6. Total motile sperm count calculation

The total motile sperm count (TMSC) was obtained by multiplying the volume of the ejaculate in by the sperm concentration and the percentage of total motility.[10]

7. Statistical analysis

The concentration, progressive motility (PR), total motile sperm count (TMSC), and morphology of the Lenshooke[TM] and WHO standard methods were analyzed statistically using correlation and Bland Altman.

Diagnostic test to measure the sensitivity and specificity of between Lenshooke[TM] SQA X1 Pro was also analyzed using the WHO standar method as the gold standard.

8. Ethical clearance

This study has been approved by ethical committee of Dr. Soetomo Hospital Surabaya with registered number 0560/KEPK/VIII/2018.

RESULTS

Data from microscopic evaluation and TMSC results using the Lenshooke[TM] SQA X1 Pro and the WHO standard methods are shown in table 1.

Table 1. Microscopic data of semen analysis using Lenshooke[TM] SQA X1 Pro and standard method

Parameter	Number of spermatozoa per HPF	Lenshooke[TM] Semen Quality Analyzer X1 Pro	Standard
Concentration (Mill/ml)	<=15	1,78 ± 1,87	2,36 ± 2,14
	16 - 100	11,33 ± 9,77	12,83 ± 8,76
	>100	72,64 ± 32,41	74,04 ± 37,59
	Total	28,58±37,05	29,74±38,70
PR (%)	<=15	7,85 ± 15,52	23,00 ± 15,59
	16 - 100	31,65 ± 17,66	34,50 ± 13,07
	>100	56,10 ± 19,71	59,25 ± 20,12
	Total	31,87 ± 26,42	38,92 ± 22,28
NP (%)	<=15	6,40 ± 17,56	11,50 ± 7,09
	16 - 100	7,30 ± 8,45	13,45± 7,86
	>100	13,10 ± 4,49	10,15 ± 4,67
	Total	8,93 ± 11,74	11,70 ± 6,70
IM (%)	<=15	85,80 ± 23,89	65,50 ± 14,93
	16 - 100	61,05 ± 18,77	52,05 ± 15,18
	>100	30,80 ± 19,70	30,60 ± 20,96
	Total	59,22 ± 30,60	49,38 ± 22,31
Normal morphology (%)	<=15	1,81 ± 2,38	0,15± 0,49
	16 - 100	3,76 ± 3,76	0,85 ± 1,18
	>100	4,7 ± 1,56	2,45 ± 1,05
	Total	3,4 ± 2,96	1,15 ± 1,35

The correlation analysis of microscopic data and TMSC results were performed. The results of the correlation analysis are shown in table 2 and figure 1. The correlation of progressive motility was not performed in the spermatozoa group <16 because some results in the Lenshooke™ SQA X1 Pro group were <1%. While the morphological data could not be divided into groups because the distribution did not spread.

Correlation analysis data from microscopic and TMSC results using the Lenshooke™ SQA X1 Pro and the standard method are shown in table 2 and figure 1.

Table 2. The correlation analysis of microscopic examination and TMSC values between Lenshooke™ SQA X1 Pro and standard method

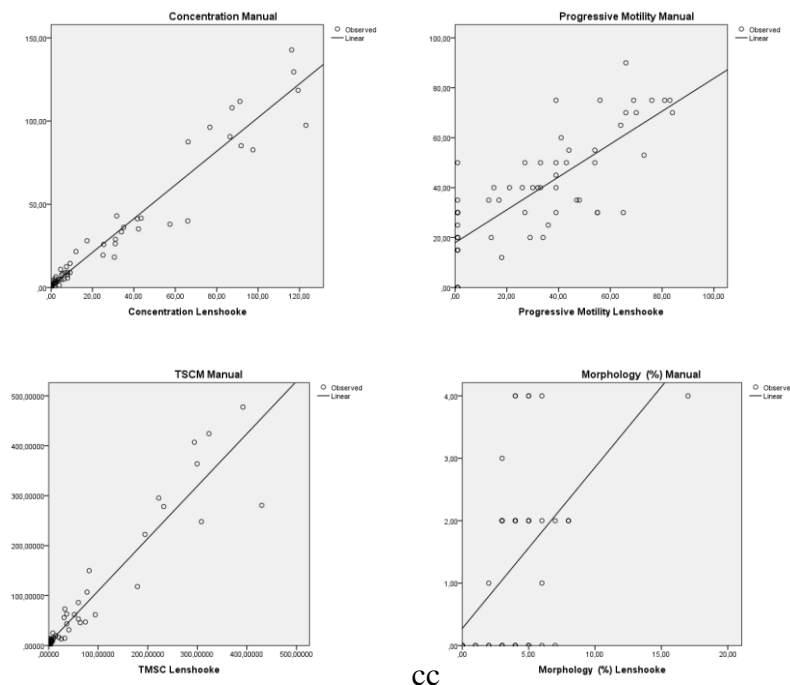| Parameter	Number of sperm per HPF							
	<16		16 – 100		>100		Total	
	r	p	r	p	r	p	r	p
Concentration	0,787	<0.0001	0,856	<0,0001	0,909	<0,0001	0,970	<0,0001
PR	-	-	0,410	0,073	0,745	<0,0001	0,781	<0,0001
TMSC	-	-	0,928	<0,0001	0,908	<0,0001	0,952	<0,0001
Morphology	-	-	-	-	-	-	0,568	<0,0001

Figure 1. Correlation analysis from microscopic examination results and TMSC values between Lenshooke™ SQA X1 Pro and standard method
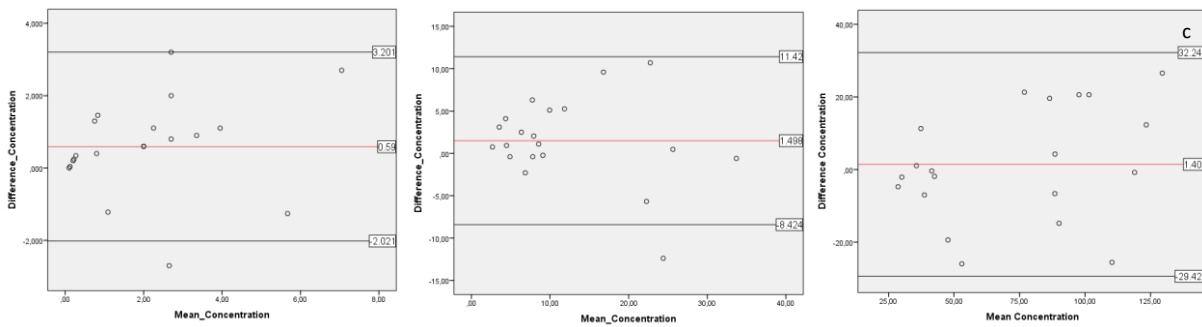
Figure 2. Bland Altman plot for concentration using Lenshooke™ SQA X1 Pro and standard methods in different groups. (a) <16 sperm/HPF, (b) 16-100 sperm/HPF, and (c) >100 sperm/HPF

Then Bland Altman analysis of the results of microscopic examination and TMSC between Lenshooke™ SQA X1 Pro and the standard method were carried out. The results of the agreement analysis are shown in Figures 2, 3, and 4.

The mean concentration difference in the three groups was 0.59 million/ml, 1.498 million /ml, and 1.407 million/ml. There was one sample with values outside the limit of agreement in groups a and b. There is no sample that is outside the limit of agreement in the group c.

The mean progressive motility difference in the two groups of spermatozoa were 2.85% and 3.15%. There was one sample with a value outside the limit of agreement in the group a. No sample was located outside of the limit of agreement in group b.
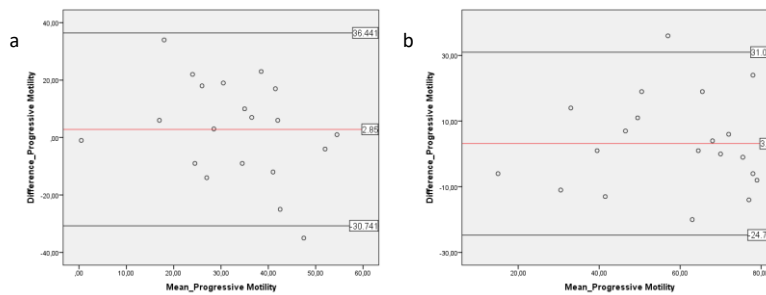

Figure 3. Bland Altman plot between the progressive motility of Lenshooke™ SQA X1 Pro and the standard method in groups of 16-100 sperm/HPF (a) and > 100 sperm / HPF (b)
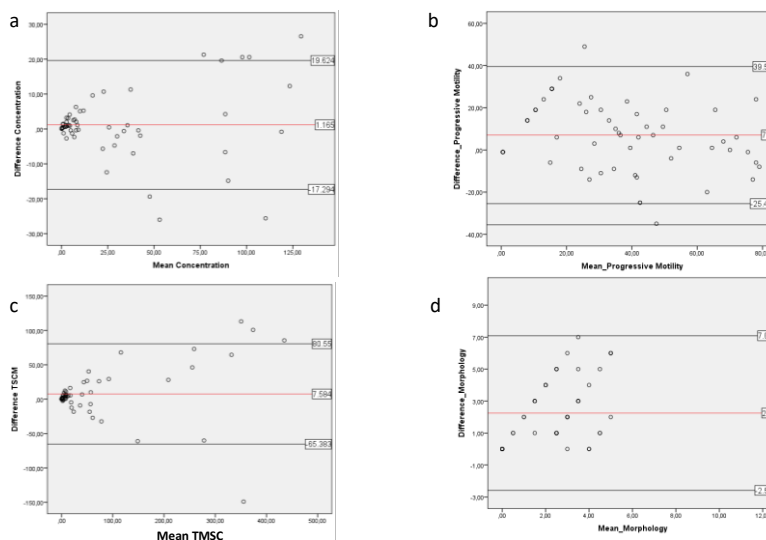

Figure 4. Bland Altman plot between concentration (a), progressive motility (b), TMSC (c), and morphology (d) of Lenshooke™ SQA X1 Pro and standard methods on all samples

The mean difference for concentration, progressive motility, TMSC, and morphology between the two examination methods were 1.165 million/ml, 7.05%, 7.584 million/ejaculate, and 2.25%.

To assess the clinical significance, the sensitivity and specificity of the Lenshooke™ SQA X1 Pro in order to diagnose abnormalities in concentration, motility, and morphology of semen was performed using standard methods as gold standard. The results are shown in Table 3.

Table 3. Diagnostic accuracy of microscopic evaluation and TMSC results using the Lenshooke™ SQA X1 Pro

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR-
Oligozoospermia	100 [89,8 - 100]	100 [87 - 100]	100 [90 - 100]	100 [87 - 100]	infinity	0
Asthenozoospermia	81 [62 - 92]	74 [57 - 85]	70 [52 - 83]	83,3 [66 - 93]	3.05 [1.69 - 5.51]	0.261 [0.12 - 0.59]
Teratozoospermia	59 [46 - 71]	100 [61 - 100]	100 [89 - 100]	21,4 [10 - 40]	infinity	0.41 [0.30 - 0.56]

DISCUSSION

The Lenshooke™ SQA X1 Pro generally gave a lower concentration value compared to the gold standard with a good correlation and agreement. Correlation test results and Bland Altman showed that correlation and agreement results were weaker in the groups with a lower number of spermatozoa per high power field. The Lenshooke™ SQA X1 Pro only evaluate the sperm in one drop and one field of view. It doesn't able to get sufficient number of sperm for reliable analysis in low number of sperm. Standard method should count until at least 200 spermatozoa in one side of improved neubauer haemocytometer and recount number of spermatozoa in another side chamber of the same neubauer haemocytometer. During counting, more than one microscope visual field should be used in order to obtain 200 spermatozoa and observe another side of the hamocytometer. It means

standard method still able to analyze sufficient number of sperm in lower number of sperm.[3] This device is not able to check all volumes of semen pellets. Thus, the absence of spermatozoa at Lenshooke™ SQA X1 Pro could not be interpreted as azoospermia. Clinically Lenshooke™ SQA X1 Pro has good sensitivity and specificity on concentration evaluation. Thus, LenshookeTM SQA X1 Pro can be used as a diagnostic tool and screening for concentration abnormality (oligozoospermia).

The Lenshooke™ SQA X1 Pro generally provide lower progressive motility value compared to the gold standard. These results had a good correlation and agreement (table 2 and figure 2). Correlation test results and Bland Altman were weaker in the group with a lower number of spermatozoa. This could happen because Lenshooke™ SQA X1 Pro had fewer spermatozoa to analyze in groups with fewer spermatozoa. In the group with number of sperm <16 per High power field, it often showed a progressive motility as "<1%". Thus, the results of motility evaluation in this group could not be used for interpretation. In another side, WHO manual method should evaluate at least 200 spermatozoa in a total 5 field in two replicate in order to achieve an acceptably low sampling error.[3] In the diagnostic accuracy test, the results of motility examinations in all groups of spermatozoa still gave good results of sensitivity and specificity because they only separated progressive motility into two groups. Thus, LenshookeTM SQA X1 Pro can be used as a good screening tool for motility abnormalities (asthenozoospermia).

The Lenshooke™ SQA X1 Pro provided a higher average normal morphology value compared to the gold standard (see table 1). Moderate correlation values (table 2) and low sensitivity (table 3) have been found in this study. This result occurred because Lenshooke™ SQA X1 Pro uses a different examination criterion, directly without staining. Although the direct morphological examination was carried out on the selection of spermatozoa for ICSI, until now morphological criteria for spermatozoa have not been applied in semen analysis.[11]

Our study is the first study which compared the Lenshooke™ SQA X1 Pro with standard WHO method. Another study used Makler chamber for concentration and motility assessment in manual method.[12] Previous study shows that the Neubauer haematocytometer is more precise diagnostic tool for counting the spermatozoa concentration in comparison to the Makler counting chamber. The assessment of sperm count by the Makler chamber is inaccurate in oligozoospermia semen samples.[13] Makler sperm counts shown generally higher than the corresponding counts obtained with the haemocytometer.[14]

CONCLUSION

The Lenshooke™ SQA X1 Pro gives a reliable result for determining oligozoospermia and asthenozoospermia, but in the situation that the clinicians need the accurate data, standard method should be used.

RECOMMENDATIONS

Lenshooke™ SQA X1 Pro can be used as a male fertility screening tool. As a diagnostic tool, it is recommended to check the number of spermatozoa per high power field at a glance using a microscope before conducting an examination with Lenshooke™ SQA X1 Pro. If the number of sperm <16 per HPF standard method should be used. It is not recommended to use Lenshooke™ SQA X1 Pro to confirm an Azoospermia.

DISCLOSURE

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