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Setting Up an Andrology Laboratory

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ABSTRACT

Male factor infertility is a sole or contributing factor in approximately 40% of the infertile couples and therefore investigating the cause of the male infertility constitutes a major part of the diagnosis and treatment of infertile couples. The andrology laboratory supports and conducts clinical assessment of the semen and helps in the diagnosis of various diseases and malfunctions related to male infertility.

Development of advanced assisted reproductive techniques in the last decade has increased the scope of today’s andrology laboratories which are also involved in cryopreservation of gametes and reproductive tissues preserving the reproductive capacities of couples for various reasons.

This chapter serves as a practical guide for setting-up an andrology laboratory for diagnostic and therapeutic purposes.

INTRODUCTION

“Andrology” is the field of medicine that deals with matters affecting the male reproductive system. The earliest use of this term appeared in 1891 in the Journal of American Medical Association when it reported on the formation of the American Andrological Association. Because male infertility is the primary or contributing factor in more than 40% of infertile couples, this field has been the object of constant research and has therefore seen advances in diagnostic tests and tools used for the investigation of male infertility.

The Andrology Laboratory encompasses clinical laboratories under both Pathology and Laboratory Medicine. It is entrusted with supporting clinical assessments of semen that may help diagnose potential diseases or malfunctions related to male fertility. Utilizing both simple and complex techniques, its evaluation provides useful information for the infertility specialist in the management of the subfertile man. Besides diagnostic activities, it should provide therapeutic procedures for patients requiring assistance in achieving pregnancy such as Assisted Reproduction techniques: Intrauterine Insemination (IUI) and In Vitro Fertilization (IVF). The Andrology laboratory also works in parallel with endocrinology, genetic, and in vitro fertilization laboratories to diagnose and assist in the treatment of patients facing difficulties conceiving or seeking preservation of their reproductive capacity by cryopreserving their gametes or reproductive tissue.

This chapter will provide readers, in a very practical way, with information on the basic requirements for setting up and operating an Andrology laboratory for diagnostic and therapeutic activities.

LABORATORY ASPECTS AND PROCEDURES

Numerous procedures can be performed in the Andrology Laboratory. Due to different applications (clinical and research) and clinical correlations in the current scientific literature, some of these procedures may not be included in this chapter.

Semen analysis is the most common test to be conducted in the Andrology Laboratory. It reveals how many spermatozoa are in the ejaculate, the vigor with which they move (quantitative motility), and how normal these cells look morphologically. Complementary assessments should include viability, the presence of
round cells and their identification (i.e. leukocytes or immature sperm cells), and detection of antibodies. Additional tests can be performed based on the results obtained from this initial analysis. Such tests may complement the initial analysis and provide more information about sperm cell functionality and structure. Biochemical markers such as Reactive Oxygen Species (ROS) and tests that assess sperm DNA damage and the inducibility of the Acrosome Reaction (AR) can provide a more specific functional assessment of these gametes. Bioassays that assess gamete interaction, such as the sperm-hamster egg penetration assay (SPA) and the human sperm-zona pellucida binding test (hemizona assay, HZA), evaluate the occurrence of spontaneous sperm AR and the capacity for penetration, respectively. They have strong diagnostic power but, like the AR test, still require better standardization before they can be introduced as clinical tools. Moreover, the bioassays require hamster eggs and non-fertilizable human oocytes, which can impose extra practical limitations for their introduction into routine laboratory practice.

The most commonly performed tests are listed below. They can be classified in two groups: diagnostic and therapeutic.

**Diagnostic Procedures**

**Complete Semen Analysis**

Macroscopic parameters (color, volume, pH, viscosity, liquefaction, and agglutination)
Microscopic parameters (sperm concentration, motility, morphology, presence of round cells, and different types of debris)

**Detection of Leukocytes in Semen**
Myeloperoxidase test (Endtz)

**Sperm Vitality and Membrane Viability**
Eosin-Nigrosin test
Hypo-osmotic Swelling test (HOS)

**Biochemical**
Fructose qualitative and quantitative

**Oxidative Stress**
Reactive Oxygen Species (ROS)
Total Antioxidant Capacity (TAC)

**Azoospermia Screen Procedure**

*Antibody Assessment*
Test for anti-sperm antibodies on the sperm surface
Test for free antibodies, directed against the sperm surface (semen plasma, serum or cervical mucus)

**Sperm DNA Damage Test**

*Sperm Chromatin Structure Assay (SCSA)*
Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)
COMET, also called single cell gel electrophoresis (SCGE).

**Therapeutic**

*Preparation for Intrauterine Insemination (IUI) or In vitro Fertilization (IVF)*

**Preparation of cryopreserved semen for IUI or IVF**

**Techniques**
Gradient method
Swim-up method
Simple wash (concentration technique)

**Cryopreservation**
Semen
Epididymal aspirate
Testicular Tissue

**LABORATORY DESIGN**

Before working on any laboratory design, local regulations must be carefully studied. These regulations may differ between countries or even between states, and compliance is not optional.

Ideally, the Andrology Laboratory should contain distinct areas for diagnostic and therapeutic procedures (Figure 16.1). Therapeutic procedures should be performed in a sterile environment, which can be achieved by using a laminar flow hood. The physical area should be determined according to a pre-estimation of the number of tests to be offered by the service and the volume of procedures expected to be performed. This will determine the number of technicians that must be employed and the different types of equipment that must be made available such as microscopes, cell counters, incubators, etc. New laboratories may not have an established range of workload due to regional and market variations and thus should always keep in mind plans for future expansion.
Even a small physical area can have its space optimized according to its disposition and organization. If the laboratory is going to offer cryopreservation, there should be space for the liquid nitrogen (LN\textsubscript{2}) containers that will store tissue samples (Figure 16.2). Due to the chemical properties of LN\textsubscript{2}, a laboratory should follow the proper safety guidelines for area ventilation and oxygen monitoring. Containers should be kept locked or in areas of restricted access. If containers are not checked daily, the laboratory should have alarms that sound when levels of LN\textsubscript{2} fall too low.

**Semen Collection Room**

The semen collection room (SCR) is a very important aspect of an Andrology Laboratory. Therefore, it must be planned with attention to design, especially in regards to location. Usually the SCR is located in proximity to the laboratory. Places that provide privacy (avoid busy corridors, lobbies or reception areas) are appropriate. The room can be equipped with a love seat or a small sofa and be supplied as needed with clean towels and sheets (Figure 16.3). There should be a sink or a washing station for use before and after collection. Not all collection rooms provide toilets and, if this is the case, a bathroom should be available nearby. The furnishings must be kept simple with preference for easy-to-clean materials or surfaces. Instructions for collection should be posted in the SCR even when they are explained to the patients by the nurse or laboratory technician. An interesting survey on patient satisfaction reported that the main areas of concern regarding the SCR were: sound privacy of the room, furnishing, cleanliness, and the availability and condition of media.\textsuperscript{4} The survey responders also rated videos as the most helpful media followed by magazines, with the preferred theme being heterosexual sex. In the same study, the responders preferred instructions on collection technique to be given face-to-face rather than in writing.

**Office and Break Room**

Clerical staff will also require office space. Patients’ charts and files need to be kept within easy access of the laboratory staff. Computers and printers should be
available for entering results and work-related issues. A very important aspect is the break room. A resting area for employees must be included in the plan. Restrooms for employees should be close by.

Workstations

For practical purposes, workstations can be pre-defined. For example:

Workstation 1: Semen wet preparation (macroscopic parameters on seminal analysis, sperm count and motility, Endtz test, HOS and Eosin-Nigrosin preparations). This area can be equipped with microscopes, cell counters, incubators, water baths or warm stage and the necessary supplies and reagents (Figure 16.4). Controlled temperature at 37°C is needed for liquefaction, count and motility assessment, and HOS incubation. This workstation can also be equipped with an automated semen analyzer, which generates a more complete assessment of sperm motion characteristics. It is important to emphasize that the automated evaluation cannot replace a manual evaluation. It is still recommended that practitioners manually count semen samples to validate the computer results.

Workstation 2: Staining and slide preparation for morphology. This bench is usually near a sink or a drain where the slides can be washed during staining (Figure 16.5). Staining solutions can be kept in plastic containers or glassware and should be verified for possible contamination daily prior to use. When ready, slides are evaluated and second on a morphology assessment bench (Figure 16.6).

Workstation 3: Biochemical and antibody tests. Due to the nature of the fructose determination, a safety hood is recommended. Reagents for these procedures are usually kept refrigerated.

Workstation 4: Semen preparation and cryopreservation procedures. As mentioned previously, these procedures should be performed in a sterile environment (Figure 16.7). The same laminar flow hood, for example, could be used for both procedures; this could be an option if the lab cannot support two separate workstations logistically or financially. When optimizing the semen preparation procedures, a CO₂ incubator can be used for sample incubation in both the pre- and post-processing periods.
EQUIPMENT, SUPPLIES AND REAGENTS

The equipment, supplies, and reagents needed for each procedure vary according to the protocols to be followed. The following list suggests what each of the following tests may require:

Semen Analysis

**Equipment**

Phase Contrast Microscope with 10 × and 20 × Objective.
Cell Counter
Counting Chamber
Centrifuge (~1600 rpm)
Refrigerator (~ 4 to – 8°Celsius)
Vortex mixer

**Supplies**

Micropipettes with different ranges and corresponding tips (5 µL, 10 µL, 20 µL, 100 µL)
Automatic Pipetor (rechargeable)
Graduated Serological Pipets (1, 2 and 5 ml)
Test Tube rack for 15 mL tubes
15 mL Polystyrene Graduated Conical Centrifuge Tubes
Specimen Cups
Glass Microscope Slides and Coverslips
pH paper Range 6-8
Disposable Transfer Pipets (Pasteur Pipets)
Microcentrifuge tubes for dilution

**Reagents**

Tyrode’s Salt Solution

**Stain for Morphology**

**Equipment**

Microscope with 100 × Objective
Differential cell Counter

**Supplies**

Micropipettes with different ranges and corresponding tips (5 µL, 10 µL, 20 µL, 100 µL)
Glass Microscope Slides and Coverslips

**Reagents**

Cytoseal Mounting Media
Papanicolaou stain or Diff-Quik (rapid staining method) or Shorr stain

**Endtz Test (Peroxidase Staining)**

* Equipment and supplies for semen analysis are required for pre-assessment

**Equipment**

Analytical Balance
Microscope 10 × objective

**Supplies**

Makler Chamber
Aluminum Foil
Dark Colored Microcentrifuge Tubes
Micropipettes with different ranges and corresponding tips (5 µL, 10 µL, 20 µL, 100 µL)

**Reagents**

Ethanol, 96%
Benzidine
3% H<sub>2</sub>O<sub>2</sub>
Tyrode’s Salt Solution

**Eosin-Nigrosin Staining**

* Equipment and supplies for semen analysis are required for pre-assessment

**Equipment**

Microscope 100 × objective
Differential cell counter
Supplies
Disposable Transfer Pipets (Pasteur Pipets)
Boerner Slides (for mixing)
Glass Microscope Slides and Coverslips

Reagents
Stain components, Eosin Y
Cytoseal Mounting Media
Immersion Oil

Hypo-osmotic Swelling (HOS) Test
* Equipment and supplies for semen analysis are required for pre-assessment

Equipment
Microscope 40 × Objective
Analytical Balance
Differential Cell Counter

Supplies
Microcentrifuge Tubes
Glass Microscope Slides and Coverslips
Micropipettes with different ranges and corresponding tips (5 µL, 10 µL, 20 µL, 100 µL)

Reagents
Sodium Citrate
β-D Fructose
Distilled water

Fructose Qualitative

Equipment
Refrigerator (−4 to −8°Celsius)
77°C water bath

Supplies
100 mL beakers
50-mL Erlenmeyer flasks
Glass funnels
Filter paper
Serological pipet (5, 10 mL)
Micropipettes with corresponding tip (100 to 1000 µL)
13 × 100 glass test tubes with caps

Reagents
Concentrated HCl
D-Fructose (Levulose)
Deionized water
Resorcinol
Ethanol 95%

Sperm Antibody Testing by Immunobead Method
* Equipment and supplies for semen analysis are required for pre-assessment

Equipment
Microscope 40 × Objective
Analytical Balance
Centrifuge (capable of 300 to 700 ×g)
56°C Water bath
Refrigerator (−4 to −8°Celsius)
pH meter
Differential Cell Counter
Covered Humidified Chamber

Supplies
15 mL Sterile Centrifuge Tubes with Caps
Micropipettes and Tips (8 µL and 200 µL)
5 mL Disposable Graduated Pipet
Glass Microscope Slides with Coverslips
### Reactive Oxygen Species

* Equipment and supplies for semen analysis are required for pre-assessment

- **Reagents**
  - Bovine Serum Albumin
  - Tyrodes Salt Solution
  - Immunobead Rabbit Anti-Human Ig (H & L) Reagent
  - IgA Immunobeads
  - IgG Immunobeads
  - IgM Immunobeads

### Sperm DNA Damage per TUNEL Technique

* Equipment and supplies for semen analysis are required for pre-assessment

- **Equipment**
  - Flow cytometer

- **Supplies**
  - Micropipettes with corresponding tips (200 µL, 1000 µL)
  - Counting chamber

### Total Antioxidant Capacity

- **Equipment**
  - Refrigerator (– 4 to – 8°Celsius)
  - Centrifuge
  - Absorbance Microplate Reader
  - Micropipettes with different ranges and corresponding tips (5 µL, 10 µL, 20 µL, 100 µL)
  - Graduated Serological Pipets (1, 2, and 5 mL)
  - Polystyrene round bottom tubes (6 mL)

- **Reagents**
  - Dimethyl Sulfoxide (DMSO)
  - Luminol (5-amino-2,3 dehydro-1,4 phthalazinedione)
  - Phosphate Buffered Saline Solution 1X (PBS-1X)

### Gradient Sperm Wash

* Equipment and supplies for semen analysis are required for pre-assessment

- **Equipment**
  - 37°C Incubator (CO₂ supplied if possible)

- **Supplies**
  - Sterile 15-mL Conical Centrifuge Tubes with Caps (tested against embryo and/or sperm toxicity)
  - Sterile Graduated Serological Pipets (1, 2, and 5 mL)
  - Micropipettes with different ranges and corresponding tips (5 µL, 10 µL, 20 µL, 100 µL)
  - Sterile Disposable Transfer Pipets (Pasteur Pipets)
  - Sterile Specimen Containers (tested against embryo and/or sperm toxicity)

- **Reagents**
  - Lower phase gradient
  - Upper phase gradient
  - Sperm Wash Media

### Setting Up an Andrology Laboratory

- **Reagents**
  - Antioxidant assay kit
  - Deionized water

- **Equipment**
  - Luminometer
  - Centrifuge

- **Supplies**
  - 15 mL Polystyrene Graduated Conical Tubes with caps
  - Micropipettes with different ranges and corresponding tips (5 µL, 10 µL, 20 µL, 100 µL)
  - Graduated Serological Pipets (1, 2, and 5 mL)
  - Polystyrene round bottom tubes (6 mL)
Semen, Epididymal Aspiration, and Testicular Tissue Cryopreservation

* Equipment and supplies for semen analysis are required for pre-assessment

**Equipment**
- Aliquot Mixer/Barnstead/Thermolyne
- 37 °C Incubator (CO₂ supplied if possible)
- –20 °C Freezer
- Liquid nitrogen container
- Liquid nitrogen container alarm
- Oxygen monitor

**Supplies**
- Sterile 15 mL Conical Centrifuge Tubes with Caps
- Sterile Graduated Serological Pipets (1, 2, and 5 mL)
- Micropipettes with different ranges and corresponding tips (5 µL, 10 µL, 20 µL, 100 µL)
- Sterile Graduated Serological Pipets (1, 2, and 5 mL)
- Sterile Specimen Containers (tested against embryo and/or sperm toxicity)
- Test Tube rack for 15-mL tubes
- Plastic Cryosleeves
- Cryovials – 2 mL
- Cryocanes
- Laboratory Cryomarkers (non-toxic)
- Liquid Nitrogen
- Cryogenic Protective Gloves
- Protective Eye Goggles

**Reagents**
- Freezing Medium

**Additional for Testicular Tissue**

**Equipment**
- Dissecting Microscope
- Inverted Microscope

**Supplies**
- Kontes Pellet Pestle
- Sterile Tissue Culture Plates, 60 x 15 mm

**Reagent**
- Sterile Mineral Oil

**SAFETY GUIDELINES**

The safety guidelines for the Andrology Laboratory include use of protective gloves and appropriate laboratory coats for all procedures. All samples should be treated as potentially contaminated and harmful. The World Health Organization (WHO) laboratory manual⁵, in its fourth edition, has described these guidelines.

**REFERENCES**