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1 Introduction to Quality Management

There have been a number of excellent texts, chapters, and reviews covering quality management in reproductive laboratories [1–7]. In this chapter, the elements required to ensure that accurate testing results are obtained and communicated to the ordering clinician by the andrology laboratory will be examined, including some examples from the author's laboratory.

In the case of andrology tests, it has long been appreciated that there is considerable variation in replicate test results within a single specimen, between technicians in a given laboratory and between different laboratories [8–18]; however, reproducible results can be obtained with strict quality control and training of personnel [3, 4, 8, 19–21]. The goal of performing evaluation of semen and sperm is to provide accurate results in a form that can be used by a clinician for patient diagnosis and treatment. Quality management is a process that ensures a consistent, high-quality product. It includes quality control (QC), quality assurance (QA), and quality improvement (QI). QC involves procedures to ensure that everything involved in the testing process is functioning correctly. QA involves ongoing assessment of the entire analytical process in order to detect and remediate problems that are resulting in substandard quality. QI is a process of making improvements in the process [22]. The field of quality management comes from manufacturing, and there is an entire literature on various methods, sometimes using different terminology. This chapter will focus primarily on QC.

As seen in the previous chapter, even a routine semen analysis involves multiple tests. For each individual test

performed, quality management is required. Many countries have governmental regulations that specify the required elements of quality management for clinical laboratories, and these should be followed as closely as possible by andrology laboratories.

The most important tool in quality management is monitoring quality indicators. Regularly scheduled measurements are performed, ensuring that equipment is functioning properly, reagents and materials have the required functionality, personnel are performing well, test results are accurate, and clinical results are acceptable. Examples of quality indicators are shown in Table 2.1. Tracking quality indicators is a key element of quality management.

By tracking quality indicators, it is possible to detect problems in the testing process that could cause poor quality results or to detect poor results allowing for investigation of possible causes. Remedial action is taken when a quality indicator does not demonstrate acceptable results, as defined by the laboratory.

A set of schedules is helpful for ensuring that each quality management task is completed on a regular basis and that completion is documented. As written standard operating procedures (SOPs) are developed and reviewed, lists can be formulated for daily, weekly, monthly, quarterly, and annual tasks with columns for the initials of the person performing the task; and times, dates, and comments are applicable. Examples are shown in Table 2.2. One of the most common comments you will hear about quality management tasks is “if it isn't documented, it didn't happen.”

2 Written Protocols

Standardization of andrology laboratory procedures requires written procedures encompassing every aspect of routine laboratory testing and management. These are often called protocols or SOPs. Accurate, up-to-date SOPs ensure that all testing personnel will produce the same results during testing

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Table 2.1 Examples of quality indicators

Process measured	Quality indicator
Media storage	Refrigerator temperature monitoring
Semen dilution for sperm counts	Pipette calibration
Counting chamber accuracy	Microbead counting
Contact material acceptability	Toxicity testing
Aseptic conditions	Microbial monitoring
Inter-technician variation	Split sample testing
Inter-laboratory variation	Proficiency testing interval (PT)
Sanitation of discarded specimens	Bleach test strip testing of discard container
Semen analysis	Time interval between collection and analysis (seminal plasma is toxic to sperm)
Semen cryopreservation	Postthaw recovery of motile sperm
IUI preparation quality	% recovery of motile sperm, intrauterine insemination (IUI) pregnancy rates
Client communication	Number of semen analyses ordered by each client per month
Patient satisfaction	Comments received from patients

Table 2.2 Documentation of regular quality management tasks

Frequency	Examples
Daily	<ul style="list-style-type: none"> Sanitize workbench at the beginning and end of each day of testing Count QC beads in each counting chamber on each day of testing Record temperatures for all instruments
Weekly	<ul style="list-style-type: none"> Check liquid nitrogen levels in all Dewars Prepare aliquots of washing media and gradients Sanitize and stock semen collection rooms Test eyewash station
Monthly	<ul style="list-style-type: none"> Supply inventory Clean centrifuge rotors and cups Discard and replace morphology stains Check for expired reagents/supplies
Quarterly	<ul style="list-style-type: none"> Calibrate thermometers Clean refrigerators Defrost freezer if needed
Biannually	<ul style="list-style-type: none"> Clean biosafety cabinet filters Have pipettors cleaned and calibrated Have line voltage checked on outlets and current leakage on instruments
Annually	<ul style="list-style-type: none"> Review and update all SOPs Have microscopes serviced Calibrate centrifuges Certification of biosafety cabinet

of a patient sample. The SOPs should cover laboratory organization, definition of services, laboratory accreditation, personnel, facilities, equipment, each test performed by the laboratory, and quality management. SOPs contain step-by-step detail that allows a staff member to carry out the procedure and allows auditors to evaluate laboratory activities. Quality management SOPs must include each step in the remediation process should a measurement fall outside accepted limits. Notes can be included to address handling of unusual specimens or provide the rationale for a procedure. Figures that illustrate procedures are also helpful. An SOP may reference governmental and institutional regulations

along with standards and guidelines from evidence-based sources. Package inserts can be appended and referenced in the SOP. Report forms, consent forms, logs, and other materials relevant to the SOP may also be appended.

SOPs should be regarded as living documents and be revised regularly. Routine review by the laboratory director is required, along with periodic review by personnel of the SOPs covering their assigned tasks. The goal is minimal deviation from the SOPs during routine laboratory activities. The current SOP should be readily accessible to workstations, in hard copy or electronic form. Personnel should not work from copies of the SOPs as they may be outdated.

An SOP should include (as appropriate):

- An SOP number indicating where the protocol belongs in the procedure manual
- A descriptive title
- The date the SOP was entered into the procedure manual
- An area to document initial approval, reviews, and removal dates
- Introduction including the purpose of the SOP and compliance with any regulations, standards, and/or guidelines
- Specimen for testing (if applicable)
- Record keeping: accessioning, records, and reporting results
- Pretesting procedures (if applicable)
- Reagents required (if applicable)
- Materials and equipment (if applicable)
- Procedures in detail
- Posttesting procedures (if applicable)
- References
- Appendices

3 Facility and Maintenance

Proper design and maintenance of the facilities in which testing takes place can have profound effects on the safety of personnel and the reliability of test results. The laboratory must be monitored for a variety of functions; examples are given in Table 2.3.

Every surface in the laboratory must be cleaned and sanitized on a regular basis. The custodial service, lighting, ventilation, plumbing, and electrical system should be reviewed regularly and maintenance performed as required.

4 Equipment

Equipment in the laboratory used to perform patient testing and the QC activities supporting patient testing must undergo regular maintenance at least as stringent as that required by

the manufacturer. Copies of equipment manuals should be kept in paper or electronic form at a location accessible to laboratory personnel during hours of operation. The original copies of the manuals should be stored in a separate location. Instrument manuals must be retained for a time period determined by the laboratory after the use of the instrument is discontinued, allowing for quality management review.

For each instrument in the laboratory, SOPs should cover the material shown in Table 2.4.

5 Reagents and Supplies

Reagents used for laboratory testing or QC should be labeled, stored, and used according to the manufacturer's instructions and any applicable governmental regulations. The laboratory manual should include SOPs with a list of all reagents and supplies used in the laboratory. An example for reagents is shown in Table 2.5. The table can also include the amount kept on hand, safety precautions, and reference to SOPs for reagent preparation or use.

A log should be kept of laboratory reagents, including the:

- Person receiving or preparing the reagent (initials)
- Date the reagent was received or prepared by the laboratory
- Lot number
- Date reagent was approved for contact with sperm (if applicable)
- Date of last use

A similar log should be kept for supplies, particularly those that contact sperm during procedures, facilitating remedial action. For example, if the sperm motility of donors is lower after a certain date, the solution used to dilute sperm for motility analysis is one possible cause and that lot number can be further evaluated. Taking a regular inventory of laboratory reagents and supplies will ensure that there is adequate stock available under any circumstances that may arise during operations.

Table 2.3 Examples of facility monitoring

Measurement	Function
Air changes	Provide acceptable air quality and ventilation
Volatile organic compounds (VOC) in air	Minimize volatile compounds that are toxic to sperm
Oxygen level in air	Prevent nitrogen gas asphyxiation when liquid nitrogen Dewars are being filled
Microbial monitoring	Protect personnel from infection, minimize cross-contamination of patient specimens, reduce microbial contamination of specimens
Lighting fixtures	Ensure personnel are working with inadequate lighting, electrical safety
Electrical outlets	Fire safety, protect instruments from inappropriate current, fire safety
Ceiling tile condition	Indicate water leakage above ceiling
Cleanliness of all surfaces	Reduce contamination of reagents and specimens

Table 2.4 Scheduling and documentation of quality control processes

SOP section	Content	Example
1. Description and normal operation	Model, serial number, date added to operations, location of use, manner of use for patient testing or QC, conditions for routine use	<ol style="list-style-type: none"> Upright refrigerator in the laboratory annex: Fisher Isotemp general-purpose refrigerator/freezer holds supplies delivered to the laboratory until they are unpackaged and released for use Model 97-926-1. Serial Number 0204-036 purchased on 03/09/10 Specifications: temperature range from 2 to 13 °C (refrigerator); 18 to 10 °C (freezer); total capacity, 18 ft³ (cubic feet); refrigerator, 13 ft³; freezer, 5 ft³. Switchable manual or automatic defrost Yellow warning label reads: “Laboratory Refrigerator” Foamed polyurethane insulation Refrigerator: two white vinyl-coated–steel slide-out adjustable shelves, two tinted slide-out bottom drawers, four door shelves Freezer: one compartment and two door shelves Two adjustable, two fixed rollers on bottom for leveling Requires air clearance of 3 in. (7.6 cm) around top, back, and sides For 115 V, 60 Hz NEMA 5–15 plug requires NEMA-15 receptacle
2. Validation	Methods and schedule for determination of accurate performance	<ol style="list-style-type: none"> Each counting chamber is validated by performing counts of QC beads on each day of sperm concentration testing. Beads consist of two solutions of sperm-sized latex beads at known concentrations Levey–Jennings analysis (see Manual § 12, <i>Statistical Analysis</i>) is conducted for each concentration of beads. If a chamber fails to be in control, it shall be inspected for damage. Wear on the pins of a Makler chamber may cause it to become inaccurate through time and use. If a chamber is judged invalid, it shall be discarded and replaced with a new chamber
3. Calibration	Adjustment of a measuring instrument to conform with an accurate standard	<ol style="list-style-type: none"> All centrifuges are calibrated annually by clinical engineering using tachometry All thermometers calibrated against a NIST standard thermometer quarterly, and the standard is serviced annually
4. Maintenance requirements	Schedule and procedures for cleaning, filter replacement, etc.	<ol style="list-style-type: none"> The biosafety hood is inspected and certified annually by a certified technician The motor is self-lubricating and must not be greased The prefilters shall be cleaned monthly and replaced as needed The HEPA filters shall be tested annually and replaced as needed The air flow velocity and air class shall be checked annually All surfaces of the inside shall be wiped with 70 % ethanol before and after use
5. Immediate action values and backup plan	If it is determined that the instrument is not functioning properly, what will be done? Backup instrument or equipment	<ol style="list-style-type: none"> The freezer must be turned off and repaired if the chamber temperature exceeds 20 °C All reagents exposed to temperatures outside the storage range given on their labels shall be discarded The refrigerator freezer serves as emergency backup for the laboratory freezer

Table 2.5 Storage conditions of reagents in the laboratory being used for patient testing or QC

Reagent	Test used	Storage requirements
Accu-beads	Sperm count QC	Package specifies, “room temperature”
Antisperm antibody (ASA) control sera	ASA QC	Refrigerator at 2–8 °C
ASA immunobeads	ASA	Refrigerator package specifies, “store at 4 °C”
Diff-quick stain kit	Sperm morphology	Room temperature at 15–30 °C
Dulbecco’s phosphate-buffered saline DPBS	Sperm washing for morphology analysis	Refrigerate packets. Refrigerator at 2–8 °C Prepared medium may be stored at 15–28 °C
70 % ethanol	All testing-sanitized surfaces	Room temperature below 80 °C Flammable storage cabinet
Glycerol	Sperm cryopreservation	Room temperature
Ham’s F-10 with human serum albumin (HSA)	Sperm wash for IUI	Refrigerator at 2–8 °C
HSA solution	Sperm washing for IUI and sperm cryopreservation	Refrigerator at 2–8 °C
ISolate sperm separation medium	Sperm washing for IUI	Refrigerator at 2–8 °C
LeucoScreen kit	Count leukocytes in semen	Refrigerator at 2–8 °C
TEST-yolk diluent	Sperm cryopreservation	Freezer at <10 °C until open. Refrigerator after thawing
Tryptic soy agar (TSA) plates	All tests used for microbial monitoring	Refrigerator at 2–8 °C before use Incubator–oven at 35 °C ± 1 °C during bacterial culture Room temperature at 20–25 °C during fungal culture
Vital screen kit	Sperm viability	Room temperature at 4–25 °C

When reagents and supplies are received into the laboratory, they should be inspected and recorded in the reagent or supply log. If a reagent was shipped to the laboratory, the outer packaging should be inspected for damage that might affect the enclosed reagents. When the packaging is opened, the shipping temperature should be assessed to ensure it is compliant with the manufacturer’s stated storage conditions. An SOP including instructions for receiving reagents should specify what actions will be taken if the condition of received reagents is out of compliance with the laboratory’s standards. If a certificate of analysis accompanies the reagent, it should be dated and initialed by the person receiving the package and stored with other documents relating to the reagent.

The primary reagent container should be inspected for damage and appropriate labeling. The label should include the name of the reagent, the date of receipt or preparation, the expiration date, and a symbol to indicate any hazards. The person receiving the reagent should ensure that the safety data sheet (SDS) for the reagent is present in the laboratory. An SDS for each reagent must be immediately accessible to workers in the laboratory, stored in a binder or electronically.

Once received, the reagent should be stored immediately under the conditions specified by the manufacturer or, for reagents prepared in the laboratory, in accordance with the laboratory SOP. Reagents exposed to non-recommended conditions should be discarded. As part of the SOPs, any changes of conditions that are acceptable should be specified. For example, after thawing, cryoprotectant medium may be stored at 2–7 °C for a specified time. Or, after removal

from the refrigerator, a medium for washing sperm may require time to reach a required temperature before use, but should not be left at room temperature for days.

6 Toxicity Testing of Reagents and Labware

The majority of the quality challenges with supplies involve contact materials: media and labware that come into direct contact with sperm during testing or processing. These materials can have detrimental effects on sperm survival, behavior, and/or function and must be monitored for toxicity. Sperm survival and function are affected differently by different brands and lots of reagents and labware, necessitating evaluation of each lot.

Much of the media and some labware used by andrology laboratories are intended for use with human sperm in a fertility clinic setting and have been extensively tested by the manufacturer. A laboratory may choose to accept these testing results when following the manufacturer’s usage instructions. If this is the case, it should be specified in the SOPs and the testing results provided with each lot should be reviewed, documented, and stored for evaluation during remedial activities.

Plastic labware can release chemicals over time. For example, bisphenol A (BPA) and some phthalates in beverage containers have reached public consciousness due to their toxic effects on human health. As release of toxins can increase over time as plastics break down, it is important to dispose of labware that has passed its expiration date.

Table 2.6 Methods of toxicity testing for materials and reagents

Method	What is measured
Constituents by gas chromatography	Purity of chemical composition
Physical properties: freezing point depression, solubility	Purity of chemical composition, generally for a single chemical
pH, osmolality	Properties of a medium with multiple constituents
Microorganism growth	Ability to support microbial growth for test plates Sterility of contact materials
Toxins, heavy metals	Specific toxin contaminates
Limulus amoebocyte lysate (LAL) test	Bacterial endotoxin or lipopolysaccharide
Rabbit pyrogen test	Rise in temperature of injected rabbits (pyrogens: endotoxins and other bacterial byproducts)
Cell culture	Survival and function of cells in culture
Sperm survival	Retention of sperm survival over time
Embryo development	Mouse embryo development

Toxicity can also be present if chemical sterilization was used during manufacture.

There are a variety of ways labware can be evaluated for toxicity; some are shown in Table 2.6. Ideally, we could use fertility outcomes as a measure of contact toxicity to sperm, but this is not feasible.

Although it may seem like embryo development is a more stringent test than sperm survival, the embryo has completely different functional requirements than sperm. The embryo is also capable of repairing molecular damage, while the spermatozoon is an extremely specialized cell with minimal cytoplasm and transcriptionally silent DNA and, thus, little capacity for self-repair. Unless ICSI is to be used, the complex organelles and surface molecules of the sperm are required to perform an unusual array of cellular functions. Even sperm survival, or more specifically motility retention, cannot measure the complex molecular systems required for normal sperm function. Nevertheless, retention of sperm motility during culture remains the most feasible method of toxicity testing for media and labware.

Quite a few methods have been published for performing this testing. One method for testing labware is shown in Table 2.7. Note that when testing new lots of media or contact materials, a control reagent or labware must be included with demonstrated low sperm toxicity.

7 Microbial Contamination

Microbial contamination in the laboratory affects the safety of personnel and the quality of sperm that are cryopreserved or prepared for insemination. Some governmental regulations mandate process control procedures that ensure that tissues for transplantation are free from contamination with infectious organisms. Although the tests described in this section are for nonpathogenic microorganisms, routine monitoring helps ensure that microorganisms in general are not

being transferred from specimens to surfaces, media, and patient samples.

Semen is exposed to the room air in the collection room and in the laboratory during testing, processing, and packaging. Routine sanitation of these areas must be performed each day semen is tested or processed. The potential for airborne microbial contamination should be routinely assessed.

There are a variety of methods for monitoring microbial contamination in air. Settle plate testing is a simple method that measures the number of microorganisms that fall passively onto a culture plate. For passive settle plate testing, the sensitivity will depend on the surface area of the plate and the length of time it is left exposed. Vacuum-assisted systems are commercially available with pumps to increase the volume of air falling on the plate over time, which decreases the time required to sample a larger volume of air.

Microorganisms in air are generally associated with skin cells or other particulates. The average-sized particle will deposit (by gravity) onto surfaces at a rate of approximately 1 cm/s. The microbial growth on plates is counted as colony-forming units (CFUs). The microbial deposition rate is reported as the CFU in a given area per unit time. The duration of the work process is generally taken as the sampling time, and microbial testing is performed during normal operations. A positive control (e.g., a high-traffic area outside the laboratory) and a negative control (e.g., within a sterile hood) must be included. A sample protocol for settle plate testing is given in Table 2.8. It can be instructive to test the incubators, semen collection room, and other areas of the laboratory.

Commercial products are available for evaluation of media for sterility. These generally involve dipping a small plate in the medium, incubation for a set time, and counting of CFUs. Tryptic soy agar (TSA) plates can be used by sampling the media with a sterile swab and streaking it on the surface of the agar or pipetting a volume of

Table 2.7 A protocol for evaluation of contact materials for toxicity to sperm

Step	Procedure
1.	Thaw semen that has been set aside for quality control; use approximately one vial of semen per type of labware to be tested, including the control tube. Run each piece of labware in duplicate
2.	Pool the thawed semen and mix well, evaluate the motility, and record
3.	Perform a gradient separation procedure, using multiple gradients as needed
4.	Combine the pellets, dilute in a minimal volume of culture medium, determine the sperm concentration, and dilute the sperm to a concentration of approximately 10 million/mL with culture medium
5.	Evaluate the percentage progressive motility and record
6.	Estimate the final volume of the washed suspension, then pipette an equal volume onto each test surface and two control tubes
7.	Cover each suspension with mineral oil to protect from evaporation and oxidative stress; if the test surface is open-ended, such as a pipette or cryostraw, then “sandwich” the sperm aliquot between two layers of mineral oil, ensuring that the sperm suspension retains contact with the test surface
8.	Place in the incubator for a minimum of 4 h at 37 °C
9.	Remove the test surfaces from the incubator one at a time for evaluation
10.	For each test surface, evaluate the percentage progressive motility and record
11.	Repeat for the remaining samples
12.	For each contact material tested, repeat this procedure three times on three different days
13.	Use a nested analysis of variance (ANOVA) to detect differences in motility retention (initial motility–final motility) between the test surfaces. Any surface that has statistically lower motility than the control tube is considered toxic to sperm

Table 2.8 Sample procedure for settle plate testing in the andrology laboratory using a biosafety cabinet as the negative control

Step	Procedure
1.	Warm three tryptic soy agar (TSA) plates to room temperature for each sampling location and label with the location
2.	The negative control, biosafety hood sample, shall be collected with the hood operating normally, given adequate cycling time for the filters before use
3.	Place the plates in the sample areas and remove each lid, leave for the duration of the process being evaluated, then replace the lids
4.	Plates are incubated media side up, within a ziplock bag with a damp paper towel
5.	Incubate two plates for 48–72 h at 35 °C ± 1 °C for bacterial detection
6.	Incubate the remaining plates at room temperature for at least 5 days for mold detection
7.	When incubation is complete, count the colonies. Record growth greater than 100 colonies as TNTC (too numerous to count). Record results on a QC-Microbial Monitoring Form
8.	The negative control plate should not have any growth. <u>Negative control remedial action</u> : sanitize the biosafety hood and retest. As an additional control, the plates may be preincubated for 24 h at room temperature without removing the lids to ensure sterility prior to use
9.	The positive control should exhibit growth of both bacteria and mold. <u>Positive control remedial action</u> : if no growth occurs, the test is invalid. Check the expiration date of the plates. Retest the positive control
10.	If more than five colonies grow from any of the work counter plates, conduct complete sanitization of the work area, evaluate sources of contamination, and make corrections to reduce future contamination. Repeat test to demonstrate improved control of contamination

medium on the agar and spreading over the surface with a sterile spreader. Standard incubation is then used for bacteria and mold.

Touch samples can be performed to assess the contamination of surfaces. RODAC (Replicate Organism Detection and Counting) plates have the agar in a convex bump on the surface that can be pressed directly onto the surface to be evaluated. Another method is to touch the test area firmly while wearing a sterile glove. The gloved fingers are then touched to the agar surface of a TSA plate for incubation and counting of CFUs. Alternatively, a drop of sterile water can be applied to the surface, then streaked or spread on the agar surface with a sterile swab.

It is well known that liquid nitrogen in Dewars can become contaminated resulting in contamination of cryovial contents and transmission of disease in domestic species [23–28]. The liquid nitrogen in Dewars can be evaluated for microbial contamination and the Dewars emptied and sanitized as remedial action. A sample procedure for evaluating microbial contamination in liquid nitrogen is shown in Table 2.9.

Process controls are also important for microbial monitoring. During the process of performing a routine sperm preparation procedure (IUI preparation or sperm cryopreservation), an aliquot of sterile medium is substituted for the semen and subjected to the entire process of preparation. At the end, the prepared process sample (e.g., IUI sample

Table 2.9 Sample procedure for testing the liquid nitrogen in a Dewar for microbial contamination

Step	Procedure
1.	Working in the sterile hood, label one 50 ml conical tube and two room temperature tryptic soy agar (TSA) plates for each Dewar to be tested and two plates each for the negative and positive controls
2.	Open the Dewar and use the measuring stick to stir up any sediment from the bottom of the tank
3.	Remove the tube lid. Using long forceps, lower the tube into the liquid nitrogen until the tube contains approximately 50 mL of liquid nitrogen
4.	Replace the lid loosely on the tube and transfer it rapidly to a tube rack in the sterile hood
5.	Allow the nitrogen to evaporate completely
6.	Working within the sterile hood, remove the tube lid and add 500 μ L of sterile water, replace the lid, and close firmly
7.	Use a vortex mixer and inversion of the tube to allow the water to rinse the entire inside of the tube
8.	Remove the tube lid and dip a sterile swab tip into the water until saturated
9.	Remove the TSA plate lid and swipe the surface of the agar with the cotton swab without disturbing the surface of the agar. Alternatively, pipette the 0.5 mL of liquid onto the agar surface and spread in a thin film across the entire surface of the agar with a sterile cell spreader. Replace the plate lid
10.	Repeat for each Dewar
11.	As a negative control, use 1 mL sterile water to rinse and sample a sterile tube. As a positive control, use contaminated water to rinse and sample a sterile tube
12.	Plates are incubated media side up, within a ziplock bag with a damp paper towel
13.	Incubate two plates for 48–72 h at 35 $^{\circ}$ C \pm 1 $^{\circ}$ C
14.	Incubate the remaining plates agar side up at room temperature (20–25 $^{\circ}$ C) for at least 5 days for mold detection
15.	When incubation is complete, count the colonies. Complete the QC-Microbial Monitoring Form
16.	If either of the following occurs, the test has failed and must be repeated: (a) the sterile water grows more than five colonies (negative control failure) or (b) the contaminated water shows no growth (positive control failure)
17.	If more than five colonies grow from any of the Dewars, decontaminate the Dewar, evaluate all sources of contamination, and make corrections to reduce future contamination. Repeat test to demonstrate decontamination

packaged in a syringe, postthaw sample) is tested for microbial contamination. This evaluates the contact materials, media, and the work environment as well as the aseptic technique of the person performing the sperm preparation.

8 Establishment and Verification of Test Methods

New test methodology, quality management procedures, types of reagents/supplies, and control materials should be introduced only after careful review and documentation. New testing methods may need to follow governmental standards, and methods used for testing or preparation of patient specimens should be well established in the medical community, with published verification of suitability for use in clinical testing or specimen preparation. The laboratory director (often in conjunction with the QA committee described below) is responsible for investigating new methods and determining their suitability for incorporation into laboratory testing. An excellent description of preparing a user requirement specification for a new methodology under consideration is given by Mortimer and Mortimer [4].

A new test method should not be used to test patient specimens until it has been validated by the laboratory. This

includes determination of the test specifications (where applicable) shown in Table 2.10. The test should be validated in the laboratory facility using the personnel, reagents, and materials that will be used once the new method is adopted.

New procedures must be performed side by side with the current method, using split donor specimens, to ensure comparable performance specifications. Process controls as described above should be replicated to ensure no increase in microbial contamination. New lots of control materials should be tested side by side on individual semen specimens before incorporation into routine use.

The variation within testing can be evaluated routinely by recording the replicate values of each test. For example, if the sperm counts from each replicate chamber are recorded, the precision of sperm counts can be determined. Table 2.11 shows an example of monitoring the precision of sperm concentration evaluation by looking at the standard deviation (SD) and the coefficient of variation ($CV = SD/\text{mean}$).

9 Personnel Competency

The requirements for laboratory staffing, as well as personnel hiring criteria, training, and assessment, are critical components of quality management. These topics are covered in the next chapter.

Table 2.10 Test specifications that should be determined for each new test

Performance specification	Explanation	Andrology example
Test accuracy	The test gives the same results as are obtained using a gold standard method	If Makler chambers or disposable chambers are to be introduced for determination of sperm concentration, they should produce the same results as hemocytometry as described in the current WHO manual
Reportable range	The reportable range is a range of values for which the test produces accurate results	The range in sperm concentration over which the test method produces accurate results. For example, if the reportable range for sperm concentration is 2–45 million/mL, then the results for a sample determined to have a concentration of 1.2 million/mL should be reported as “<2 million/mL.” Samples more concentrated than 45 million/mL should be diluted before counting
Linearity	The linearity means that there is a linear relationship between the gold standard method results and the results using the new method over the reportable range of the test	There should be a linear relationship between the actual sperm concentration (produced by serial dilution of a sample of known concentration) and the concentration determined by the new method, e.g., CASA. If linearity is not achieved, the reportable range must be adjusted to include only the linear portion of the range
Reference range	The normal range of values for persons who do not have the disease	For andrology tests, this means the normal range of values for fertile men. Ideally, this should be determined by each laboratory using the facilities, reagents, materials, and personnel in use by the laboratory
Analytical sensitivity	The proportion of patients with the disease for whom the test will give abnormal results	These are determined by receiver operating characteristics (ROC) analysis, preferably using laboratory-specific data for known fertile and infertile men. In three studies looking at prediction of natural fertility [29–31], sperm morphology had a specificity of about 80 % and sensitivity of about 70 %, with an area under the curve (AUC) of about 80 %, giving it “fair” to “good” accuracy as a diagnostic test. Several studies have produced analogous data for DNA fragmentation assays [32–35], finding an AUC of 80–90 %, making these tests “good” to “excellent” diagnostic tests of whether a man will be infertile
Analytical specificity	The proportion of patients without the disease for whom the test will give normal results	

10 Maintaining the Integrity of Patient Specimens

It is crucial that steps be taken to maintain the integrity of sperm samples destined for insemination. There have been quite a few, well-publicized cases in which patients received the wrong sperm due to a laboratory’s failure to prevent either contamination of the husband’s specimen with other sperm or insemination with the wrong specimen. There is the additional concern that patient semen can become contaminated with pathogens originating from other specimens.

To prevent such contamination, strict procedures must be followed to ensure the integrity of sperm samples intended for insemination. This includes specimens prepared for IVF, IUI, and specimens cryopreserved for sperm banking.

The basic principles employed to ensure sample integrity are:

- Separation in space: strict physical separation of different sperm specimens with restriction of each specimen to a discrete specimen preparation area.
- Separation in time: the time of semen collection should ensure that only one semen specimen at a time is handled in a designated work area.

- Use of sterile, disposable, single-use contact materials.
- Unique labeling of all containers and contact materials used for each specimen.
- Sanitation of each preparation area before and after use.

Before bringing a specimen into the work area, lay out and label all of the materials to be used for that specimen. All contact materials should be labeled with the patient’s name and a secondary identifier unless it is a disposable labware that will not leave the hand of the worker from the time it contacts the semen until it is discarded into a bio-hazardous waste container. Once the specimen, in its sealed container, is placed in its preparation area, no other specimens or contact materials may enter the designated area unless they are sterile material still in their packaging or sealed containers labeled with the patient’s name. When a specimen is removed from its designated preparation area for centrifugation or incubation, each aliquot must be contained in a labeled, sealed container. Semen specimens entering the laboratory for evaluation that are not destined for insemination are handled in the same manner, except that unlabeled contact materials (e.g. slides) may be brought into the area.

Table 2.11 Precision of sperm motility and concentration of whole semen determined from replicate counts of each specimen

Month	Number of specimens	Motility		Count		Year	Number	Motility		Count	
		Mean	Mean	Mean	Mean			Mean	Mean	Mean	Mean
		CV (%)	Diff. ^a (%)	CV (%)	Diff. ^a			CV (%)	Diff. ^a	CV (%)	Diff. ^a
Jan-12	20	12	10	8	6						
Feb-12	23	18	13	7	5						
Mar-12	21	9	7	11	6						
Apr-12	27	16	14	10	10						
May-12	29	17	14	10	7						
Jun-12	18	14	11	8	6						
Jul-12	27	17	14	11	7						
Aug-12	29	19	16	18	13						
Sep-12	21	25	18	17	11						
Oct-12	23	26	19	16	9						
Nov-12	25	23	17	18	10						
Dec-12	27	19	14	15	13	2012	290	18	10	13	9
Jan-13	35	19	15	17	11						
Feb-13	33	22	17	15	14						
Mar-13	35	20	16	14	12						
Apr-13	23	16	13	16	8						
May-13	29	21	16	16	8						
Jun-13	23	26	20	15	8						
Jul-13	33	22	17	19	13						
Aug-13	34	19	15	11	10						
Sep-13	30	18	13	18	10						
Oct-13	28	23	17	16	10						
Nov-13	29	24	17	13	11						
Dec-13	27	25	18	19	15	2013	358	21	11	16	11
Jan-14	78	24	17	18	13						
Feb-14	28	25	18	13	9						
Mar-14	54	27	19	16	9						
Apr-14	35	23	16	15	9						
May-14	42	26	19	14	8						
Jun-14	34	24	18	20	12						
Jul-14	42	19	15	18	12						
Aug-14	41	18	14	13	8						
Sep-14	33	20	16	15	8						
Oct-14	35	21	15	21	12						
Nov-14	44	29	21	12	6						
Dec-14	27	28	20	20	8	2014	493	24	11	16	10

^aMean differences (Diff.) use the absolute value of the difference for each determination

11 Quality Control Procedures for Andrology Tests

Quality control of tests require standardized control materials, and testing of a control material must yield consistent results over time. While this is straightforward for most clinical tests, such as endocrine fertility assays, it is more difficult to identify and obtain appropriate control materials for andrology.

As for any clinical test, testing of the controls must be performed for andrology tests with acceptable results on each day of testing before testing of patient specimens. SOPs

for testing QC should detail daily procedures and the remedial actions to be taken if unacceptable results are obtained. Remediation should be completed before testing resumes and patient results are reported.

Results of a clinical test must be both accurate and precise. Accuracy means that the value measured and recorded by the testing personnel is the correct value compared with what would be obtained using a gold standard method. For example, for determination of sperm concentration, hemocytometry, as described in the WHO laboratory manual for the examination and processing of human semen [36], is widely accepted as the gold standard. Some disposable chambers differ significantly from hemocytometry, and this must be

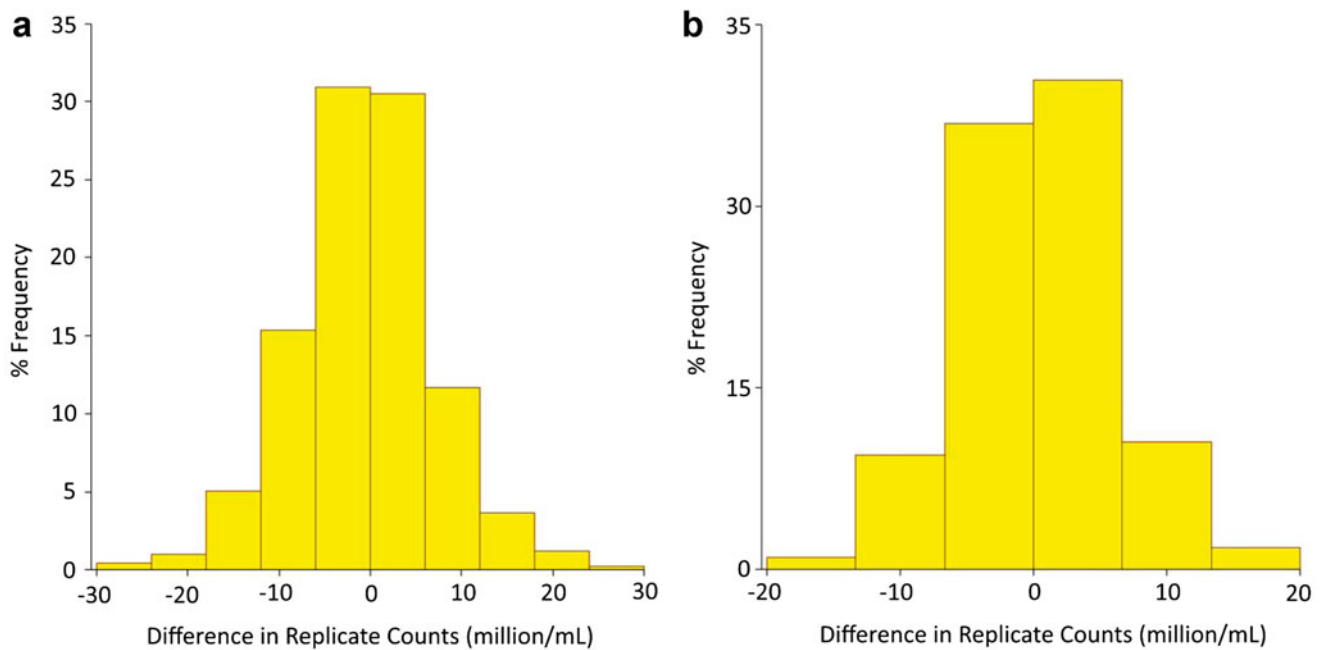


Fig. 2.1 Histograms showing the differences in bead counts between the upper and lower rows of a single Makler chamber. These counts were made over 495 days of testing. (a) High bead concentrations. (b) Low bead concentrations

taken into account when calculating and reporting results [37]. Swan et al. [38] found that MicroCell chambers produced sperm concentration values that were significantly lower than those for hemocytometry, and some microbead control products give separate determined values for the hemocytometer and MicroCell chambers. Precision is a measure of the ability of a test to give the same result repeatedly for the same sample. For example, precision evaluation of Makler cell chambers can be seen in Table 2.11. The standard deviations and coefficients of variations of replicate counts of individual patient specimens are measures of the precision of sperm count determination. MicroCell chambers are more precise than hemocytometers [38], while Makler chambers require very strict QC to achieve accurate and precise results. If loaded correctly, with the cover glass seated firmly on the silicone posts, the Makler chamber produces slightly lower concentration values than hemocytometry, although the precision is comparable if daily QC is performed.

There are two types of error you are looking for when performing longitudinal (i.e., daily) tests on control materials: random error and systematic error.

Random error is least concerning because it occurs by random chance and is not related to equipment failure or to mistakes made by personnel. For example, if the same person counts the top row and bottom row of a Makler chamber filled with sperm-sized latex beads, you generally will not get the same answer for the two rows due to random arrangement of the beads in the chamber. Data showing this are pre-

sented in Fig. 2.1. The difference between the upper and lower rows for 495 days of testing counts was 0.11 ± 7.6 (mean \pm standard deviation) for the high bead concentrations and 0.76 ± 5.8 for the low bead concentrations. These histograms show the normal distributions typical of random error.

Systematic error is what we are really looking for when making daily measurements on the same control material. Examples of systematic error are:

1. One technician consistently obtains a higher value than another technician when determining sperm motility daily from a video recording of sperm.
2. One technician often obtains a higher value than the other technician when determining sperm concentration using hemocytometry.
3. One counting chamber consistently obtains a higher sperm count than another chamber on daily determinations of sperm concentration using a suspension of fixed sperm.
4. The daily percentage of normal sperm determined from all morphology control slides gradually declines.

Systematic error can suggest the type of remedial actions to be taken. In the first two cases, evaluation of technician performance is indicated. The second case could involve inaccurate dilution of the semen for hemocytometry or inaccurate counting. In the third case, the Makler chambers should be evaluated against a hemocytometer. In the fourth case, which has been reported in a number of laboratories,

there may be a drift in how morphology is counted by the laboratory's technicians [39]. The SOP for each control procedure should include a list of the steps to be taken in the event of each anticipated detection of systematic error.

11.1 Levey–Jennings Charts

When using control materials for any clinical test, there must be a mechanism to evaluate the results and determine if the testing process is in control and testing may proceed. A common method for achieving this employs a Levey–Jennings (L-J) graph for each control [40]. This is often done automatically by the instrument when endocrine tests are run, and a judgment may even be made by the instrument, notifying the operator if remedial action is required before running patient samples. This judgment is made by applying rules to the results [41, 42]. For example, it is common to use:

A Process Is Out of Control If

- $1_{3\sigma}$: One control value exceeds the expected value (EV) ± 3 SD.
- $2_{2\sigma}$: Two consecutive control values exceed the EV + 2 SD limit or the EV - 2 SD limit.
- $R_{4\sigma}$: One control observation in a run exceeds the expected value + 2 SD, and a consecutive value exceeds the EV - 2 SD limit.
- $4_{1\sigma}$: Four consecutive control values exceed the same EV + 1 SD or the EV - 1 SD limit.
- 10_x : Ten consecutive control values fall on one side of the mean.

For andrology tests, the control charts can be prepared by hand or using statistical software. Figure 2.2 shows L-J charts for counting standardized control preparations of sperm-sized latex beads over a 100-day test period for a single Makler chamber. A high- and low-concentration control was counted each day of testing. In this case, the mean for the 100 days is taken as the expected value, shown by the horizontal black line. The red points indicate that a rule has been violated and the Makler chamber has failed QC, requiring remedial action. The first red point in Fig. 2.2b for the low control shows that the value is more than 3 SD above the expected value ($1_{3\sigma}$). The sixth red point in Fig. 2.2a for the daily high control shows that 4 days in a row, the values were more than 1 SD above the expected value ($4_{1\sigma}$). The rest of the red points on each plot show days when more than ten

sequential values are either above the expected value or below the expected value (10_x). On these L-J charts, an abrupt shift in values occurred on the 52nd day of testing, when a new lot of control beads was started. Figure 2.3 shows the L-J charts separated out by bead lot. Notice that the results are more precise using the expected value published by the manufacturer: for the high bead control, the CV has decreased from 19 to 13 % and 11 % for the two bead lots, respectively. For the low bead control, the CV decreased from 19 to 15 % and 12 %, respectively. The red point in Fig. 2.3c shows a day when two rules were violated: the value is more than 3 SD above the expected value ($1_{3\sigma}$), and on 2 successive days of testing, the value was more than 2 SD below the mean on the first day and then more than 2 SD above the mean on the second day ($R_{4\sigma}$).

Before changing the lot of control materials, for at least 10 days, the old and new lot should be run side by side. If the new lot differs in expected value, the ten values can be used as the beginning of a new L-J chart for that lot, using the new expected value.

A trend in values (decreasing or increasing) suggests different QC problems during remedial action than the QC shift seen in Fig. 2.2 [43]. A trend could indicate gradual deterioration of the control materials or deterioration in the counting chamber.

11.2 Semen Volume

Although many andrology laboratories use serological pipettes to measure semen volume, the WHO manual [37] recommends measuring semen volume by weight. Specimen cups are pre-weighed before collection, then reweighed containing the specimen. This is the gold standard for accurate determination of semen volume, and other methods should be routinely compared with specimen weight.

11.3 Sperm Concentration

One of the first aspects of both sperm concentration and sperm motility determination that must be appreciated is that semen is not a homogeneous material. Even in a well-mixed specimen, the actual sperm concentration and motility are different in different parts of the sample. This is particularly true when hyperviscosity is involved. Indeed part of the variation seen between counts in different squares in a counting chamber, or between counts from replicate counting chambers, results from true biological variation in different regions of a sample [5]. Mixing the specimen well before sampling is the best method for reducing this variation; however, it is likely to persist, even after extensive mixing. Often

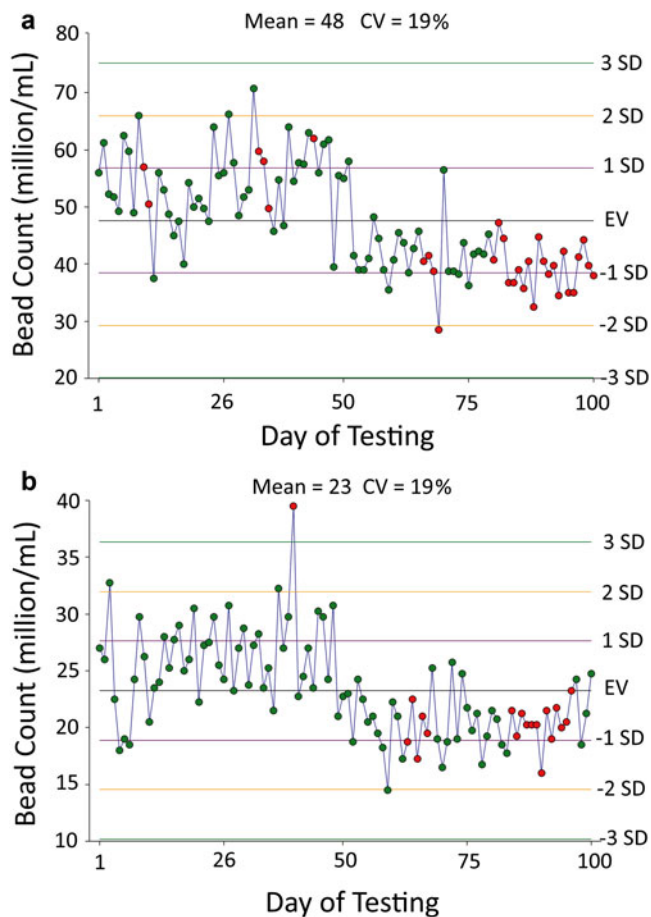


Fig. 2.2 Levey–Jennings charts showing daily counts of sperm-sized latex bead at two concentrations in a single Makler chamber. (a) High bead concentration and (b) low bead concentration. The expected value (EV), taken as the mean value for the 100 days, is shown as a horizontal black line. The purple lines show one standard deviation (SD) from the EV, the orange lines indicate 2 SD from the EV, and the green lines indicate 3 SD from the EV. Red points indicate days on which the Makler chamber failed QC because one of the specified rules was violated. A change in bead lot occurred on day 51 of testing

areas with different sperm concentration can be visualized by scanning a chamber, even after extensive mixing of the semen before sampling.

A variety of control materials are available for the evaluation of sperm concentration testing. Ideally, this involves counting of actual fixed sperm in the counting chambers to be used for patient specimens. Although counting known concentrations of sperm-sized beads is a good control for the counting chambers and is amenable to daily counts, it is less rigorous than sperm suspensions for QC of sperm concentration determination because latex beads do not resemble spermatozoa.

Although sperm-sized beads used as controls for sperm counts or for the counting chambers do not have the biological variation seen in semen, there is still considerable variation between replicated counts as seen in the standard

deviations and coefficients of variation ($CV = SD/\text{mean}$) shown in Table 2.12. This can be due to random error or can indicate that the counting chambers differ.

11.4 Sperm Motility

Although in the past, products were available involving lots of frozen sperm that could be used for motility QC, in most studies, the variation in the motility of thawed aliquots of a single frozen specimen has proven excessive for routine QC [3, 9, 21, 44, 45], although Cooper et al. [46] achieved reasonable results. Today, most laboratories use routine motility determination from videomicroscopy of sperm. Videos can be evaluated by testing personnel on each day of testing and L-J charts used to determine if motility evaluation is in control. For those participating in proficiency testing programs using video for assessment of sperm motility, videos can be retained for daily QC. If computer-assisted sperm analysis (CASA) is used by the laboratory to evaluate sperm motility in patient specimens, then videotapes should be used as a routine control of the CASA determinations.

11.5 Sperm Morphology

Sperm morphology evaluation has undergone an enormous decrease in the percentage of normal forms counted from a specimen. This decrease, which has not been uniform between laboratories, has led to serious problems in accuracy of morphology results. This is a case for which inter-laboratory testing (i.e., proficiency testing described below) is critical.

Sperm morphology testing requires at least two control procedures: one to ensure that the staining of the slide is acceptable and one to ensure accurate determination of the percentage of normal forms from a well-stained slide. Multiple lots of staining controls can be prepared from donor semen. For example, 100 slides can be prepared using the procedure in routine use, and these smears are stored in the refrigerator to be run as daily stain controls. For morphology determination, stained slides or micrographs can be purchased (e.g., the plates following Chapter 2 in the WHO manual) [36] or retained after proficiency testing.

11.6 White Blood Cell Concentration

The methods used for sperm concentration determination can be adapted to act as controls for the determination of round cells in semen. Round cells, which are any spherical cell larger than a sperm head, are counted under light microscopy before staining. Determination of the white blood cell

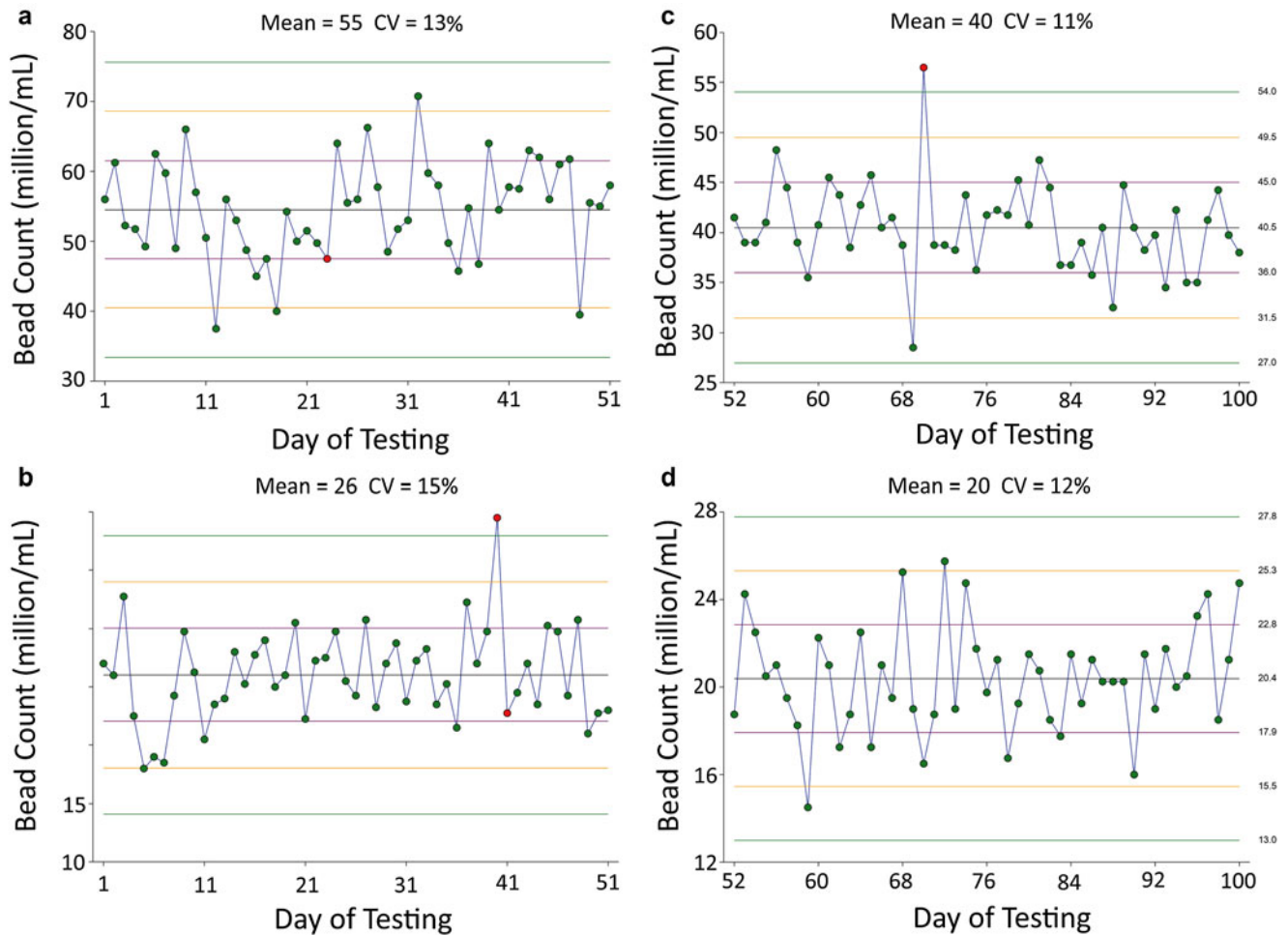


Fig. 2.3 The Levey–Jennings charts of latex beads shown in Fig. 2.2 with the two bead lots separated. (a) High bead concentration, lot 1; (b) low bead concentration, lot 1; (c) high bead concentration, lot 2; and (d) low bead concentration, lot 2. The expected value (EV) is shown as a horizontal black line. The purple lines show one standard deviation

(SD) from the EV, the orange lines indicate 2 SD from the EV, and the green lines indicate 3 SD from the EV. Red points indicate days on which the Makler chamber failed QC because one of the specified rules was violated. Note that the Makler chamber is in control on most days of testing

count must be performed if the round cell concentration exceeds the value set by the laboratory for excessive leukocyte concentration.

If a peroxidase method is used for leukocyte determination (e.g. Endz’s test), a peroxidase positive control and water or medium negative control should be run on each day of testing. The proportion of positively staining cells is multiplied by the concentration of round cells to determine the concentration of leukocytes. It should be noted that this method only detects peroxidase positive cells, such as polymorphonuclear (PMN) leukocytes. As PMNs are the predominant leukocytes in semen, this method is acceptable in most cases. Fluorescent staining followed by fluorescence microscopy or flow cytometry can be used to differentiate the leukocytes in semen.

White blood cells can also be detected from the stained slide used for sperm morphology counts. A differential count of leukocytes can be conducted along enumeration of sperm cells, and the proportion of leukocytes of various types per

sperm, along with the sperm concentration, can be used to calculate leukocyte concentration.

There is no acceptable control material available that is appropriate for daily QC of round cell counts or leukocyte staining. Ideally, aliquots of frozen semen with known round cell and leukocyte concentration could be used as routine controls. If flow cytometry is used, bead preparations are commercially available as control material to evaluate each fluorescent stain used.

11.7 Antisperm Antibodies

Preparation of control materials for antisperm antibody (ASA) testing is performed by the laboratory on the day of testing. Sperm that are free of ASA are treated with serum containing ASA for the positive control and not containing ASA for the negative control. After washing to remove

Table 2.12 Counts of a high and low concentration of sperm-sized latex beads for each counting chamber on each day of testing showing the variation in the replicate counts

Bead lot	Chamber	Mean	Mean daily SD	Mean daily CV (%)	Number of counts
Bead lot 1		High control			
	4	43.1	4.7	11	38
	5	43.6	6.0	14	38
	6	43.1	5.3	12	267
	7	43.3	5.4	12	267
		Low control			
	4	24.3	2.9	12	38
	5	23.9	3.8	16	38
	6	22.7	3.3	15	267
	7	22.8	3.4	15	267
Bead lot 2		High control			
	6	43.0	4.9	11	81
	7	43.3	4.3	10	158
	8	43.3	4.9	11	158
		Low control			
	6	20.4	2.7	13	81
	7	21.2	3.3	16	158
	8	21.6	3.1	14	158
Bead lot 3		High control			
	7	36.9	5.0	13	228
	8	37.6	4.5	12	287
	9	37.1	3.9	11	110
		Low control			
	7	18.6	3.2	17	228
	8	18.4	3.4	19	287
	9	18.0	3.3	18	110

unbound antibodies, these specimens are run along with the patient sample. Control failure is detected if the positive control sperm fails to bind immunobeads or the negative control sperm does bind immunobeads. A semen sample known to be free of ASA must be used for preparing controls (or the negative control will fail). Discarded or donated semen can be cryopreserved for this purpose.

12 Proficiency Testing

Intra-laboratory variation (e.g., difference between technicians) should be routinely determined by side-by-side testing as discussed in the next chapter. This must be documented and can be used to detect when retraining is required. Interlaboratory evaluation or proficiency testing is mandated by some governments, including the Clinical Laboratory Improvement Act (CLIA) in the USA. A central laboratory prepares testing material and sends replicates to the member laboratories for evaluation. The proficiency testing (PT) specimens are analyzed in exactly the same manner as patient specimens. PT of semen analysis is available for sperm count, sperm motility, sperm viability, sperm morphology, and antisperm antibodies. Alternatively, a group of laboratories can set up a PT program.

13 Data Management

13.1 Clerical Errors

The laboratory must have in place a mechanism for detection and documentation of clerical errors. One method is to conduct a monthly audit of a given percentage of specimens tested or preparations performed by the laboratory. The reported results should be compared with the information provided by the patient and values recorded in the laboratory during testing. Calculations should be checked. It is a good practice to review other documentations accompanying the test or procedure, such as consent forms, logs, and screening materials. Remedial action should be taken when errors are detected.

13.2 Computer Data Security

The laboratory must, to the best of its ability, protect the confidentiality of patient medical records. The processes in place to protect patient health information should be reviewed regularly by the laboratory director as this is an area of rapid transformation.

14 Quality Assurance and Quality Improvement

QA and QI activities involve looking at the entire process, which includes QC, but also includes tracking endpoints of testing and procedures. Not only are results reviewed, but the laboratory director is responsible for ensuring that methodology is current and evidence based. For example, sperm preparation for IUI involves reviewing quality indicators that look at the pretesting, testing, and posttesting components of IUI preparation. Pretesting indicators could include the number of times specimens are rejected by the laboratory due to patient failure to label his specimen container or failure failure to collect in a sterile container. Testing indicators would include patterns of QC failure and instrument performance. Posttesting indicators include assessment of personnel, investigation of patient complaints, and the pregnancy rate for the IUI program. Each laboratory or the fertility program should have a QA committee that reviews the available information, researches solutions, and suggests actions to be taken to maintain and improve the quality of each test and sperm processing procedure in the laboratory.

Quality management in fertility clinics has lagged behind these procedures for other pathology testing, in part due to the difficulty in developing appropriate quality measures for the tests performed on reproductive tract cells and tissues. The andrology laboratory is no exception, and the lack of QC is partly responsible for the current impression that semen analysis is a poor measure of male fertility. Although significant resources are required for initiation of QC procedures, the cost and time required decrease as the work becomes a routine. Scrupulous attention to ensuring a quality product pays off by protecting the andrology laboratory from the burden of liability that may arise when quality guidelines are ignored and reduce the expense of recovering from catastrophic failures. In this chapter I have reviewed QC procedures required to provide a facility, equipment, supplies, and reagents that will support accurate and reliable test results and production of high-quality sperm preparations. Together with QC procedures for each semen test and procedure, the process of quality management will build confidence in the laboratory's results among staff, patients, and referring physicians.

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