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Technical Report

Impact of the presence of debris on semen analysis using an automated system (SQA-V)

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Abstract

Objectives: This study examined the influence of the manual assessment of debris on the results of sperm concentration, progressive motility, and normal morphology measured using the SQA-V system (Medical Electronic Systems LLC, Los Angeles, CA, USA).

Methods: Sixty samples were analyzed simultaneously and independently by different operators using the manual technique and the SQA-V analyzer. Three measurements (sperm concentration, progressive motility, and normal morphology) of each sample were assessed using the 4 debris assessment levels: none/few, moderate, many, and gross.

Results: Given an optimal assessment of debris, the study data indicate that the SQA-V provided results that had very good agreement with those of the manual method: sperm concentration (rho=0.987, regression analysis formula: y=0.961x+1.962, p<0.0001), progressive motility (rho=0.949, regression analysis formula: y=0.989x+0.418, p<0.0001), and normal morphology (rho=0.694, regression analysis formula: y=0.678x+4.061, p<0.0001). Underestimation of debris increased sperm concentration and decreased motility and normal morphology, while overestimation of debris decreased sperm concentration and increased motility and normal morphology.

Conclusion: The results indicated that the performance of the SQA-V remains subject to the competency of the operator.

Keywords: Automated system, interference of debris, semen analysis, SQA-V

Semen analysis is one of the first diagnostic methods used to evaluate male infertility. While manual microscopic semen analysis by qualified staff remains the reference technique, significant intra- and inter-laboratory variability can occur [1]. Spermatozoa motility, morphology, and concentration can also be analyzed using automated methods. Semen analyzer systems have been used in veterinary sperm analysis for many years, and have become established as an alternative approach in human biology laboratories. However, some characteristics of human semen led to a lack of accuracy in the results. For example, animal ejaculate is generally "clean," containing few other cells or debris, while human ejaculate often contains numerous particles and other debris, which create significant background noise [2]. Two types of detection technology are used in the in vitro diagnostic market: computer-assisted sperm analysis (CASA) [2] and sperm quality analyzer (SQA) systems (Medical Electronic Systems LLC, Los Angeles, CA, USA) [3]. The SQA-V automated analyzer is capable of measuring sperm motility, kinematics, and sperm concentration. The SQA automatically compensates for debris according to a level selected by the operator; however, this assessment of debris is subjective. The objective of this study was to evaluate the impact of the debris evaluation on the results of 3 semen measurements: sperm concentration (106/mL), progressive motility (%), and normal morphology (%).

Materials and Methods

This study was performed in accordance with Article L.1211-2 of the French Public Health Code. The research was conducted

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at an andrology laboratory using 60 samples collected for semen analysis between October 2020 and February 2021 as part of an infertility workup. Each ejaculate sample was collected via masturbation at the laboratory after 2 to 8 days of sexual abstinence. After liquefaction within 60 minutes at 20 to 37°C, viscosity and pH were evaluated. The volume of the ejaculate was then measured using the gravimetric method.

Sperm analysis, i.e., sperm concentration, progressive motility, and normal morphology, was performed simultaneously and independently by different operators using the manual technique and an SQA-V automated analyzer. The SQA system is pre-calibrated to conform to the World Health Organization Fifth Edition guidelines [4] and regular quality control protocols were performed in accordance with the ISO 15189:2012 [5].

The SQA-V system is based on the analysis of the detection of electro-optical signals in a sample (about 500 µL) of ejaculate that is subsequently analyzed with a spectrophotometer to define the concentration of spermatozoa and motile spermatozoa, and ultimately determine the concentration of immobile spermatozoa. The sperm morphology is based on an estimate calculated using a proprietary algorithm.

In this study, the manual results were used as the reference. SQA-V results for each sample were obtained using each of the 4 debris assessment levels (Fig. 1):

- None/few: <10% (for every 10 sperm 1 or less piece of non-sperm debris).
- Moderate: 10-30% (for every 10 sperm there are 1-3 non-sperm debris).
- Many: 30-99% (for every 10 sperm there are 3-9 non-sperm debris).
- Gross: >100% (for every 10 sperm there are 10 or more non-sperm debris).

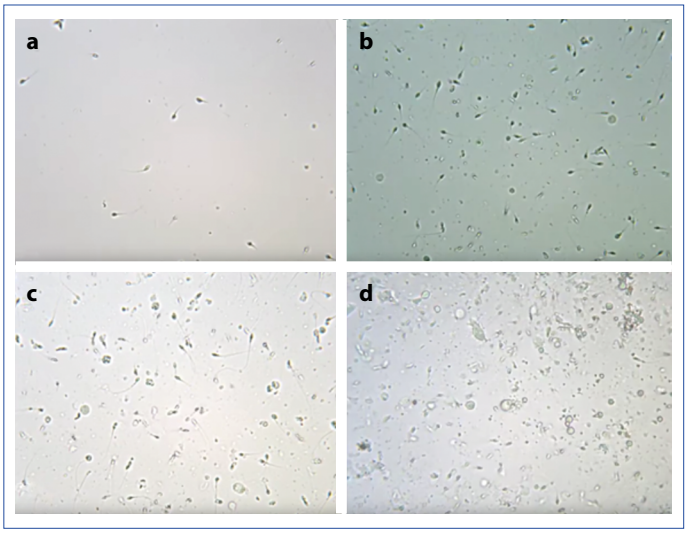

Figure 1. Classification of debris. (a) None/few: <10%; (b) Moderate: 10-30%; (c) Many: 30-99%; (d) Gross: >100%.

To evaluate the impact of debris, 5 grades were used:

- optimal assessment (results in agreement with the manual analysis)
- underestimation of 1 threshold
- overestimation of 1 threshold
- underestimation of 2 thresholds
- overestimation of 2 thresholds

A Shapiro–Wilk test demonstrated that the distribution of results was non-parametric. The Wilcoxon-rank test was used to compare dependent variables once it was determined that the data did not conform to normal distribution. A p value of <0.05 was considered a statistically significant difference. Descriptive data were presented as the median (1^{st}-3^{rd} quartile). Passing-Bablok regression analysis and Spearman correlation analysis were used to evaluate the compatibility of the 2 methods ($p<0.05$ was considered statistically significant). Boxplots of the percentage difference were created to illustrate the impact of the change according to the debris assessment. The relative deviation (%) of the median result was evaluated against the findings reported by Ricos et al. [6] and using the total allowable error limit formula developed by Frazer [7]. The total error limit defined was +/-43.5% for sperm concentration, +/-25% for progressive motility, and +/-32.8% for normal morphology.

Results

Of the 60 semen samples, 6 were classified as mild to moderate oligozoospermic ($5–15\times10^6$/mL) and the remainder were considered normal ($>15\times10^6$/mL).

The data indicated that the SQA-V results demonstrated very good agreement with those obtained using the manual method: sperm concentration (rho=0.987, regression analysis formula: y=0.961x+1.962, p<0.0001), progressive motility (rho=0.949, regression analysis formula: y=0.989x+0.418, p<0.0001), and normal morphology (rho=0.694, regression analysis formula: y=0.678x+4.061, p<0.0001). Underestimation of debris increased sperm concentration and decreased motility and normal morphology, while overestimation of debris decreased sperm concentration and increased motility and normal morphology (Table 1). Using the Ricos minimum total error as a limit of variation, the study results suggested an accuracy allowance of +/-1 threshold in the assessment of debris. Outside this allowance, particularly in the case of underestimation, the discrepancies with the manual method were too significant (Fig. 2).

Discussion

In the present study, the sperm concentration, motility, and normal morphology measured using manual analysis and SQA-V were significantly correlated. Several studies have demonstrated that the SQA-V automated analyzer could

Table 1. Statistical description of the impact of the manual assessment of debris on the SQA-V results for 3 semen measurements: sperm concentration, progressive motility, and normal morphology

Statistical information	Sperm concentration manual vs SQA-V (%)						Progressive motility manual vs SQA-V (%)						Normal morphology manual vs SQA-V (%)					
	Manual results for sperm concentration (10^6/mL)	Correct assessment	Underestimation of debris – 1 threshold	Underestimation of debris – 2 thresholds	Overestimation of debris – 1 threshold	Overestimation of debris – 2 thresholds	Manual results for progressivemotility (%)	Correct assessment	Underestimation of debris – 1 threshold	Underestimation of debris – 2 thresholds	Overestimation of debris – 1 threshold	Overestimation of debris – 2 thresholds	Manually results for normal morphology (%)	Correct assessment	Underestimation of debris – 1 threshold	Underestimation of debris – 2 thresholds	Overestimation of debris – 1 threshold	Overestimation of debris – 2 thresholds
Minimum value	6	-28	-33	53	-51	-70	15	-40	-53	-62	-33	-33	1	-50	-100	-62	-50	-50
Maximum value	182	22	85	127	21	152	65	27	10	50	53	48	20	200	200	200	300	200
1st quartile	28	-7	9	59	-27	-44	40	-5	-33	-48	6	4	6	-7	-29	-50	10	14
Median	41	-3	26	76	-20	-30	50	0	-27	-40	14	13	9	10	-11	-42	29	31
3rd quartile	76	5	43	98	-11	-15	55	5	-19	-10	22	22	12	33	0	-22	58	73
Wilcoxon-rank test p value		0.619	<0.0001	<0.0001	<0.0001	<0.0001		0.752	<0.0001	<0.0001	<0.0001	<0.0001		0.103	0.004	<0.0001	<0.0001	<0.0001

provide an accurate and highly correlated alternative to manual sperm analysis [2-4]. Nonetheless, any technology that analyzes live, motile cells has inherent limitations. The presence of debris in raw semen may impact test results, regardless of the analysis method, and should be taken into consideration [2, 3, 8, 9]. The SQA-V manufacturer has recommended the use of test strips (QwikCheck Test Strips; Medical Electronic Systems LLC, Los Angeles, CA, USA) prior to testing to detect high concentrations of leukocytes in the sperm (>1 million per mL). However, leukocytes are only 1 component of debris that may interfere with the sperm parameters when using an SQA-V analyzer. The visualization of the sample on the SQA-V screen was an important step. While the SQA-V software has an algorithm to adjust the measurement based on the debris assessment provided by the operator, the results of this study demonstrated that the operator's expertise was very important. An overestimation or an underestimation of debris could have a significant impact on the accuracy of the measured values. Our laboratory has conducted special training and uses operator debris assessment to address this issue and uses the SQA-V analyzer in conjunction with manual semen analysis to determine sperm concentration, motility, and morphology.

The SQA-V analyzer offers several benefits: standardization, speed, automated data recording, and less need for highly skilled professionals to perform semen analysis, but the need for a manual assessment of debris is a limitation.

Conclusion

The results of this study demonstrated that the manual assessment of debris had a significant impact on the results of 3 semen measurements: sperm concentration, progressive motility, and normal morphology. When there is an accurate determination of debris, the SQA-V demonstrated very good agreement with the manual method. Staff training for the appropriate use the SQA-V must be rigorous. It is not simply an easier substitute or alternative means to perform accurate semen analysis. The quality of the SQA-V results is subject to the competency of the operators.

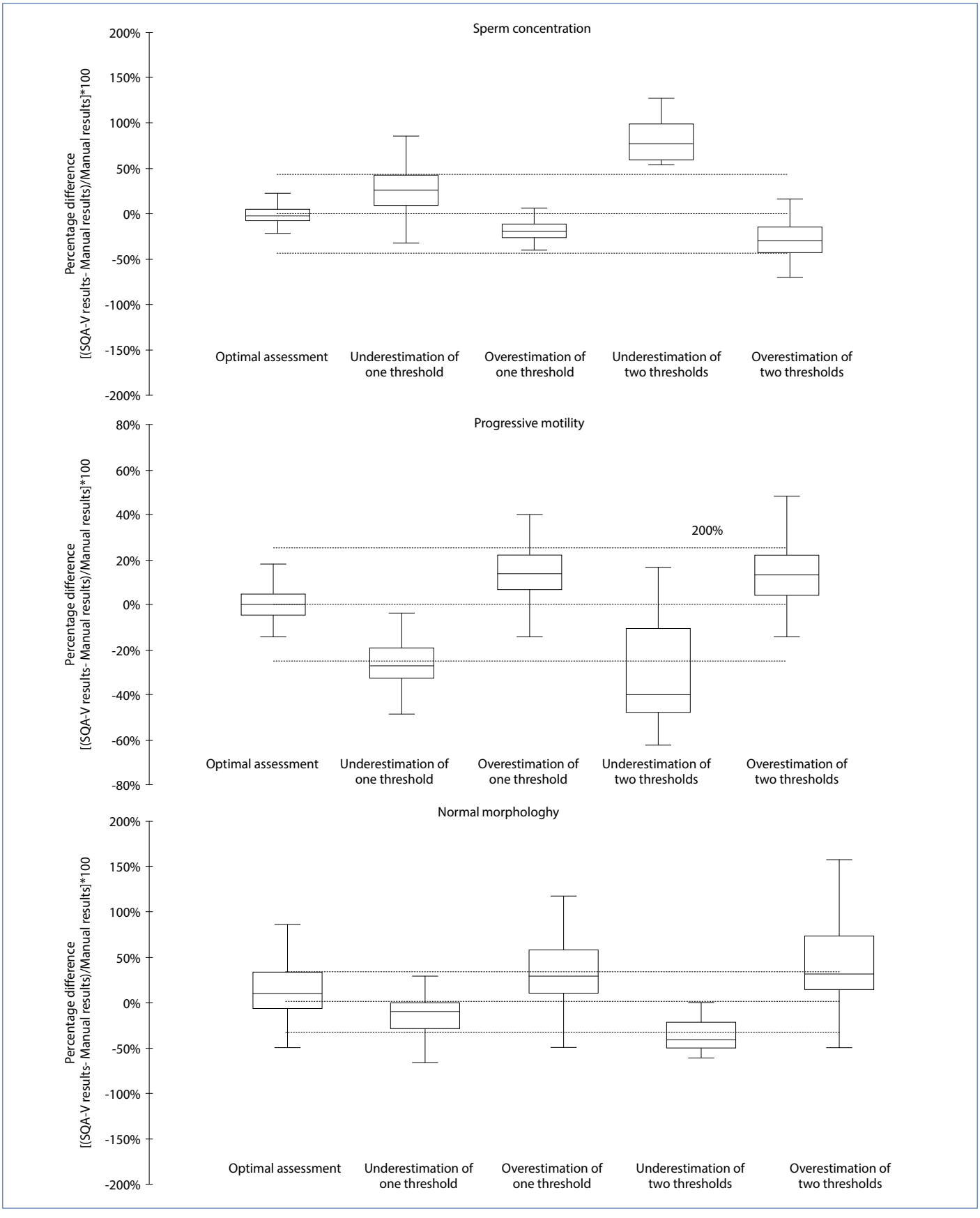

Figure 2. Boxplots of percentage difference between the results of the manual method and the SQA-V analyzer according to debris assessment.

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