Scandinavian Journal of Clinical and Laboratory Investigation

ISSN: (Print) (Online) Journal homepage: www.tandfonline.com/journals/iclb20

Retrospective study investigating the performance of the SQA-vision analyser compared with manual semen analysis

Claudio Ilardo, Naomi Defort, Anna Gala, Violaine Ostengo, Gilles Regnier Vigouroux, Guillaune Quere & Pierre Sanguinet

To cite this article: Claudio Ilardo, Naomi Defort, Anna Gala, Violaine Ostengo, Gilles Regnier Vigouroux, Guillaune Quere & Pierre Sanguinet (15 Aug 2024): Retrospective study investigating the performance of the SQA-vision analyser compared with manual semen analysis, Scandinavian Journal of Clinical and Laboratory Investigation, DOI: 10.1080/00365513.2024.2392245

To link to this article: https://doi.org/10.1080/00365513.2024.2392245

Published online: 15 Aug 2024.

SCANDINAVIAN JOURNAL OF CLINICAL AND LABORATORY INVESTIGATION
https://doi.org/10.1080/00365513.2024.2392245

Taylor & Francis
Taylor & Francis Group

TECHNICAL NOTE

Retrospective study investigating the performance of the SQA-vision analyser compared with manual semen analysis

Claudio Ilardo, Naomi Defort, Anna Gala, Violaine Ostengo, Gilles Regnier Vigouroux, Guillaune Quere and Pierre Sanguinet

INOVIE LABOSUD laboratory (Inovie member), Montpellier, France

ABSTRACT

The objective of this study was to compare the results of semen analysis using the manual method and the SQA-Vision sperm analyser after four years of practice and with a large cohort of patients. This was a comparative study of 1130 cases collected for semen analysis between October 2019 and October 2023, which were analysed simultaneously and independently by different operators using the manual microscopic method and an SQA-V automated analyser. For each sample, sperm concentration, progressive motility, motility, normal morphology, and round cells count were performed. There was no significant difference between the SQA-V method and manual assessment for all sperm parameters (Mann–Whitney test $p > 0.05$). According to the parameter studied, there was a strong correlation (rho = 0.81) and a very high correlation (rho = 0.98) between manual assessment and the SQA-V method. In the analysis of sperm concentration, the sensitivity and specificity were 0.90 and 0.99, respectively. The sensitivity and specificity for the analysis of sperm progressive motility were 0.98 and 0.99, respectively, while the sensitivity and specificity for the analysis of sperm motility were 0.87 and 0.99, respectively. The sensitivity and specificity for the analysis of normal morphology were 0.88 and 0.99, respectively. Regarding the analysis of round cells, the sensitivity and specificity were 0.98 and 0.99, respectively. The results of this retrospective study indicate that the SQA-V system offers satisfactory performance for routine sperm analysis.

ARTICLE HISTORY
Received 23 February 2024
Revised 9 July 2024
Accepted 11 August 2024

KEYWORDS
Automated system; semen analysis; SQA-v; method comparison; male infertility

Introduction

A standard laboratory test, semen analysis provides basic information on spermatogenesis, gonadal secretory activity, and male genital tract patency [1]. The results may indicate the absence of spermatozoa, a severe or slight deviation in sperm parameters, or normal values for semen volume, sperm count and concentration, sperm motility and morphology of the spermatozoa. According to Keel et al. [2], most laboratories perform sperm analysis using a manual method of microscopic analysis. The recommendations provided by the WHO guidelines were intended to standardise practices in the performance of sperm analysis, through adherence to well-defined technical processes. However, substantial inter-observer variability persisted, according to EQA results [3]. Moreover, semen analysis using the WHO criteria was very time consuming, particularly for assessing sperm motility and morphology. In the last four decades, the emergence of automated semen analysis systems on the market has demonstrated that they can be considered an alternative to the manual method used as a routine reference to improve standardisation in the laboratories. Initially, these systems were not effective in measuring sperm concentration as it was difficult to distinguish between spermatozoa from debris or round cells. Newer generations of systems have contributed to enhanced analysis by detecting the flagellum and enabling measurement of sperm mobility, as well as by improving the measurement of sperm concentration in the presence of debris or round cells. The Sperm Quality Analyser Vision (SQA-V) is a fully automatic system based on the detection of electro-optical signals generated by motile spermatozoa and then interpreted using algorithms. Signal processing to measure sperm motility is coupled with spectrophotometric technology to determine sperm concentration. Several studies have demonstrated that automated semen analysis systems offer a more accurate and reproducible alternative to the manual method, which is renowned for its high variability and insufficient standardisation [4–6]. The aim of our retrospective study was to evaluate the performance of SQA-V after four years of practice with a large cohort of patients.

Materials and methods

Inclusion of specimens

This monocentric study was conducted using data collected between October 2019 and October 2023. On each day, a sample was selected at random and analysed independently by different operators using two distinct methods: the

CONTACT Claudio Ilardo calogero.ilardo@inovie.fr INOVIE LABOSUD laboratory (Inovie member), Montpellier, France

manual microscopic method and an SQA-V automated analyser (MES Medical Electronic Systems, Encino, CA 91316, USA). As the laboratory systematically excludes samples with sperm concentrations below 1 M/mL delivered by the SQA-V, this exclusion criterion has been included in the study.

The retrospective data were utilised in accordance with the ethical standards of EU Regulation 2016/676 on the protection of natural persons and the processing of personal data. All data were subsequently anonymised.

Analysis

A total of 1130 samples were collected by masturbation after 2-8 days of sexual abstinence for semen analysis. Following liquefaction between 30 and 60 min at temperatures between 20 and 37 °C, viscosity and pH were evaluated, and the volume of the ejaculate was measured by weighing. For each sample, sperm concentration, progressive motility, motility, normal morphology, and round cells count were performed.

The SQA-V system is based on the analysis of electro-optical signals in a sample (approximately 500 µL) of ejaculate, which is subsequently analysed with a spectrophotometer to define the concentration of spermatozoa and motile spermatozoa. This ultimately determines the concentration of immobile spermatozoa. The estimation of sperm morphology is based on a proprietary algorithm. The SQA-VISION visualisation compartment is employed for the purpose of viewing and performing manual round cell assessment.

Regarding the manual methods employed, sperm concentration was evaluated using a Neubauer counting chamber, while motility was assessed through observation of cells under phase contrast microscopy between slide and coverslip. The morphology of the sperm was evaluated on a slide by staining the cells, allowing for the examination of their size, shape, and general appearance.

The manual methods were performed in accordance with the WHO 5th Edition (2010) [7]. For the evaluation of sperm count, semen dilutions were complex. However, the observation of 0–4 spermatozoa per field at 400 magnifications (or the observation of 0–16 spermatozoa per field at 200 magnifications) could provide sufficient indication for the assessment of concentration. The 5th edition of the WHO manual recommended the use of strict criteria for identifying a normal spermatozoon, and provided the following precise definition of a normal spermatozoon: Regarding categories of sperm motility, the motility of each spermatozoon is graded as follows:

- Progressive motility (PR) is defined as spermatozoa moving actively, either linearly or in a large circle, regardless of speed.
- Non-progressive motility (NP) is defined as all other patterns of motility with an absence of progression, i.e. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed.
- Immotility (IM): no movement.

The results of each parameter studied by the manual and SQA-V methods were reported daily in software to verify the accuracy of the automated system.

Statistical analysis

The conformity of the numerical values to a normal distribution was evaluated using a Shapiro–Wilk test, which demonstrated that the distribution of results was non-parametric. The comparison between populations was evaluated using a Mann–Whitney test ($p < .05$ was considered significantly different). Passing Bablok regression analysis and Spearman correlation analysis was used to evaluate the compatibility between the two methods. $p < .05$ was statistically significant. The dashed lines represent a confidence interval that contains 95% of the expected observations.

Analytical agreement

The sensitivity and specificity of SQA-V were calculated based on the WHO Manual, 6th edition reference values [8] for semen parameters and using the microscopic manual method as the reference standard. This new version of the WHO incorporated a new dimension, adding new data on fertile men in Southern Europe, Asia, and Africa. The percent agreement and kappa coefficients with a 95% CI were calculated to estimate the agreement of evidence and recommendation levels between all paired samples [9]. According to Landis and Koch [10], kappa coefficients can be interpreted as one of the following six degrees of agreement: poor ($\kappa < 0$), slight (0.01–0.20), fair (0.21–0.40), moderate (0.41–0.60), substantial (0.61–0.80), and almost perfect (0.81–1.00). The percent agreement between the paired samples was calculated as the proportion of concordant sample sets divided by the total number of samples.

Results

Sperm concentration

Of the 1130 semen samples, three demonstrated severe oligozoospermia (sperm count <5 M/mL), 99 had mild to moderate oligozoospermia (sperm count 5–15.9 M/mL), and 1028 were considered normal (sperm count ≥16 M/mL). The median sperm count values according to manual and SQA-V were 51.0 and 49.9 M/mL, respectively, and there was no statistically significant difference between the two values ($p = 0.298$) (Table 1). There was a very high correlation between the two measurement methods (rho = 0.98), and the Passing-Bablok regression analysis formula was $y = 0.91x + 2.79$ (Table 2 and Figure 1). The SQA-V method demonstrated 98.3% agreement and a perfect Kappa coefficient of 0.90, while sensitivity and specificity were 0.90 and 0.99, respectively (Table 3).

Progressive motility

A total of 1050 semen samples presented normal progressive motility, while 80 samples revealed a progressive motility

Table 1. Statistical description of the semen parameters results, according to the two measurement methods (manual microscopic and SQA-V).

	Sperm concentration M/mL			Progressive motility %			Motility %			Normal Forms % (morphology)			Round Cells M/mL		
	Manual microscopic	SQA-V	Mann–Whitney test Manual *vs* SQA-V	Manual microscopic	SQA-V	Mann–Whitney test Manual *vs* SQA-V	Manual microscopic I	SQA-V	Mann–Whitney test Manual *vs* SQA-V	Manual microscopic	SQA-V	Mann–Whitney test Manual *vs* SQA-V	Manual microscopic	SQA-V	Mann–Whitney test Manual *vs* SQA-V
Nb. Specimens	1130	1130	$p = 0.298$	1130	1130	$p = 1.000$	1130	1130	$p = 1.000$	1130	1130	$p = 0.251$	1130	1130	$p = 0.225$
Minimum	1.8	1.4		5	4		5	6		1	1		0.0	0.0	
Maximum	240	280		74	89		85	100		60	65		45	46	
1st Quartile	30.0	30.0		45	43		55	53		7	9		0.4	0.4	
Median	51.0	49.9		50	51		60	60		9	11		0.7	0.7	
3rd Quartile	80.0	77.7		55	57		65	65		11	13		1.6	1.6	

Table 2. Correlation results between manual microscopic and SQA-V methods.

	Pearson correlation coefficient (r)	Regression slope	Intercept
Sperm concentration	***r* = 0.98**	**0.91**	**2.79**
	[95% CI]:	[95% CI]:	[95% CI]:
	0.96–0.99	0.90–0.93	1.81–3.77
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Progressive motility	***r* = 0.95**	**1.00**	**0.66**
	[95% CI]:	[95% CI]:	[95% CI]:
	0.92–0.96	0.98–1.00	−0.281–1.61
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Motility	***r* = 0.94**	**0.98**	**0.31**
	[95% CI]:	[95% CI]:	[95% CI]:
	0.93–0.97	0.97–1.03	−0.94–1.55
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Normal Forms (morphology)	***r* = 0.81**	**0.79**	**3.72**
	[95% CI]:	[95% CI]:	[95% CI]:
	0.78–0.85	0.75–0.82	3.37–4.08
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Round Cells	***r* = 0.98**	**1.03**	**0.03**
	[95% CI]:	[95% CI]:	[95% CI]:
	0.96–0.99	1.01–1.04	−0.01–0.06
	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

below 30%. The median values were 50% and 51%, respectively, and no statistically significant difference was observed ($p = 1.000$) (Table 1). A high correlation was observed between the two measurement methods (rho = 0.95), and the Passing-Bablok regression analysis formula was $y = 1.00x + 0.66$ (Table 2 and Figure 1). The SQA-V method demonstrated a 99.0% agreement rate and a perfect Kappa coefficient of 0.93, while sensitivity and specificity were 0.98 and 0.99, respectively (Table 3).

Motility

A total of 981 semen samples demonstrated normal motility, while 149 samples exhibited motility below 42%. The median values were 60% and 60%, respectively, and no statistical difference was observed ($p = 1.000$) (Table 1). A high correlation was observed between the two measurement methods (rho = 0.94), and the Passing-Bablok regression analysis formula was $y = 0.98x + 0.31$ (Table 2 and Figure 1). The SQA-V method demonstrated 97.0% agreement and a perfect Kappa coefficient of 0.88, while sensitivity and specificity were 0.87 and 0.99, respectively (Table 3).

Normal forms (morphology)

A total of 996 semen samples presented normal morphology values (≥ 4%), while 134 were considered abnormal (<4%). The median values for manual and SQA-V were 9 and 11%, respectively, and no statistically significant difference was observed ($p = 0.251$) (Table 1). A strong correlation was observed between the two measurement methods, with a coefficient of determination ((rho) of 0.81 (Table 2 and Figure 1)). The Passing-Bablok regression analysis formula was $y = 0.79x + 3.72$ (Table 2 and Figure 1). The SQA-V method demonstrated 98% agreement and a perfect Kappa coefficient of 0.92. Regarding the sensitivity and specificity for the detection and accurate classification of normal sperm, the values were 0.88 and 0.99, respectively (Table 3).

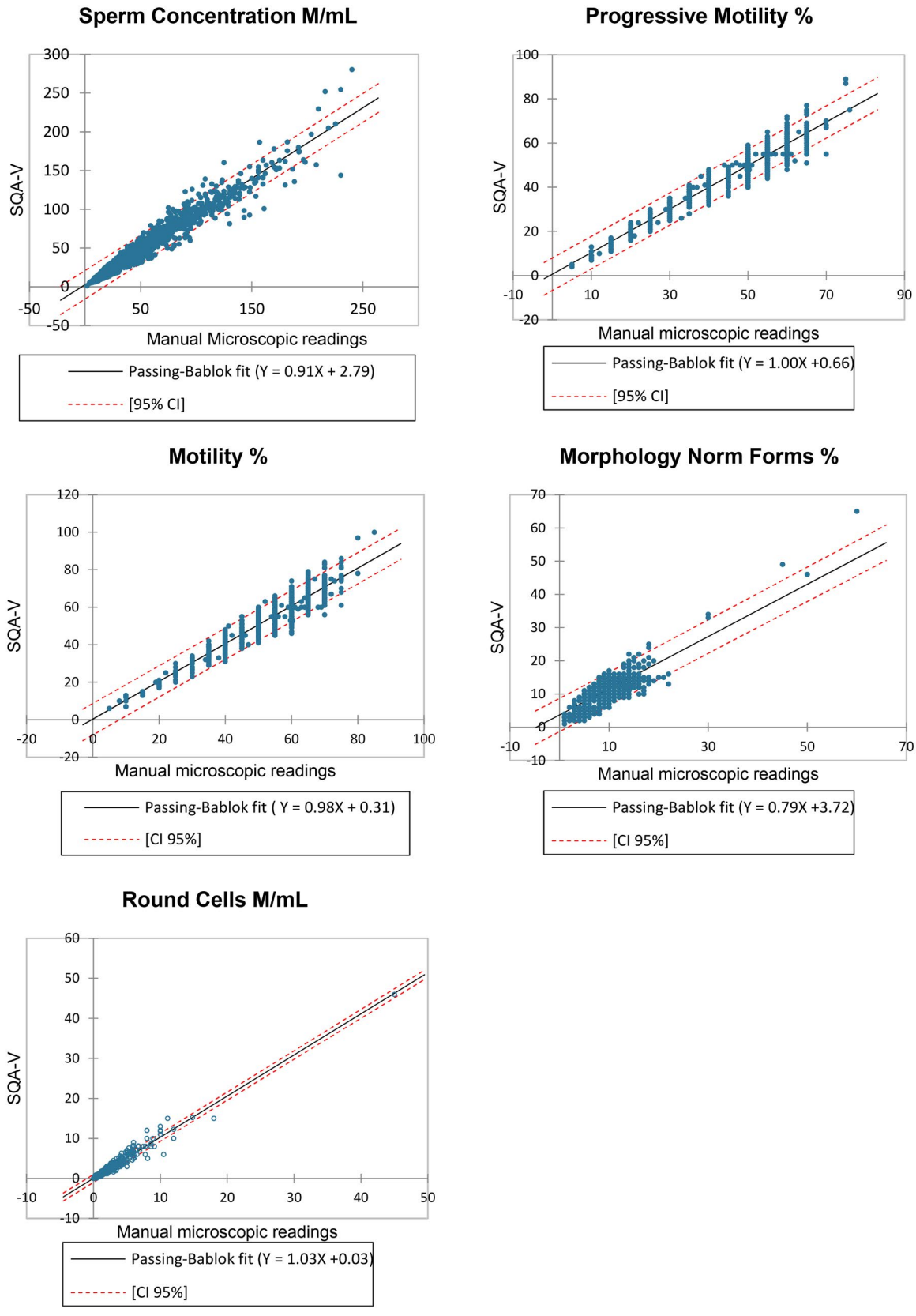

Figure 1. Passing-Bablok Regression plots comparing manual microscopic readings versus automated (SQA-V).

Round cells count

Of the 1130 semen samples, 360 presented a round cell count exceeding 1 million per millilitre. The median values for manual and SQA-V were 0.7 and 0.7 M/mL, respectively, and no statistical difference was observed ($p = 0.225$) (Table 1). A high correlation was observed between the two measurement methods (rho = 0.98), with the Passing-Bablok regression analysis formula being $y = 1.03x + 0.03$ (Table 2

Table 3. Clinical agreement of results between the manual microscopic and the SQA-V methods.

Sperm concentration	Reference value ≥ 16 M/mL WHO 6th Edition Reference I	Manual microscopic / < 16 M/mL	Manual microscopic ≥ 16 M/mL	Agreement rate (%)	Kappa [95% CI]	Sensitivity [95% CI]	Specificity [95% CI]
	SQA-V count < 16 M/mL	92	9	98.3	0.90 (0.85–0.94)	0.90 (0.84–0.96)	0.99 (0.98–1.00)
	SQA-V count ≥ 16 M/mL	10	1019				
Progressive motility	Reference value ≥ 30% WHO 6th Edition Reference	Manual microscopic < 30 %	Manual microscopic / ≥ 30%	Agreement rate (%)	Kappa [95% CI]	Sensitivity [95% CI]	Specificity [95% CI]
	SQA-V count < 30 %	78	9	99.0	0.93 (0.89–0.97)	0.98 (0.94–1.00)	0.99 (0.98–1.00)
	SQA-V count ≥ 30 %	2	1041				
Motility	Reference value ≥ 42 % WHO 6th Edition Reference	Manual microscopic <42 %	Manual microscopic ≥ 42%	Agreement rate (%)	Kappa [95% CI]	Sensitivity [95% CI]	Specificity [95% CI]
	SQA-V count < 42 %	129	9	97.0	0.88 (0.84–0.93)	0.87 (0.81–0.92)	0.99 (0.98–1.00)
	SQA-V count ≥ 42%	20	972				
Normal Forms (morphology)	Reference value ≥ 4 % WHO 6th Edition Reference	Manual microscopic <4 %	Manual microscopic ≥ 4%	Agreement rate (%)	Kappa [95% CI]	Sensitivity [95% CI]	Specificity [95% CI]
	SQA-V count < 4 %	119	3	98.0	0.92 (0.88–0.96)	0.88 (0.83–0.94)	0.99 (0.98–1.00)
	SQA-V count ≥ 4%	15	993				
Round Cells	Reference value < 1 M/mL WHO 6th Edition Reference	Manual microscopic ≥ 1 M/mL	Manual microscopic < 1 M/mL	Agreement rate (%)	Kappa [95% CI]	Sensitivity [95% CI]	Specificity [95% CI]
	SQA-V count ≥ 1 M/mL	353	2	99.0	0.98 (0.96–0.99)	0.98 (0.97–0.99	0.99 (0.99–1.00)
	SQA-V count < 1 M/mL	7	768				

and Figure 1). The SQA-V count demonstrated 99% agreement and a perfect Kappa coefficient of 0.88, while sensitivity and specificity were 0.98 and 0.99, respectively (Table 3).

Discussion

Several studies have been conducted to investigate the correlation between the results obtained by different automated systems currently on the market. However, most of these studies have used a limited number of samples. For sperm counts, the SQA systems have been demonstrated to be particularly reliable, as evidenced by studies [11,12]. Regarding total sperm count, progressive motility, and total motility, our results demonstrated a robust correlation and aligned with those previously reported by Lammer and colleagues [6]. In terms of normal forms, the SQA-Vision demonstrated a moderate correlation with the manual method (rho = 0.81), with only a small proportion of samples (2%) showing discordant interpretations of sperm morphology and a perfect Kappa coefficient (0.92).

In comparison to the existing literature, a notable strength of this work was the evaluation of the performance of SQA-V after four years of practice in a large cohort of patients. This feedback over several years has demonstrated the analytical robustness of the SQA-V system for long periods of use. The sensitivity and specificity of the system for the different parameters have been calculated using a large cohort of data. Furthermore, previous studies have not evaluated the performance of the SQA-V automated system in assessing round cells.

The collective findings of the various studies indicate that SQA can be employed for the routine analysis of semen in the investigation of male infertility. This approach offers rapid, accurate, and objective results, and positive impact at the laboratory level through the standardisation of results [4–6,11,12]. Our study has corroborated these assessments. Nevertheless, the SQA-V system is subject to certain limitations, which are inherent to the characteristics of human sperm. In particular, the presence of numerous cells and debris in the sample can be mistakenly counted as cells by the software. In such circumstances, it is incumbent upon the operator to identify these discrepancies and implement appropriate corrective measures [13]. The sample volume of 0.5 ml for analysis is undoubtedly more representative; however, it corresponds to a considerable volume for ejaculate volumes of 2–3ml on average. Moreover, its functionality is limited when the sperm parameter values are situated beyond the measurement range, and it cannot fully replace manual methods.

Furthermore, the analytical performance demonstrated in this study revealed numerous advantages of the SQA-V

system. Firstly, the SQA-V system was faster than manual analysis, adhering strictly to WHO recommendations. Secondly, the computer-assisted software enabled the analysis to be carried out on a larger number of cells, resulting in more accurate results than the manual method. Thirdly, IT support was used to store and archive sperm analysis videos, providing a practical tool for training new staff. The influence on the evaluation of the cells under study was reduced, and the results of the parameters studied were more objective and more reproducible. However, it was important to remember that the quality of the SQA-V results was subject to the competency of the operators, and staff training in the use of the analyser had to be rigorous from both theoretical and practical points of view to ensure that a critical eye was kept on the results delivered.

The findings of our study indicate that the routine use of SQA-V could ensure the quality of the results while improving the laboratory's management. Further investigations should be conducted to examine the potential benefits of using SQA-V on sperm viability and sperm DNA fragmentation, beyond the basic parameters of sperm.

Conclusion

In this retrospective study, we demonstrated that the SQA-V systems and the manual method exhibited a high degree of concordance for routine sperm analysis. This instrument has proven to be efficacious during the four-year investigation period.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

[1] Andrade-Rocha FT. On the origins of the semen analysis: a close relationship with the history of the reproductive medicine. J Hum Reprod Sci. 2017;10(4):242–255. doi: 10.4103/jhrs.JHRS_97_17.

[2] Keel BA, Quinn P, Schmidt CF, et al. Results of the American Association of bioanalysts national proficiency testing programme in andrology. Hum Reprod. 2000;15(3):680–686. doi: 10.1093/humrep/15.3.680.

[3] Neuwinger J, Behre H, Nieschlag E. External quality control in the andrology laboratory: an experimental multicenter trial. Fertil Steril. 1990;54(2):308–314. doi: 10.1016/S0015-0282(16)53709-1.

[4] Akashi T, Mizuno I, Okumura A, et al. Usefulness of sperm quality analyzer-V (SQA-V) for the assessment of sperm quality in infertile men. Arch Androl. 2005;51(6):437–442. doi: 10.1080/014850190959081.

[5] Agarwal A, Sharma RK. Automation is the key to standardized semen analysis using the automated SQA-V sperm quality analyzer. Fertil Steril. 2007;87(1):156–162. doi: 10.1016/j.fertnstert.2006.05.083.

[6] Lammers J, Splingart C, Barrière P, et al. Doubleblind prospective study comparing two automated sperm analyzers versus manual semen assessment. J Assist Reprod Genet. 2014;31(1):35–43. doi: 10.1007/s10815-013-0139-2.

[7] World Health Organization. WHO Laboratory manual for the examination and processing of human semen. 5th ed. Geneva. World Health Organization; 2010

[8] World Health Organization. WHO Laboratory Manual for the examination and processing of human semen. 6th ed. Geneva. World Health Organization; 2021

[9] Cohen J. A coefficient of agreement for nominal scales. Educ Psychol Meas. 1960;20(1):37–46. doi: 10.1177/001316446002000104.

[10] Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159–174.

[11] Chaurasia AK, Sinha AK, Upahdyay P. Comparison of semen analysis by manual and automated method. J Pathol Nep. 2016;6(12):990–993. doi: 10.3126/jpn.v6i12.16278.

[12] Lammers J, Chtourou S, Reignier A, et al. Comparison of two automated sperm analyzers using 2 different detection methods versus manual semen assessment. J Gynecol Obstet Hum Reprod. 2021;50(8):102084. doi: 10.1016/j.jogoh.2021.102084.

[13] Ilardo C, Ostengo V, Eon L, et al. Impact of the presence of debris on semen analysis using an automated system (SQA-V). Int J Med Biochem. 2021;4:211–215. doi: 10.14744/ijmb.2021.97658.