Review Article
Male reproductive health and infertility

pISSN: 2287-4208 / eISSN: 2287-4690
World J Mens Health Published online Apr 21, 2021
https://doi.org/10.5534/wjmh.210022

The World Journal of MEN's HEALTH

Standardized Laboratory Procedures, Quality Control and Quality Assurance Are Key Requirements for Accurate Semen Analysis in the Evaluation of Infertile Male

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Semen analysis is a basic test for evaluating male fertility potential, as it plays an essential role in driving the future management and treatment of infertility in couples. Manual semen analysis includes the evaluation of both macroscopic and microscopic parameters, whereas automated semen analysis is conducted through a computer-aided sperm analysis system and can include additional parameters that are not evaluated by manual analysis. Both quality control (QC) and quality assurance (QA) are important to ensure reproducible results for semen analysis, and represent fundamental checks and balances of all stages (pre-analytical, analytical, and post-analytical) of semen analysis. To ensure accuracy and precision, the laboratory technicians' performance should be evaluated biannually. This narrative review aims to describe standardized laboratory procedures for an accurate assessment of semen parameters that incorporate both QC and QA practices.

Keywords: Semen analysis; Standardization; Quality assurance; Quality control

Received: Feb 20, 2021 **Revised:** Mar 5, 2021 **Accepted:** Mar 7, 2021 **Published online** Apr 21, 2021
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INTRODUCTION

Infertility, defined as "failure of a couple to achieve pregnancy after at least 12 months of regular well-timed intercourse without contraception", is a clinical condition that affects 13% to 18% of couples worldwide [1]. Both the male and female partner contribute to infertility, however, male factors either partially or solely underlie an estimated 50% of the infertility cases [2]. In recent years, a decline in semen quality across Africa, Europe, North America, and Asia has been reported [3,4]. This seems to suggest that male infertility is a growing global problem.

Identifying and diagnosing male infertility consists of a physical examination along with standard semen analysis, performed according to the World Health Organization (WHO) criteria, the latest of which is the 5th edition in 2010 [5]. However, this diagnostic procedure may not always identify the cause of male infertility as 25% of infertility cases worldwide are considered as unexplained [6]. Nevertheless, semen analysis remains the cornerstone for laboratory evaluation of male factor infertility and plays a vital role in understanding the cause of infertility [7,8]. As a diagnostic test, semen analysis may help predict natural conception and help clinicians to manage couple infertility using assisted reproductive technology (ART) [9,10].

Laboratory personnel are responsible for ensuring the quality of services provided. This is achieved through establishment of quality control (QC) and quality assurance (QA) plans, which are fundamental to monitor the process rigorously [5,11]. The reliability and validity of semen analysis depend solely on the expertise and skills of laboratory personnel [12]. Hence, frequent training and continuous education of trainees and future practitioners in the field of andrology is of great importance to ensure precision and accuracy in the results obtained [13].

However, the COVID-19 pandemic quickly became an obstacle to acquire such learning and made it impossible to conduct any form of in-person training. Consequently, the use of digital media, in the form of webinars and online presentations, became the only option to spread knowledge remotely without any physical contact [14].

With this in mind, the American Center of Reproductive Medicine (ACRM; Cleveland Clinic, Cleveland, OH, USA) recently launched a virtual ART Training program consisting of 14 bimonthly modules. The fundamental goal of this program is to train clinicians, biologists, and researchers on a global scale about various routine and advanced laboratory tests in andrology,

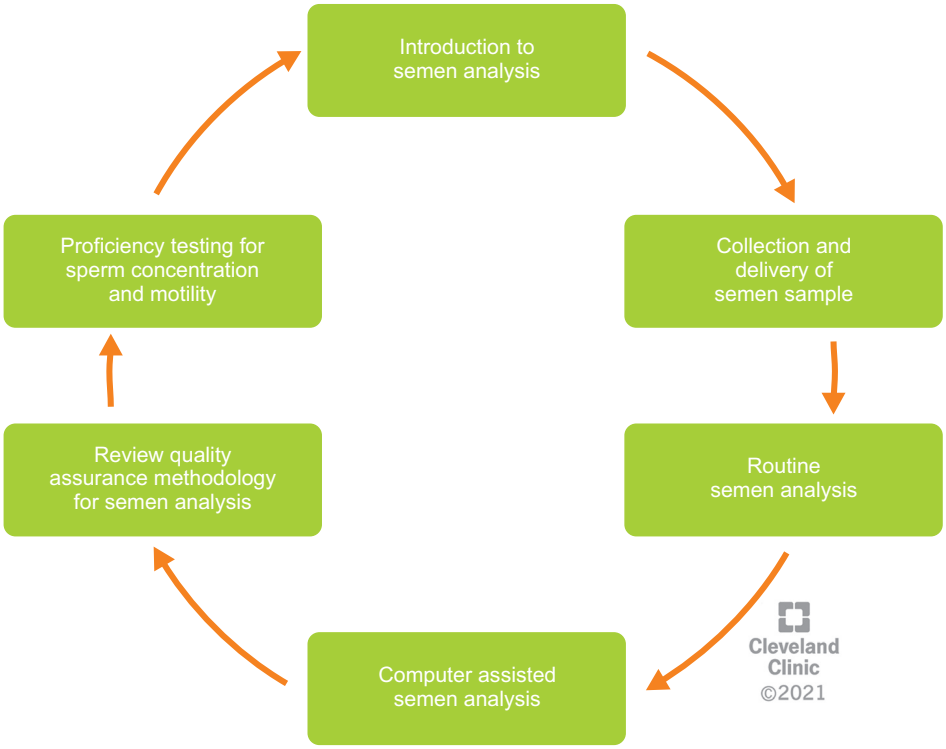


Fig. 1. Course content of the Module 1A–Online assisted reproductive technology (ART) Training.

cryopreservation of gametes and assisted reproduction. The first session of the online ART Training course in November 2020 saw participation from more than 1,400 candidates from 66 countries. The module content was delivered, through six lectures on standard semen analysis, delivered by experts in the field among the ACRM faculty *via* the WebEx platform (Fig. 1). Specifically, the course encompassed a) the theoretical and practical bases of manual semen analysis according to WHO 5th edition guidelines, b) the importance of QC and QA in maintaining high quality andrology services, c) semen analysis conducted *via* computer-aided semen analysis (CASA) systems, and d) the clinical interpretation of semen analysis results.

This review summarizes the information disseminated to the trainees during ACRM's first online ART Training course. This will be elaborated in two parts: in the first, we describe the standardized laboratory procedures essential for proper evaluation of semen quality, and the established QC and QA plans in the context of managing the quality of a busy clinical andrology laboratory. In the second part of this paper, we describe several clinical scenarios in which reliable semen analysis results have played a significant role in the management of the infertile male.

MANUAL SEMEN ANALYSIS

Prior to providing a sample for analysis, the patient should be provided with clear instructions for collecting the semen sample [5,15]. The initial fraction of the ejaculate contains the highest concentration of sperm, and so if any fraction is lost, this must be duly recorded. After 2 to 7 days of abstinence, the entire semen sample is collected in a sterile cup. After collection, the sample is placed in an incubator at 37°C for 30 to 60 minutes to allow liquefaction before being analyzed (Fig. 2, 3). The analysis is then conducted based on the reference values reported in the WHO 5th edition manual. These are calculated on the lower fifth per-

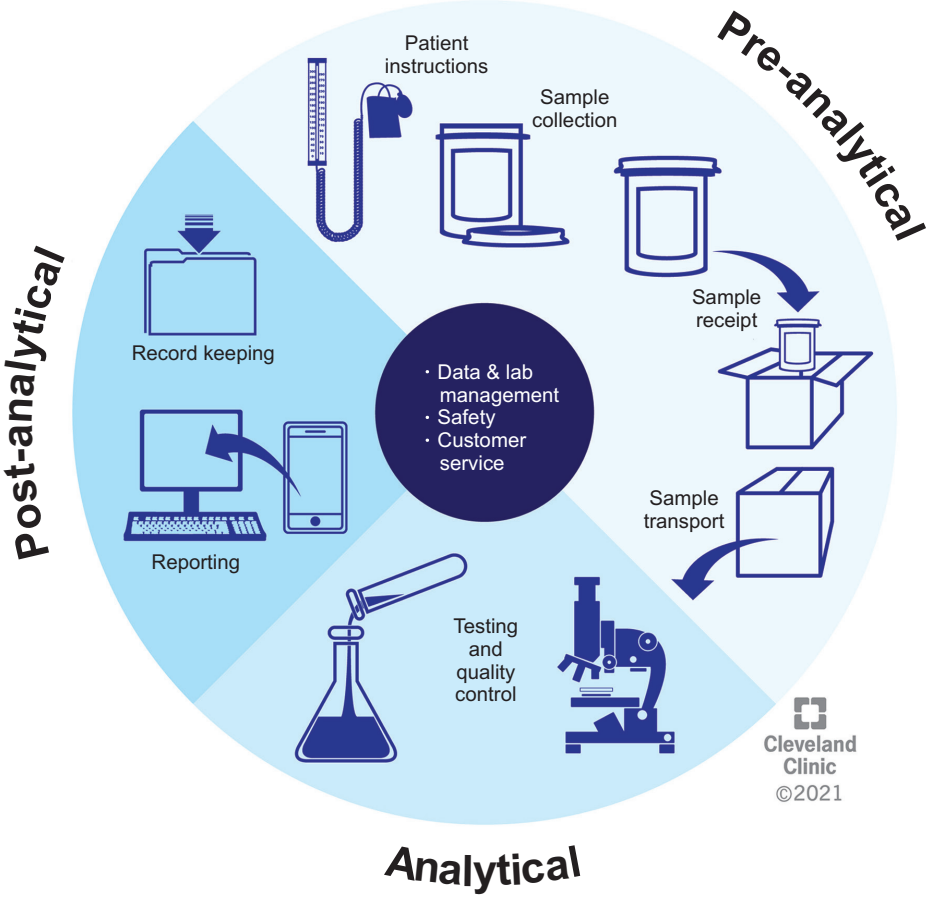
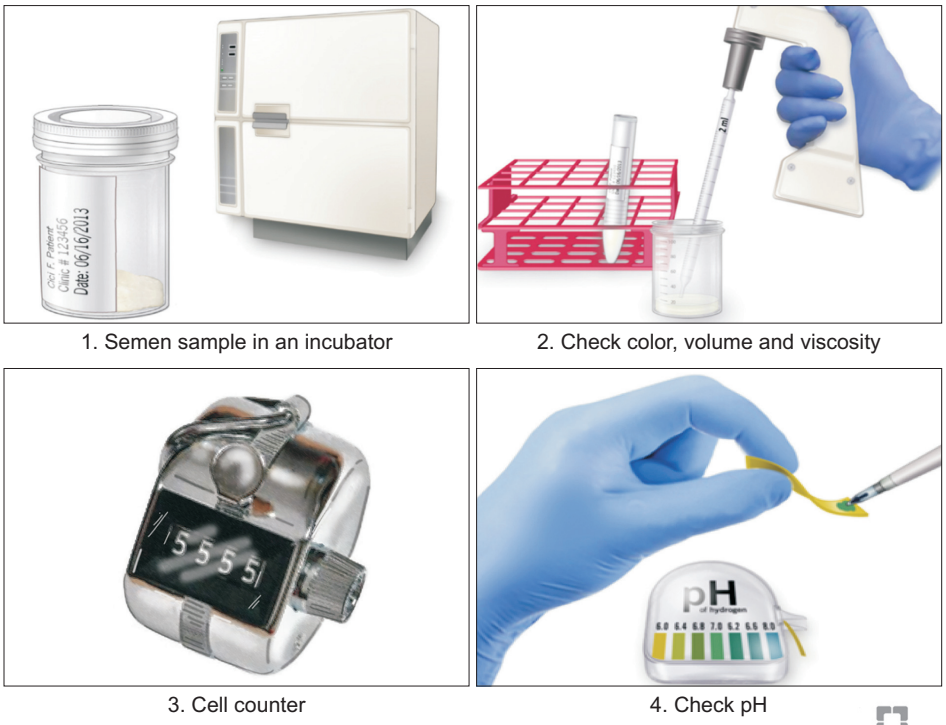


Fig. 2. Graphical illustration representing the workflow for semen analysis in an andrology laboratory.



1. Semen sample in an incubator
2. Check color, volume and viscosity
3. Cell counter
4. Check pH

Fig. 3. Main steps in standard semen analysis.

centile and are lower compared to those proposed in the 4th edition of the WHO manual [5,16,17].

QUALITATIVE AND QUANTITATIVE TEST OF SEMEN

A semen examination should be performed immediately after liquefaction and no longer than 60 minutes after ejaculation [5]. A manual semen analysis includes macroscopic and microscopic evaluation. It is important to mix the sample well using a vortex mixer before any examination, to resuspend the cellular fraction.

1. Macroscopic examination

The ejaculate consists of secretions from seminal vesicle (70%), prostate (25%), epididymis, vas deferens, bulbourethral, and urethral glands (~5% in total) and sperm (~5%). The macroscopic evaluation includes liquefaction, viscosity, appearance of the ejaculate, volume, and pH [5,15]. Viscous samples can be treated with a proteolytic enzyme such as α-chymotrypsin or bromelain, but not by vigorously forcing the ejaculate through a needle with a syringe [5,15].

2. Microscopic examination

The sample is examined under a phase-contrast microscope and an ocular micrometer is placed in the eyepiece. Microscopic evaluation allows the calculation of sperm concentration, total sperm count and sperm motility. The presence of round cells, white blood cells, and sperm agglutination are also examined during microscopic evaluation [5,15].

SIGNIFICANCE OF QUALITY CONTROL IN AN ANDROLOGY LABORATORY

QC is a system of procedures which allows laboratories to establish quality indicators for laboratory equipment, standard operating procedures (SOPs) and testing personnel, thus ensuring that they meet the established criteria of accuracy. The importance of QC in semen analysis was introduced for the first time in the 4th edition of the WHO manual [17]. QC is an integral part of any laboratory committed to provide quality service as per ISO (International Organization for Standardization) standards or local regulatory bodies' guidelines. ISO standards were set in 1947 as the

standard requirements to ensure quality, consistency and safety of any product or service internationally. ISO 9001 (2015) is the most widely accepted standard for quality management systems (QMSs) [18].

Quality indicators are the measures of QC to monitor the accuracy and precision of a particular test or procedure and to identify any out-of-range values. Some commonly used quality indicators in the pre-analytical phase are: specimen identification, test order accuracy, time to analysis, patient's waiting time for the appointment, and waiting times in the laboratory. For any unsatisfactory outcomes, each andrology laboratory should formulate and strictly follow a predefined specimen rejection criterion such as incorrect sample collection, exposure to extreme temperature during the transport and delay in the sample analysis (>60 minutes). The first step to initiate a QC program in the laboratory is to create SOPs for all applicable processes involved in the workflow of a laboratory, including implementation of QC. The SOPs contain clear systematic instructions to ensure uniformity of a particular process when executed at different times or by different persons [18]. The main objective for integrating SOPs are to reduce errors by controlling variations, improve the work efficiency by continuous updates, help in staff training, provide guidance when alert values arise, follow-up in reporting alert values and lastly, to maintain a safe environment in the lab. The lab should ensure that every lab personnel follows the SOPs.

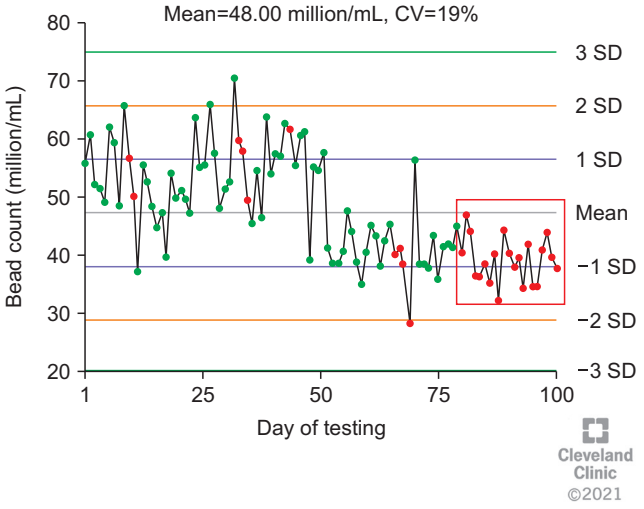


Fig. 4. Levy–Jennings plot for high range of QC beads. There are specific rules for declaring that an instrument's function is out of control: (1) a single point lies outside the 3 SD; (2) two out of three successive points lie outside the 2 SD; (3) four out of five successive points lie outside the 1 SD; (4) two successive results lie above the upper, or below the lower, 1 SD; (5) two successive results lie one above the upper, and one below the lower, 1 SD; (6) eight successive points are on the same side of the centerline. SD: standard deviation, CV: coefficient of variation, Red dots: error, 1 SD: warning control limit, 2 SD: action control limit.

QC in the analytical phase includes instrument function checks, instrument calibration and maintenance checks (Fig. 4). Competency and proficiency of testing personnel is an important component of the analytical QC.

To ensure quality in the post-analytical phase, the laboratory personnel should double check that all results on the worksheet are correctly reported both manually and in the electronic medical records (EMRs). The results in the EMR also need to be verified, validated and monitored for turnaround time [19].

There are two equally important ways of doing a quality check in the laboratory:

(1) Internal quality control (IQC): it is defined as a system to assess and minimize the variability of an existing procedure within a laboratory. These tests assess day-to-day reproducibility and the detection of errors. Implementing IQC at all stages requires checking all the critical points during the work routine such as temperature control, equipment maintenance and the technical performance by individual technologists as well as in comparison of results with that of other team members. The information collected can then be recorded in a chart for monitoring. IQC is useful for detecting random variation (accuracy assessment) and intra-laboratory variations. An in-house proficiency testing is performed for sperm motility, hypo-osmotic swelling and qualitative fructose and the results are reported for each semi-annual period.

(2) External quality control (EQC): it is defined as a method to check the laboratory performance by an external agency, as a tool to assess the accuracy and detect systematic variations [5]. This procedure is commonly carried out by evaluating the same sample by different laboratories, so that the laboratory can compare its results with those of others, allowing for different assessment methods. It is very important that this sample is evaluated by testing it as a routine patient sample by the designated laboratory technologist. Laboratory technologists are put on a rotation schedule to perform EQC testing. This ensures that all laboratory technicians participate in the EQC exercise.

The frequency of conducting QC assessment is in-

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fluenced by multiple factors such as the guidelines issued by national or local accrediting bodies, and the workload of the laboratory. Frequent assessment of QC points is essential to ensure that all the equipment work to the required standards. QC samples are used to monitor technicians and trainees, and to validate new equipment, supplies and procedures. Therefore, each laboratory has to set up their own schedule for QC. Table 1 shows an example of a schedule with important quality indicators that must be checked daily, weekly, monthly, or annually [5,19,20].

Review and interpretation of QC data is an essential component of any QC program, which is performed by reviewing the various quality indicators. Quality indicators can also be checked by creating Bland–Altman or X-bar charts to compare two technologists' results [5], as in the example provided in Fig. 5, where the inter-technician agreement for sperm motility is assessed [5,21]. Corrective action must be included in the SOPs and applied every time a non-conformity is recognized (Table 2) [5,22].

To ensure accuracy and precision of test results, it is

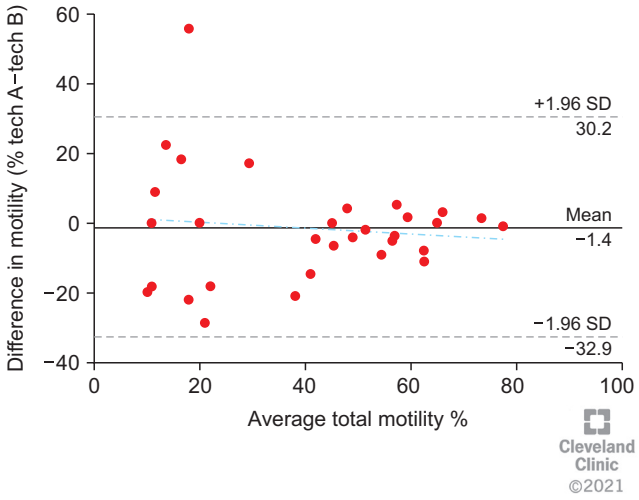

Fig. 5. Bland–Altman plot to assess inter-technician agreement for sperm motility assessment. The standard deviation (SD) for sequential measurements of difference in motility are plotted on the graph with the mean value and 2 SD limits, which were previously measured. The difference between two measurements (Tech A-Tech B) and the mean ([Tech A+Tech B]/2) is constructed respectively on the vertical and horizontal axis with 95% confidence interval (CI). Within CI, 95% of the data points should fall within ±2 SD of the mean difference. Therefore, the CI allows one to assess the range of variability between the two techniques.

Table 1. Periodic schedule for quality control (QC) in the andrology laboratory [5,19,20]

Frequency	QC steps
Daily	- Monitor the temperature of all instruments
	- Count QC beads in counting chamber on each day of testing
	- Check the level of liquid nitrogen tanks. Fill as necessary
	- Microscope cleaning, checking the optic setting, cover after use
	- Cleaning laboratory surfaces
	- Check morphology staining solutions
Weekly	- Analysis of results replicability among technicians
	- Maintenance of the pH
	- Change the water in the incubator tray
	- Calibrate automated Semen Analyzer
	- Check and change solutions (Endtz working solution, Tyrodes buffer solution)
	- Restocking reagents and inventory
	- Testing eyewash station
Monthly	- Clean centrifuge rotors
	- Check supplies inventory
	- Checking and removing all expired reagents or supplies
	- Internal and external cleaning of the incubators
	- Microscope maintenance
	- Clean automated Semen Analyzer
	- Check and change solutions (Eosin-Nigrosin staining solutions, Hypoosmotic Swelling [HOS] Test solution)
Bi-annually/annually	- External Quality Control (EQC)
	- Calibration of pipettes, thermometers and timers
	- Verification of counting chambers
	- Semi-annual environment cultures

Table 2. Grades of non-conformity or error [5,22]

Grade	Outcome description
A	Impacted patient care
B	Potential to impact patient care
C	No impact on patient care
D	Regulatory requirement

essential to perform an annual competency test (Table 3) to assess whether the testing personnel have the necessary training and skills to perform a given test correctly. A qualified supervisor should conduct the assessment by quizzing the personnel for knowledge as well as directly observing the entire steps including specimen collection, interaction with patients, macroscopic and microscopic examinations, calculations and reporting. The supervisor provides relevant constructive feedback, training, or repeat the competency test if required [19].

Our laboratory in the Andrology Center (Cleveland Clinic) participates in the semi-annual Proficiency Testing Survey provided by the American Association of Bioanalysts (AAB) and College of American Pathologists (CAP), with survey materials shipped to the laboratory twice in May and October. Andrology labs can subscribe to the following surveys from AAB and CAP: sperm count, anti-sperm antibody, sperm morphology (Strict Morphology and WHO Morphology), and sperm viability and post vasectomy semen analysis [23]. According to the AAB, the criteria for a satisfactory performance is to score a minimum of 80%. When in a same specialty of the proficiency testing, the score is an overall of all analytes [24]. When the results obtained are outside of the acceptable limits of 2 SD (standard deviation), corrective action is required and must be recorded, before further testing is performed [5]. These can include instrument calibration or staff retraining [18].

QUALITY ASSURANCE IN ANDROLOGY LABORATORY

While QC determines quality standard to measure the precision of a particular test [25,26], QA is the lab's overall surveillance to ensure the quality of each and every process in the laboratory [5]. Although the definition seems to overlap, they have a different perspective as QC ensures the test result's reliability and accuracy

Table 3. Example of the competency checklist of laboratory procedures for basic semen analysis

Test parameters	Scores		Comment/ recommendations
	Yes	No	
Quiz			
Specimen collection			
Patient interaction			
Macroscopic examination			
Microscopic examination			
Calculations			
Reporting			

by detecting errors, and QA determines a quality goal to deliver overall reliable test results and services. QA is a requirement per the licensing and accreditation agencies to maintain licensure of the reproductive lab. To summarize, QC is a part of QA and both are equally important components of the entire QMS operating in a laboratory. Various QMS models have been proposed but ISO is the most recognized worldwide.

The QA program ensures that the quality indicators are met for the andrology services, focusing on identifying possible non-conformities at all phases of the process: pre-analytical, analytical, and post-analytical (Fig. 6).

All non-conformities need to be investigated to identify the cause and then be corrected and documented. The monitoring and correction of deviations will enhance the laboratory's quality indicators [18,27].

The recommendations made by the Code of Federal Regulation (42 CFR 493) requires written policies and procedures to define a comprehensive QA program for continuous assessment of the quality in the testing process. In the USA, in order to obtain certification from the Clinical Laboratory Improvement Amendments (CLIA) as well as the previously described agencies, the laboratory needs to follow various standards as prescribed by the CFR. Following the standards and procedures will help the laboratory to report accurate and precise tests results, report reliable results promptly, maintain staff competency and QA documentation as required [28]. Each laboratory must follow standardized and reproducible methods to guarantee the high quality of the results. As such, to conceive the core of the QA plan and ensure such quality is desired, there must be ongoing management, administration, statistical analysis, preventive and corrective action of all the laboratory items [5].

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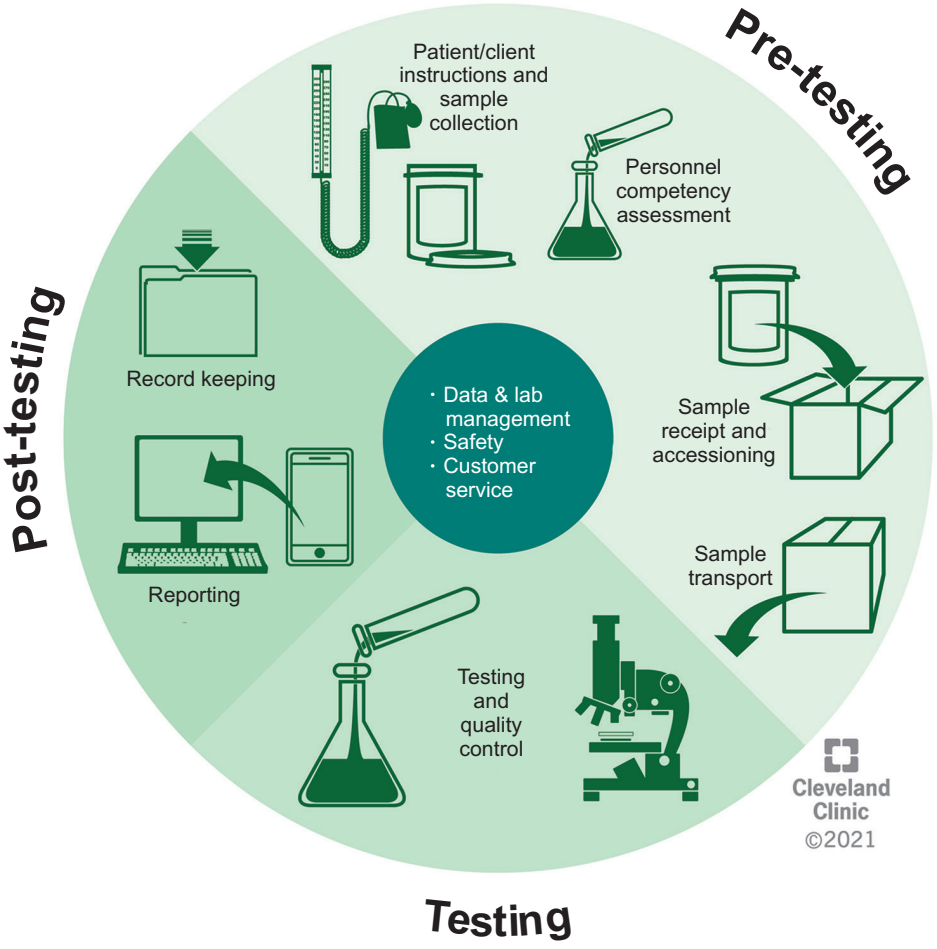

Fig. 6. Quality assurance cycle in semen analysis.

1. Quality assurance procedures: pre-analytical, analytical, and post-analytical

The pre-analytical phase involves providing correct instructions to the patient before collecting a semen sample, ensuring correct abstinence time, test requisition, sample container and transportation, criteria for specimen rejection, and correct patient identifiers. The analytical phase include the standardized performance of a semen analysis, in order to demonstrate its ability to provide a real and accurate diagnosis for the patient. The post-analytical phase results are finalized, and reports are then produced. All parameters evaluated in the previous phases must be monitored and documented, along with the corrective action taken to correct any errors (Fig. 7).

2. Implementing a quality assurance plan in an andrology laboratory

Formulating and incorporating a comprehensive QA plan into the routine practice of an andrology laboratory is an ongoing, multivariate process requiring strategic team effort and commitment of each laboratory staff to provide the most reliable services [11,29] and to obtain quality accreditation, which is a requirement for high complexity andrology labs (Fig. 7) [30].

Due to the laboratory procedures' complexity and subjectivity of the laboratory procedures, errors are inevitable [18]. Therefore, each laboratory should define its evaluation mechanism to identify non-conformity areas, *i.e.*, the deviation from the set thresholds or SOPs [11,18,31]. The determination of the quality of testing is based on quality indicators selected for all aspects of testing. The best way to set the critical value is to conduct well-planned independent audits [32]. A weekly audit of laboratory worksheets and a monthly assessment of laboratory data collected for all applicable procedures are performed to verify any deviation from the expected identified quality standards. Monthly QA reports are prepared to review the laboratory's entire process, including semen preparation, cryopreservation,

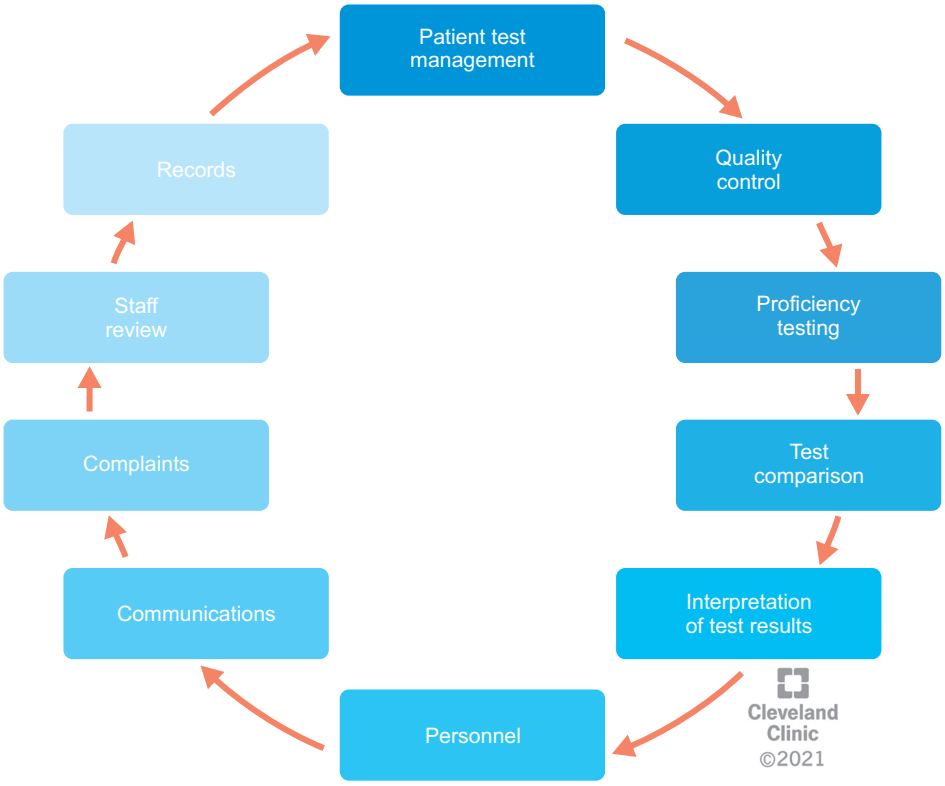

Fig. 7. The core elements of a quality assurance plan.

frozen sperm inventory checks, and the turnaround time for andrology tests. Any incident, error, or deviation is also recorded in a laboratory occurrence report with a detailed description of the error [29]. Proper documentation of each step of the audit is equally important to record both compliance, deficiencies, and corrective actions taken. Any identified error is then adequately documented.

AUTOMATED SEMEN ANALYSIS

The online ART Training module included a section on introduction to automated semen analysis. In andrology and ART laboratories, accuracy and precision of the results are important for ensuring correct infertility diagnosis, defining further treatment, and providing management advice to infertile couples [33]. As inter- and intra-technician variability are the primary source of error in manual semen analysis results, CASA systems have been increasingly used to improve the reliability of laboratory results and reduce errors [7,34]. CASA systems are automated or semi-automated instruments which are able to identify the movement of sperm and interpret this information thanks to an algorithm [34,35]. More precise results are hence provided in a faster way compared to manual semen analysis. Commonly used CASA systems in andrology laboratories are produced by several different companies, including Microptic (SCA), Hamilton Throne (IVOSII and CEROSII), or MES (SQA-Vision, SQA-V Gold) (Fig. 8).

Considering that each system uses different mathematical algorithms, there is a possibility of unreliable results when comparing semen parameters across different devices [33]. One of the most recently produced device is the LensHooke X1 Pro (Bonraybio, Taichung, Taiwan), a fully automated instrument that uses an artificial intelligence optical microscope (AIOM)-based technology to analyze seminal pH value, concentration, total motility and morphology, within a very short range of time (between 3-5 minutes). A study comparing X1 Pro and IVOS CASA systems with manual semen analysis demonstrated that both automated systems provided comparable results with manual analysis [36]. The X1 Pro was able to differentiate samples with abnormal semen concentrations and motility, with a positive predictive value (PPV) of 100% and 86.5% respectively. Whereas the IVOS CASA system showed a PPV value of 100% for differentiating abnormal concentrations, but lower PPV value (71.7%) for abnormal

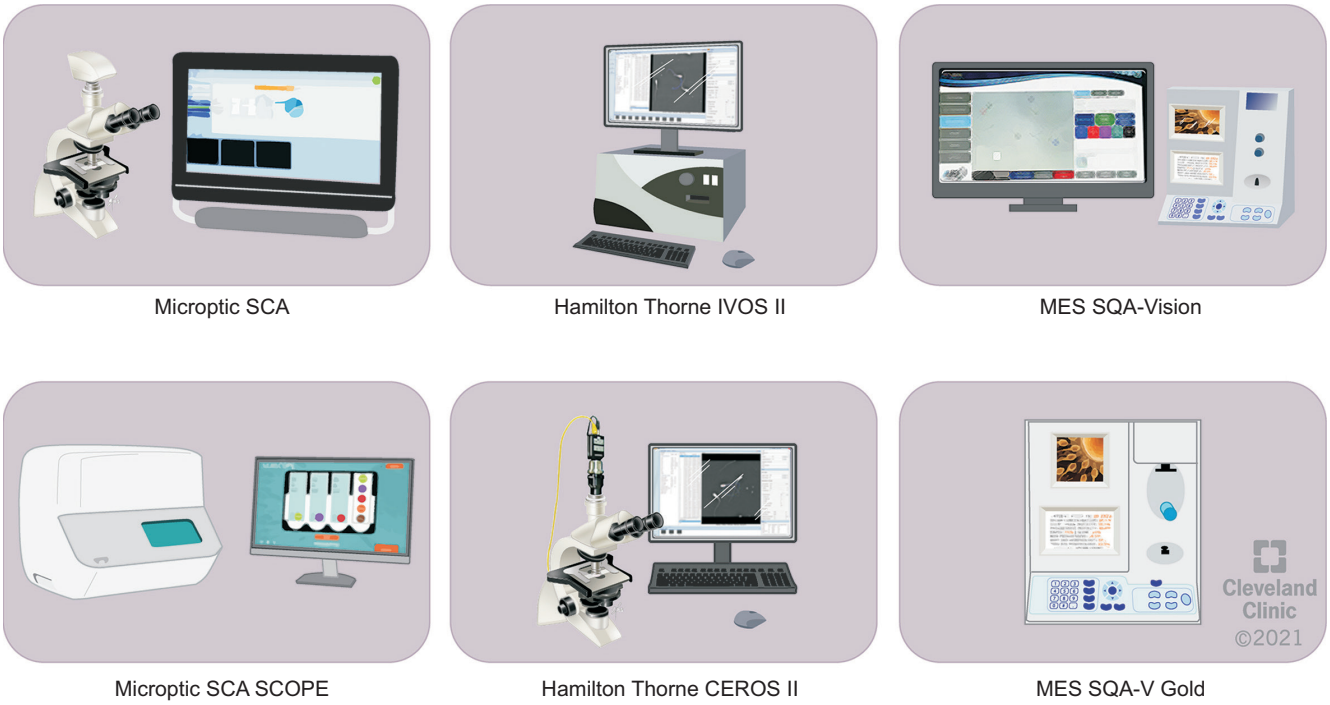

Fig. 8. Currently used computer-aided semen analysis (CASA) systems in clinical practice produced by companies, such as Microptic (SCA, SCA SCOPE), Hamilton Throne (IVOS II, CEROS II), Medical Electronic Systems (MES) (SQA-Vision, SQA-V Gold).

motility.

CLINICAL INTERPRETATION OF SEMEN ANALYSIS: CLINICAL CASE SCENARIOS

At this stage of the ACRM Online ART Training module, the trainees had received in depth information regarding manual and CASA and were able to appreciate the vital role of QC and QA in ensuring that the andrology services offered to patients were of the highest quality. In the next part of the course, the trainees were exposed to relevant case scenarios that illustrate how the interpretation of semen analysis results weigh in on the diagnosis and management of infertile male patients. Once again, emphasis was placed on why obtaining standardized test results is so critical and why measures should always be taken to ensure the precise and accurate reporting of results.

1. Case A

1) Scenario

Mr. M is a 28 year-old gentleman complaining of primary infertility of 1-year duration. His past medical and surgical histories were insignificant. His wife is 26 years old and has regular menses and normal gynecological investigations. The patient was of normal build with a body mass index (BMI) of 28 kg/m^2. His genital examination showed normal testicular size and consistency, full epididymides bilaterally, palpable vasa differentia and no palpable varicoceles. Semen macroscopic assessment showed a volume of 0.4 mL, normal viscosity, pH of 6.4. Microscopic analysis revealed absence of sperm which was confirmed following centrifugation at 3,000 g for 15 minutes. Fructose test was negative. On repetition of the semen analysis, the same findings were obtained.

2) Interpretation

The clinical presentation and the semen analysis result of Case 1 suggest the diagnosis of ejaculatory duct obstruction (EDO). This clinical diagnosis is suspected to be based on the presence of low ejaculate volume, acidic pH and negative fructose associated with azoospermia. Moreover, the seminal vesicle secretion is alkaline and contains fructose which aids in sperm nutrition [5,37]. Therefore, in case of EDO, with absence of seminal vesicle and testicular secretions, the ejaculate will only be formed of the prostatic secretions,

resulting in low volume, acidic ejaculate and absent of fructose. In such cases, further investigations would be required to confirm the diagnosis including transrectal ultrasound and/or prostatic MRI. Most cases can be treated by transurethral resection of the ejaculatory ducts to alleviate the obstruction.

2. Case B

1) Scenario

Mr. J is a 32 year-old gentleman complaining of primary infertility of 2-year duration. His past medical and surgical histories were insignificant. His wife is 28 years old and has regular menses and normal gynecological investigations. The patient was obese with a BMI of 33 kg/m^2. His genital examination demonstrated normal testicular volume and consistency, normal epididymides bilaterally, palpable vasa differentia and no palpable varicoceles. Semen macroscopic assessment showed a volume of 2 mL, normal viscosity and pH of 7.8. Microscopic analysis revealed a sperm concentration of 8.5 million/mL, total motility of 30%, progressive motility of 12%, normal morphology of 2%, and round cells of 0.2 million/mL. A comparable result was obtained on repeating the semen analysis.

2) Interpretation

This is a case of oligoasthenoteratozoospermia (OAT). In such cases the search for correctable causes of this semen abnormality should be the first step in clinical management. This includes varicocele, leukocytospermia, lifestyle factors (smoking, obesity), and environmental exposures (excessive heat exposure, pesticides, and heavy metals). In Mr. J's case, obesity is the only factor contributing to his altered semen result. Obesity has been recognized as an important risk factor for infertility through a number of pathophysiologies including aggravated oxidative stress levels, altered hypothalamo-pituitary-gonadal axis, and increased testicular temperature from excessive fat deposition in the groin area [38,39]. Several studies have revealed a significant positive impact with weight loss on semen parameters [38].

3. Case C

1) Scenario

Mr. K is a 44 year-old gentleman complaining of secondary infertility of 5-year duration. He is married for 10 years and has 1 child, 5 years of age. He was diagnosed with diabetes mellitus 5 years ago and it is well-controlled with oral medications. There was no significant past surgical history. His wife is 38 years old and has regular menses, however her gynecological evaluation revealed low anti-Mullerian hormone (6.3 pmol/L). The patient has a normal phenotypic appearance with a BMI of 29 kg/m^2. His genital examination showed normal testicular size and consistency, normal epididymides bilaterally, palpable vas deferens and a clinically palpable grade 3 left varicocele. Semen macroscopic assessment showed a volume of 3.5 mL, normal viscosity and pH of 8.0. Microscopic analysis revealed a sperm concentration of 20 million/mL, total motility of 35%, progressive motility of 5%, and normal morphology of 5%. A comparable result was obtained on repeating the semen analysis.

2) Interpretation

This is a case of isolated asthenozoospermia associated with left clinical varicocele. Varicocele is the most common correctable cause of infertility prevalent in up to 80% of men with secondary infertility [40]. This clinical diagnosis can impair spermatogenesis through testicular hyperthermia, ischemia, increased oxidative stress, and reflux of adrenal metabolites [41]. Varicocelectomy has been found to significantly improve semen parameters in 60% to 80% of cases resulting in spontaneous pregnancy in up to 60% of cases [42]. Varicocele ligation is indicated in patients with clinically palpable disease, abnormal semen parameters and the absence of female fertility factors [43]. In Mr. J's case, his wife's age and low ovarian reserve may require the use of ART instead of varicocelectomy. However, the couple may be counseled that correcting the varicocele before ART, may improve semen quality and ultimately ART outcome.

4. Case D

1) Scenario

Mr. S is a 24 year-old gentleman complaining of primary infertility for 3 years. His past medical and surgical histories were unremarkable. His wife is 24 years old and has regular menses and normal gynecological investigation. The patient has normal build with a BMI of 24 kg/m^2. His genital examination showed nor-

mal testicular size and consistency, normal epididymes bilaterally, palpable vasa differentia and no varicoceles. Semen macroscopic assessment showed a volume of 2.5 mL, normal viscosity and pH of 8.0. Microscopic analysis revealed a sperm concentration of 28 million/mL, total motility of 55%, progressive motility of 34%, and normal morphology of 8%. A comparable result was obtained on repeating the semen analysis.

2) Interpretation

Mr. S has normal semen parameters. In the absence of female factors of infertility, the diagnosis of unexplained infertility is made which is prevalent in about 10% to 30% of couples seeking fertility [44]. Advanced tests of sperm function including sperm DNA fragmentation and oxidative stress testing is indicated in such cases. Recently, we coined the term Male Oxidative Stress Infertility (MOSI) after revealing elevated oxidative stress measures in about 30% to 40% of cases of unexplained infertility [2]. Furthermore, an elevated sperm DNA fragmentation level can also be detected in up to 30% of cases of unexplained infertility [45]. These patients may benefit from antioxidant supplementation with/without ART [46].

CONCLUSIONS

This review has highlighted the fundamental topics that were discussed in ACRM's first online ART Training course. The understanding of the principles and procedures for the collection and analysis of semen specimens plays a major role in clinical practice and in the management of infertile patients. In this context, it is essential to enforce strict QC in the andrology testing to ensure reproducible semen analysis results. Participation in proficiency tests for reproductive laboratories engaged in moderate or high complexity testing is required by all accrediting agencies. Finally, understanding the reference ranges for normal semen parameters and definitions of various categories of abnormal semen parameters is important in the clinical interpretation of laboratory results.

ACKNOWLEDGEMENTS

Authors are thankful to the artists from the Cleveland Clinic's Center for Medical Art & Photography for their help with the illustrations. This study was supported by the American Center for Reproductive Medicine.

Conflict of Interest

The authors have nothing to disclose.

Author Contribution

Conceptualization: AA, MKPS, RF, SG, Rakesh S. Project administration: AA. Supervision: AA, RF, MKPS, RH, SG, Rakesh S. Writing – original draft: all the authors. Writing – review & editing: all the authors.

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