

Equipment for your andrology laboratory

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Introduction

Andrology is the field of medicine that deals with physiology and disease of the male reproductive system. For any practitioner offering theriogenology services to clients, this potentially represents half of your caseload! An andrology laboratory that is adequately equipped and well maintained can improve productivity and quality of the services provided. Sufficient equipment must be available for the number of staff working in the laboratory and for the laboratory to function efficiently at maximum caseload; this will be dependent on the individual practices' volume. Consideration should also be given to having backup equipment where appropriate to ensure that the andrology laboratory can continue to function in the event of equipment failure or repairs. Practitioners should also be aware of the need for quality control measures and equipment maintenance to ensure that results obtained are accurate, as it has been demonstrated in other species that adherence to strict quality control guidelines are necessary for consistent results.¹⁻³

Basic laboratory equipment

Basic laboratory equipment is necessary for macroscopic parameter assessment, including color, volume, and pH assessment. Supplies and consumables that may be of use include semen collection cones/receptacle, microcentrifuge tubes, glass microscope slides and coverslips, micropipettes with different ranges and corresponding tips, stage or slide warmer, beakers, graduated cylinders, 15mL conical tubes with caps, disposable 3mL graduated pipettes, an analytical balance, centrifuge, and water bath. Semen handling supplies can be stored in a laboratory incubator set to 37°C. If frozen semen is to be stored on premises, a liquid nitrogen dewar will be needed for storage.

Stains for semen analysis

Buffered formol saline can be helpful for preparation and storage of semen samples for phase-contrast or differential interference contrast microscopy. Approximately 1mL of buffered formol saline can be added to a microcentrifuge tube, with a few drops of raw ejaculate added until the solution appears cloudy. These samples can be labelled and stored in the refrigerator after morphological assessment for future review if required. Alternately, eosin-nigrosin (Hancock's stain) can be used for routine morphology as well as assessment of live vs. dead sperm. Dead sperm cells, with a damaged plasma membrane, are colored by eosin; living cells do not absorb the stain. The background is stained dark from nigrosin. Other stain options, such as Romanowski stains (Diff-Quick®) or Spermac may also be helpful depending on the results of clinical examination or morphologic assessment. Foreign cells (erythrocytes, leukocytes, round cells) are readily identified on air dried smears after staining with Romanowski stains, and Spermac allows clear visualization of the head, acrosome, equatorial region, centrepiece, and tail of the sperm.

Microscopy

The microscope is one of the most used pieces of equipment in the andrology laboratory, and both bright field and contrast microscopy have their advantages in the laboratory for different applications. A bright field microscope will be ideal for examining stained preparations, such as for morphology, and can be used for evaluating motility if the field diaphragm is closed to enhance contrast and improve sperm visualization.

Phase microscopy is an important technology that allows one to view live organisms and internal structures without staining. A microscope with good quality phase contrast optics is extremely helpful in the andrology laboratory. Ideal specifications of a microscope configured for andrology include 10x, 20x, 40x objectives (all positive low phase contrast), 100x oil immersion objective, phase telescope for proper alignment of phase rings, wide-field eyepieces (12.5x preferred), an extra eyepiece of built-in grid reticle,

and heated stage.⁴ Systems with video capability will also need intermediate magnification capability and camera oculars/camera adapters (C-mount for video camera).

Differential interference contrast (DIC) produces an image with more uniform greys, as opposed to the flatter image with darker cellular features seen with phase contrast.⁵ With DIC, Wallaston prisms are utilized that result in very sharp, 3-D like images, making it very useful for studying sperm. In bulls, morphologic assessment with eosin-nigrosin stained slides using bright field microscopy was deemed adequate when compared to formal saline wet mounts examined with DIC, although DIC was preferred as it was more effective in visualizing major defects.⁶

Tools for determining sperm concentration

Sperm concentration can be determined by manual or automated methods. The hemocytometer is considered the gold standard for determining concentration of canine sperm, as well as other species.^{7,8} It consists of a specialized microscope slide with a grid system; sperm are counted using one of several methods within the grid.⁹ With this counting method, semen samples must be diluted prior to use to allow identification of individual cells within the grid. Once the hemocytometer is loaded and allowed to equilibrate, sperm on both sides of the counting grid should be assessed to ensure that both sides are within 10% of each other. If greater than 10% discrepancy between the two sides is noted, the hemocytometer should be reloaded and the counting process repeated.

Automated devices include the NucleoCounter®, photometric devices (ie Spermacue® or Densimeter®), and computer-assisted sperm analysis (CASA) systems. The NucleoCounter® has been assessed in canine sperm and provides assessment of not only sperm concentration, but also viability with reasonable repeatability.¹⁰ This device provides automated cell counting with a built-in fluorescence microscope using propidium iodide, and provides a hardware-based image analysis. Photometric devices, on the other hand, determine concentration of sperm by determining the percentage of light transmission. Poor sample handling (ie, fingerprints on cuvettes) or non-sperm material such as other cell types or debris will result in inaccurate results as these artefacts will also reduce light transmission through the sample. Semen samples with concentrations at either extreme (highly concentrated or dilute) will yield significantly less accurate results.

Computer-assisted sperm analysis (CASA)

Manual semen analysis does not provide any information on the kinematics of sperm motion. However, CASA systems should not be viewed as a panacea for poor semen analysis techniques, as accurate assessment is dependent on the specific computer algorithm and the initial setup and validation process. Manual assessment is much more accurate in discerning among debris or immotile/dead sperm heads.¹¹ Dependent on the specific CASA system purchased, program functions may include assessment of motility, sperm morphology, concentration, DNA fragmentation, acrosome status, and vitality.

Computer-assisted sperm analysis systems typically consist of a phase-contrast microscope, a camera, stage warmer, an image digitizer, and a computer to save/analyze data.¹² This allows the system to analyze cell motion from successive image frames. Most systems have a playback feature to allow the user to validate whether cells were properly identified by the software. Computer-assisted sperm analysis systems have the significant advantage of providing accurate, rapid, and simultaneous analysis of multiple sperm parameters such as concentration, motility/velocity assessment, linearity of sperm movement, beat cross frequency, and amplitude of the lateral head displacement.^{13,14} The main disadvantage to these systems is the need for standardization and validation of the system; the choice of internal image setting such as minimum cell size, frame rate, and analysis time clearly influences the results obtained.^{12,15} Thus, these systems are only as good as the user defined settings. Dilution of the semen sample, the type of diluent used, temperature, and the type of counting chamber may also result in variability of results.^{13,14}

Cryopreservation units

Semen cryopreservation can be performed using incredibly low-tech equipment. A polystyrene box can be utilized to hold liquid nitrogen (LN₂). A floating rack can be purchased commercially or

made from polystyrene box lids or metal test tube racks. With a little imagination, many items readily available around the clinic can be re-purposed for semen freezing. Controlled rate freezing units (biological freezers) may improve post-thaw motility in some dogs.¹⁶

Freezing of canine semen in a polystyrene box has been compared to a protocol using an ultra-low temperature freezer (152°C).¹⁷ After freezing and thawing, there were no significant differences in sperm motility or the percentage of live or abnormal spermatozoa between the two techniques. This led the authors to conclude that ultra-freezers could be used as potential alternative to liquid nitrogen for storage.

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