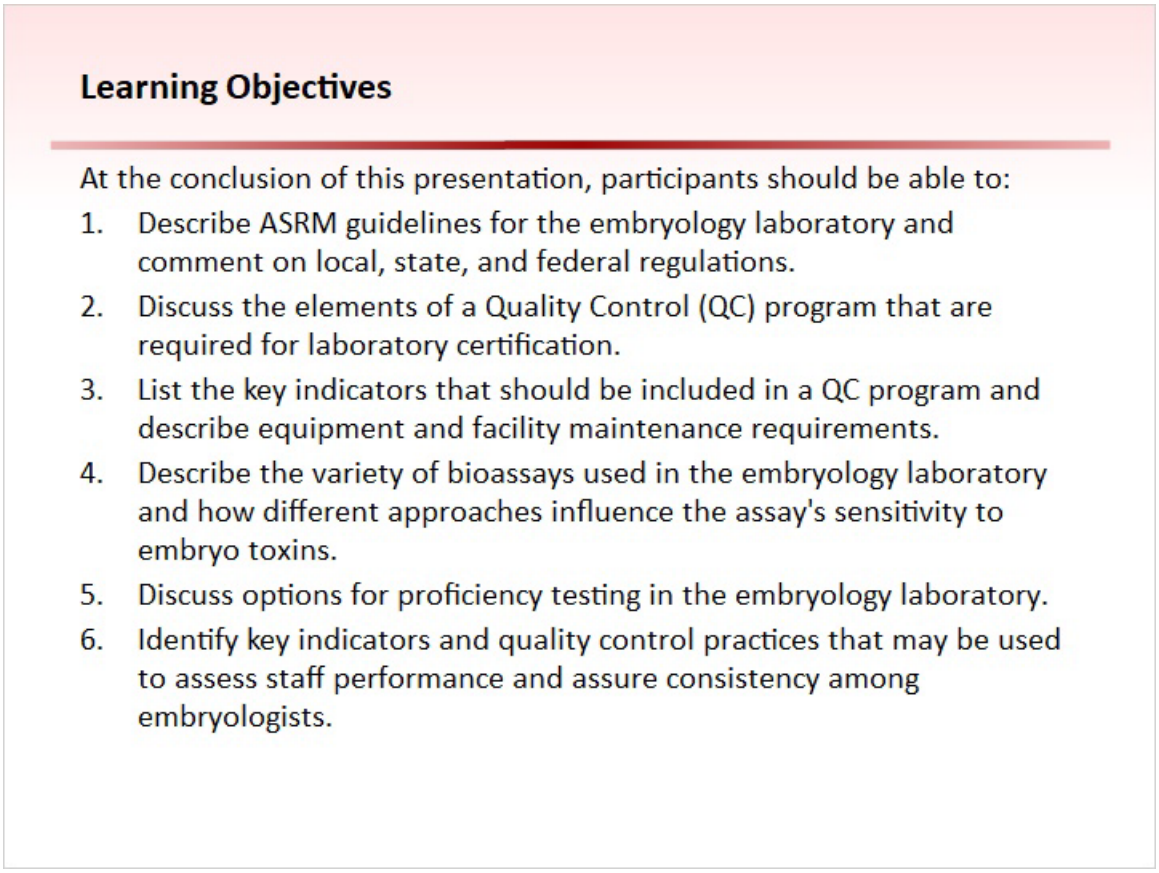
LABCC100 Lesson 34

1.1 Quality Control in the Embryology Laboratory

Notes:

Welcome to the American Society for Reproductive Medicine's eLearning modules. The subject of this presentation is Quality Control in the Embryology Laboratory.

1.2 Learning Objectives

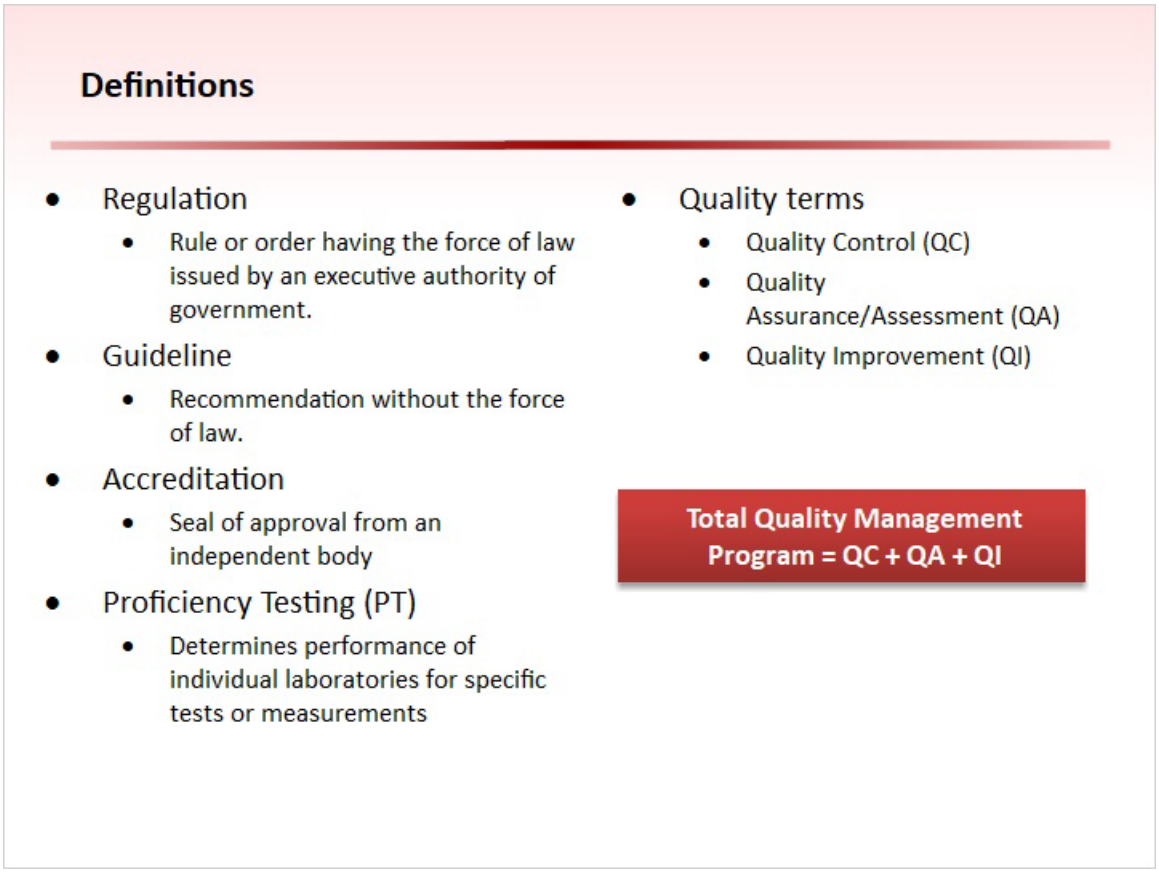


Notes:

At the conclusion of this presentation, participants should be able to:

1. Describe ASRM guidelines for the embryology laboratory and comment on local, state, and federal regulations.
2. Discuss the elements of a Quality Control (QC) program that are required for laboratory certification.
3. List the key indicators that should be included in a QC program and describe equipment and facility maintenance requirements.
4. Describe the variety of bioassays used in the embryology laboratory and how different approaches influence the assay's sensitivity to embryo toxins.
5. Discuss options for proficiency testing in the embryology laboratory.
6. Identify key indicators and quality control practices that may be used to assess staff performance and assure consistency among embryologists.

1.3 Definitions



Notes:

It is important to distinguish regulations from guidelines.

A Regulation is a rule or order having the force of law issued by an executive authority of government.
A Guideline refers to a recommendation without the force of law.

Accreditation is a seal of approval from an independent body. In the United States, there are no regulations that require embryology laboratories to be accredited, but guidelines accreditation of the embryology laboratory agencies may be deemed by regulatory agencies. This means that a successful voluntary on-site survey from the accrediting agency may be sufficient for the regulatory agency.
Proficiency Testing (PT) determines the performance of individual laboratories for specific tests or measurements and is used to monitor laboratories' continuing performance. Proficiency testing is also called interlaboratory comparison. As this term implies, proficiency testing compares the measuring results obtained by different laboratories.
QUALITY TERMS

Quality Control (QC) is the use of operational control techniques, particularly end-product testing or inspection, to ensure that a product or service satisfies its stated or implied role, and to ensure regulatory compliance and requirements for accreditation. In the laboratory, quality control materials are used for analytical activities.

- **Quality Assurance (QA)** consists of planned and documented systematic activities to provide confidence that an organization fulfills requirements for quality. **Quality Assessment** is the monitoring and evaluating of all activities in the laboratory to ensure accuracy of results, including preanalytical, analytical and postanalytical. Proficiency testing is a common method for assessing quality.
- **Quality Improvement** is the process used to enhance all phases of laboratory performance.

- **Total Quality Management Program** is a systematic process-oriented approach that combines QC, QA and QI to meet quality objectives. This presentation will focus on the quality control portion of the quality management program and will touch upon proficiency testing.

1.4 Embryology Laboratory

Embryology Laboratory

- Integral part of an assisted reproductive technology (ART) program
- Services offered by embryology laboratories may include but are not limited to:

• Culture media preparation	• Evaluation of fertilization and zygote quality
• Examination of follicular aspirates	• Embryo culture and grading
• Assess oocyte quality and maturity	• Oocyte and embryo micromanipulation
• Semen analysis* and sperm preparation for ART	• Gamete and embryo cryopreservation, storage and thawing
• Insemination of oocytes	• Embryo transfer

**Semen analysis is the only service in this list classified as a high complexity test and regulated by Clinical Laboratory Improvement Act (CLIA '88).*

Notes:

The embryology laboratory is an integral part of an assisted reproductive technology (ART) program. Services offered by embryology laboratories may include but are not limited to the services listed here. In contrast to the ART program's andrology and endocrinology laboratories that are both classified as high-complexity laboratories under the Clinical Laboratory Improvement Act (CLIA '88), the embryology laboratory remains unclassified and essentially unregulated. Embryology laboratories performing semen analysis prior to preparing sperm for ART must be in compliance with CLIA '88 regulations and will be subjected to on-site inspections by a regulatory agency or an accreditation agency deemed by regulatory agencies.

1.5 Embryology Laboratory Accreditation

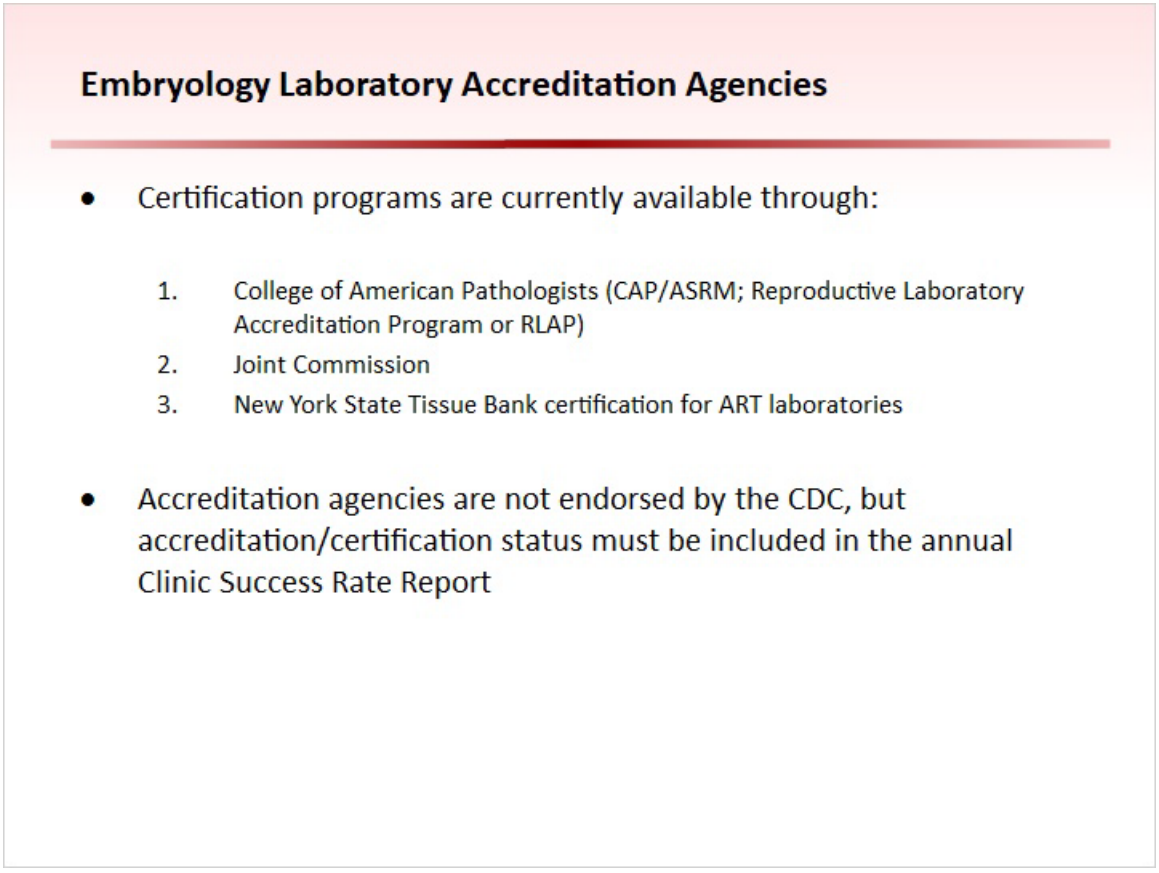
Notes:

The Centers for Disease Control and Prevention (CDC), which is concerned with public health and safety, provides education and information to enhance health care decisions, including addressing best practices in laboratory medicine. In this role, the CDC was

charged with developing a Model Embryology Laboratory under Public Law 102-493 (Fertility Success Rate and Certification Act of 1992 [FCSRCA]), which includes semen preparation for ART procedures.

The FCSRCA mandates that the Secretary of Health and Human Services (HHS), through the CDC, develop a model program for the certification of embryo laboratories, to be carried out voluntarily by interested states. The requirements for reporting the certification status of embryo laboratories, including definitions, administrative requirements, and embryo laboratory standards are described in the Federal Register Notice.

1.6 Embryology Laboratory Accreditation Agencies



Notes:

Currently, certification of embryo laboratories can be done by one of the 3 nonfederal laboratory accreditation programs:
(1) The College of American Pathologists (CAP/ASRM), RLAP accreditation programs are created with the primary objective of improving the quality of clinical laboratory

services. RLAP was established in collaboration with ASRM to develop an accreditation program specifically designed for the unique needs of reproductive laboratories (andrology, embryology, endocrinology, genetics). The program has been approved by Centers for Medicare and Medicaid Services (CMS) for the accreditation of those andrology tests regulated by CLIA'88. It employs voluntary participation, professional peer review, education, and compliance with established performance standards. RLAP is widely associated with excellence in ART clinical laboratory services.

(2) The Joint Commission has collaborated with health care organizations for more than 50 years. It is a nonprofit organization of professional inspectors established to improve the delivery of patient care. It was granted deeming authority through the CMS in 1966. The Joint Commission offers a patient-centered and process-focused survey that is conducted every 3 years.

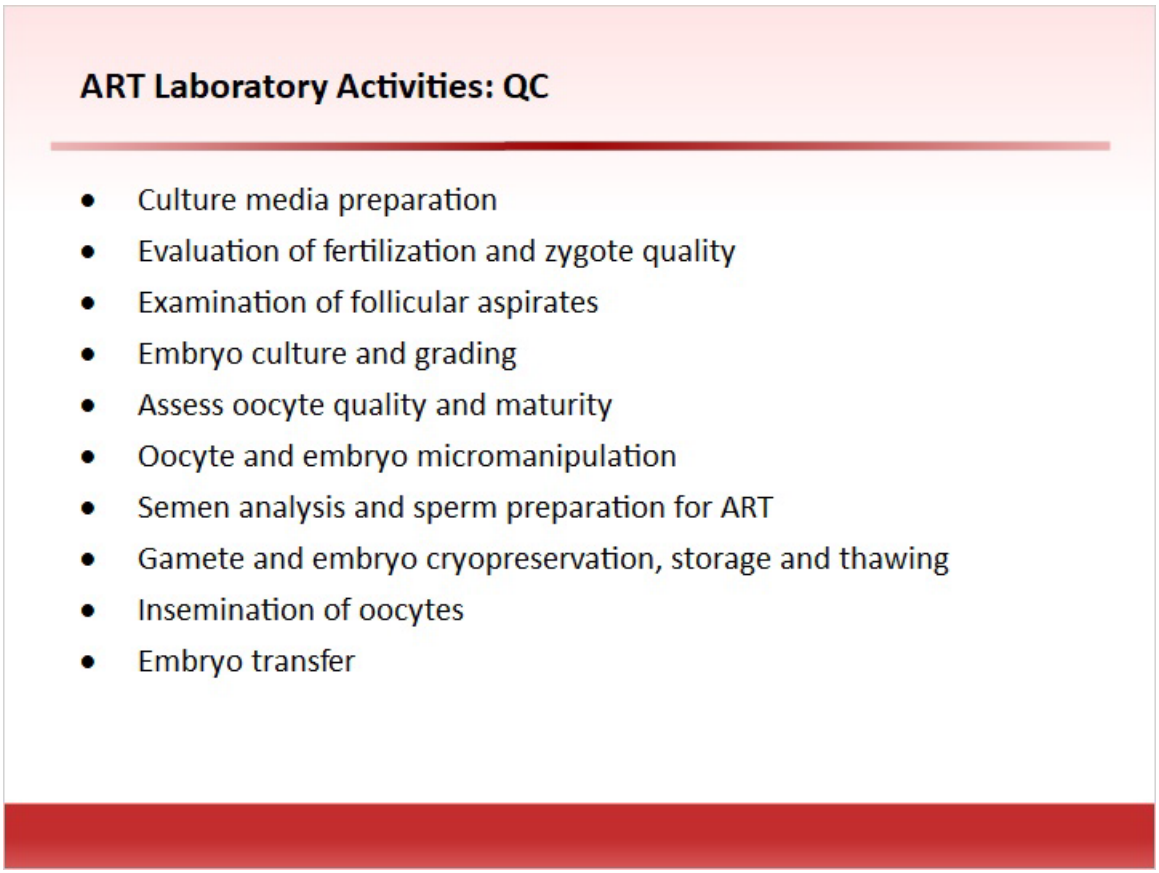
(3) The New York State Tissue Bank certification for ART laboratories (NYSTB). The CDC does not endorse these accreditation agencies, but rather is providing available laboratory accrediting information in all annual Clinic Success Rates Reports.

1.7 Quality Control Program

Notes:

The CAP General and Reproductive Laboratory Accreditation Checklists and ASRM Guidelines for Human Embryology list minimum requirements and recommendations for QC in embryology laboratories, leaving much of the QC program design up to the discretion of the laboratory director.

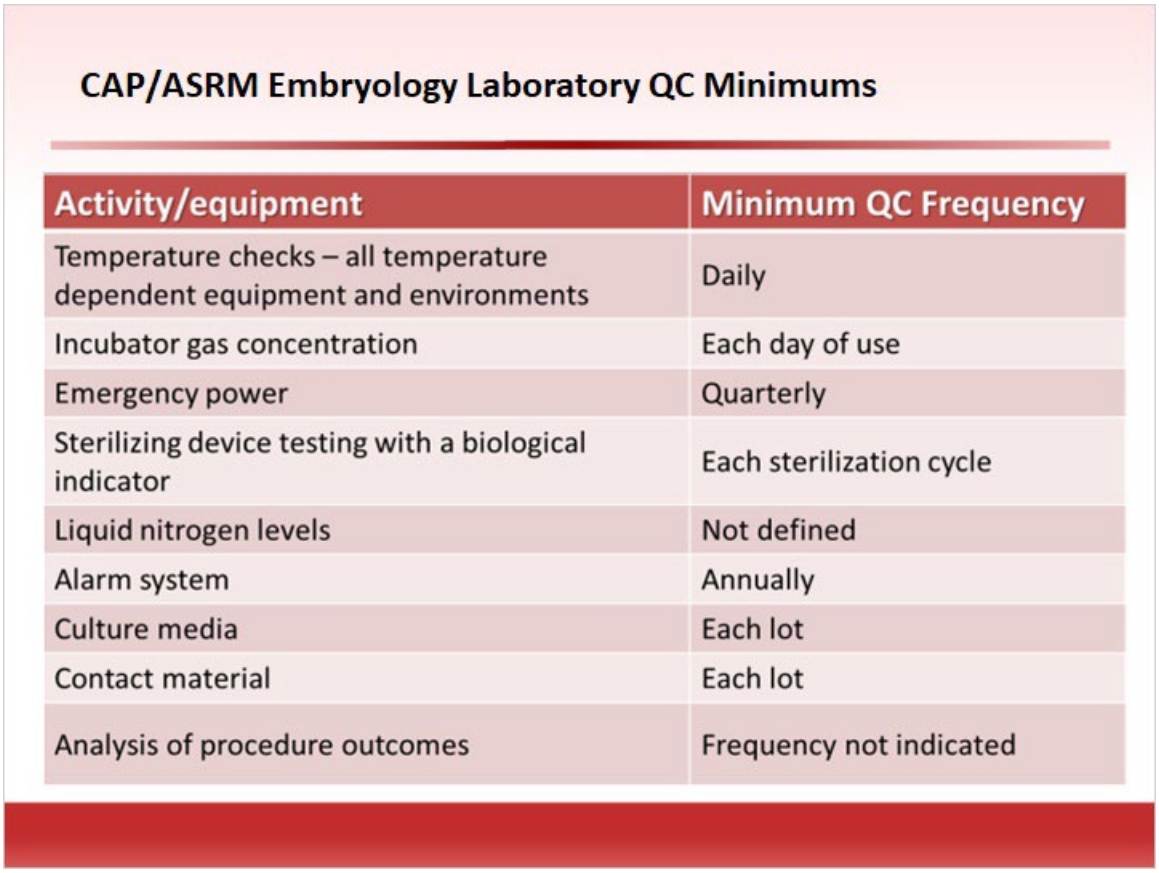
1.8 ART Laboratory Activities: QC



Notes:

Regardless of regulatory agency from which the laboratory wishes to seek accreditation, the laboratory must have a QC plan in place that will assure that the services listed are performed in optimal conditions. The QC program must measure the quality and performance of all products, equipment, procedures, and environments associated with these activities to ensure that every aspect of the laboratory is functioning correctly.

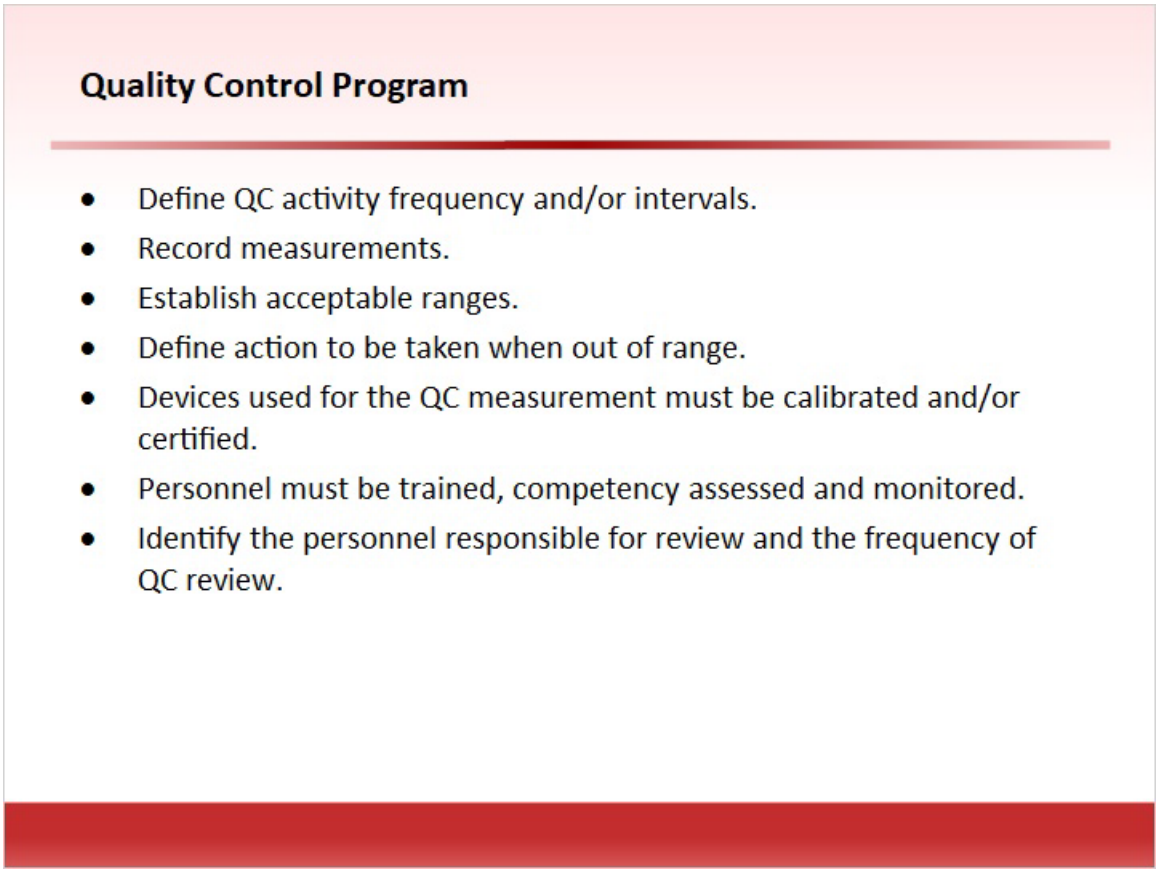
1.9 CAP/ASRM Embryology Laboratory QC Minimums



Notes:

Accreditation by CAP requires the embryology laboratory to include the QC activities listed here. Laboratories may choose to perform the QC measures more frequently than the minimum requirements and the selection of the methods to obtain these measures are left to the discretion of the laboratory director.

1.10 Quality Control Program



Notes:

The quality control program for the entire laboratory must be documented. It must include general policies and assignments of responsibilities and address the following points:

The program must clearly state the frequency when each measure is made. The frequency needs to reflect the activity in the laboratory and must be performed at defined intervals that could detect a problem before it compromises patient care.
Measurements or readings must be recorded. It is difficult to see a drift in performance if the QC activity is documented by a check mark indicating that the measurement was "in range."
The laboratory must validate that the values within the acceptable range yield optimal results and, if applicable, comply with manufacture's recommendations. Laboratories may opt to aim for a tighter range within the manufacturer's recommendations.
The QC program must define what action must be taken when a measurement is out of range.
Devices used for the QC measurements must be calibrated and/or certified.
Just as accuracy of the devices used for QC must be assured, the personnel performing

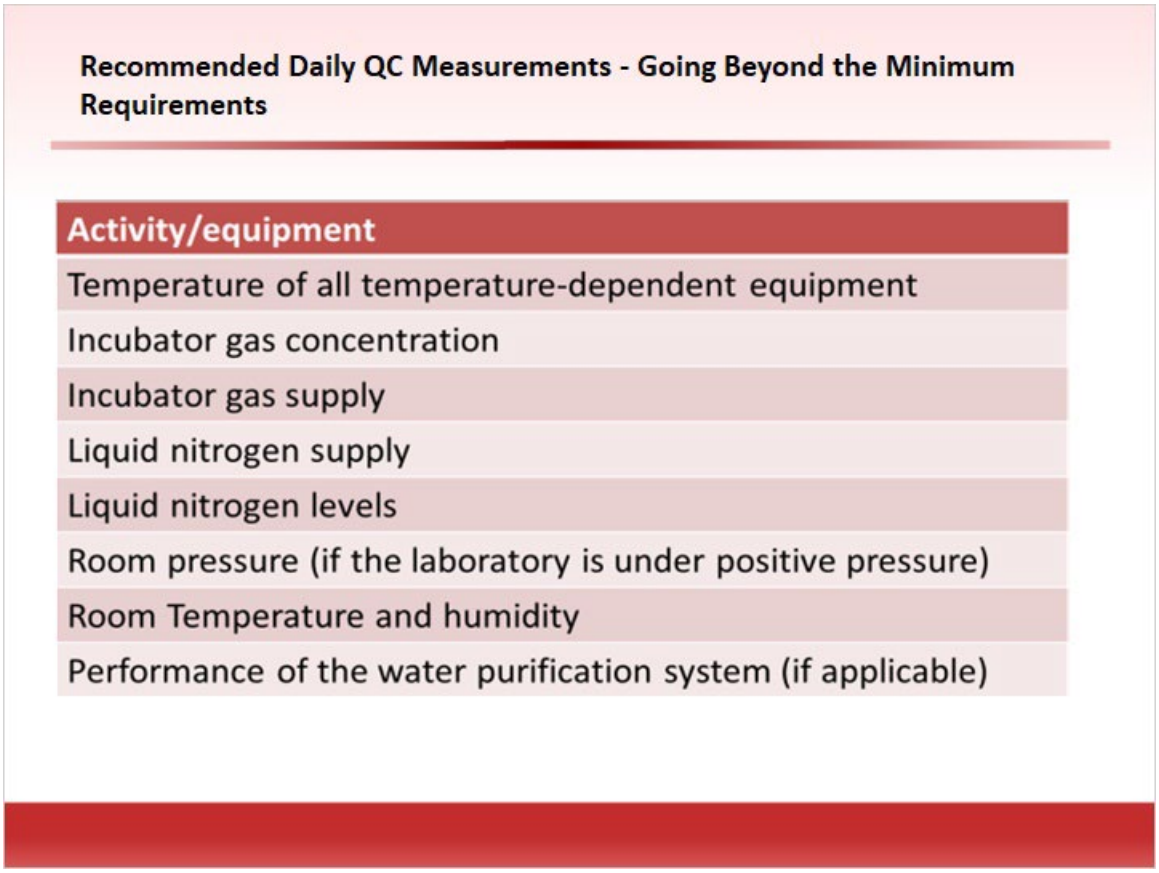
the QC activities must be trained, their competency assessed, and their performance of QC activities must be monitored. The identity of the staff member performing must be documented along with each QC measurement.
Quality control records should be well-organized with a system to permit regular review by appropriate supervisory personnel (laboratory director, supervisor, or laboratory QC coordinator).

1.11 Methods of Quality Control Measures and Their Frequency

Notes:

A detailed description of methods of quality control measures and their frequency will be presented in this section, starting with daily QC activities and laboratory maintenance followed by activities that are required, but with less frequency, including HVAC maintenance and air quality monitoring. Specific attention will be paid to the testing and handling of culture media and contact materials.

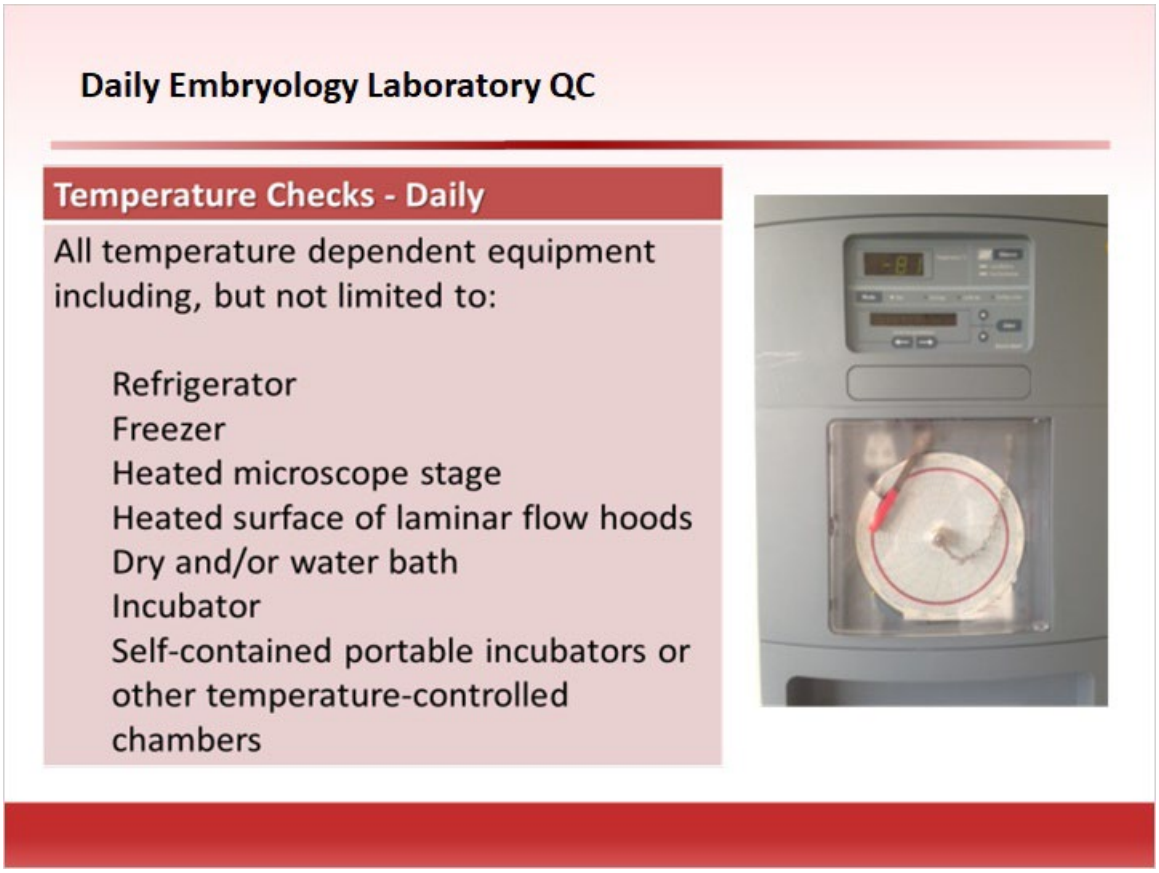
1.12 Recommended Daily QC Measurements – going beyond the minimum requirements



Notes:

The QC activities required for accreditation by CAP are minimums and the laboratory will likely need to add additional QC measures or perform the measures more frequently than the minimum frequency. Here is an example of a typical list of daily QC activities performed in embryology laboratories.

1.13 Daily Embryology Laboratory QC



Notes:

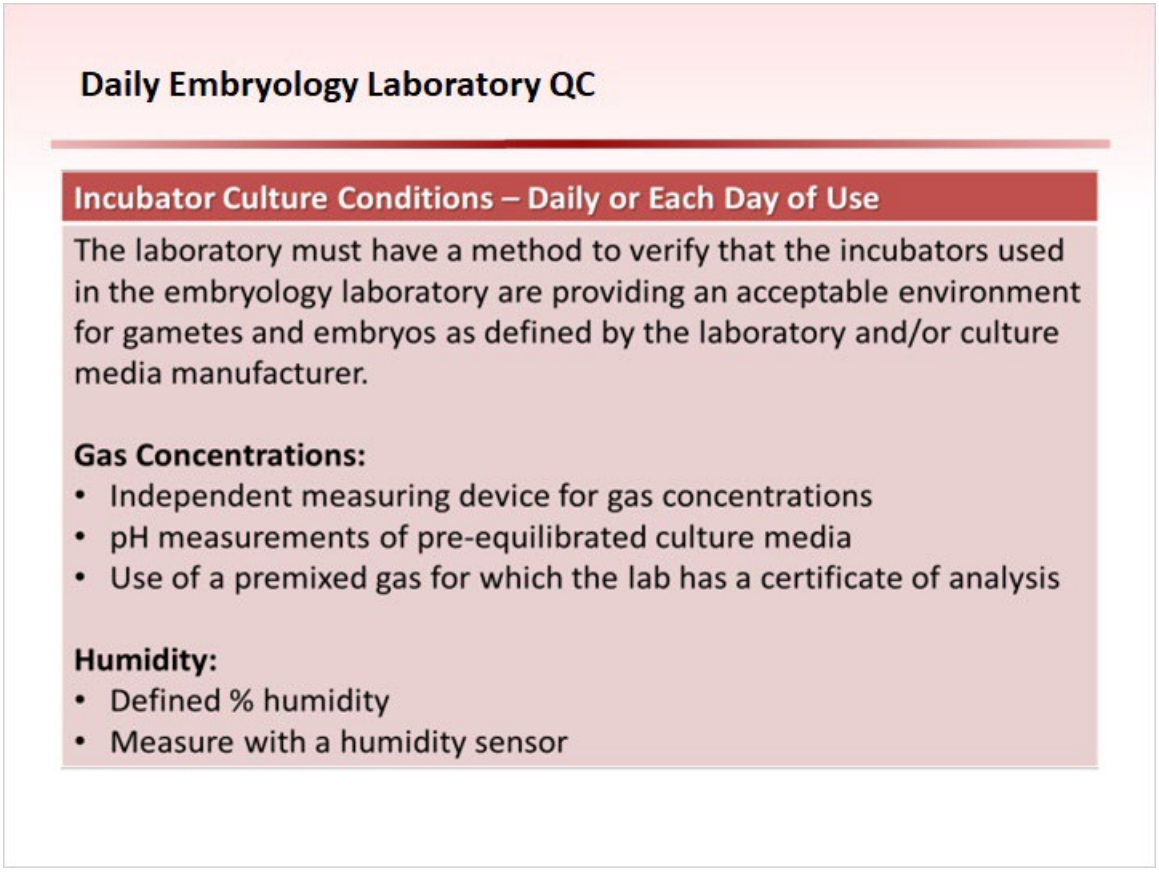
Temperatures of any temperature-dependent equipment should be monitored daily. This includes: refrigerators, freezers, heated microscope stages, heated surfaces of laminar flow hoods, dry and/or water baths, incubators, self-contained portable incubators, or other temperature-controlled chambers.

Internal temperature measurement in refrigerators, freezers, baths, incubators, and self-contained portable incubators can be accomplished by placing a thermometer in a test tube filled with water or culture oil. Acceptable temperature ranges for surfaces of heated microscope stages or laminar flow hoods should be established by placing a calibrated probe into a culture media control drop and determining what surface temperature is required to achieve the desired temperature in the culture media drop and ultimately the location of the oocyte or embryo.

The two acceptable ways of recording temperature for compliance with the CAP checklist includes: 1) recording the temperature or 2) placing a mark on a graph that corresponds to a numerical temperature (either manually or using a graphical recording device). The use of an automated (including remote) temperature monitoring system is

acceptable, providing laboratory personnel have ongoing immediate access to the temperature data, so that appropriate action can be taken if a temperature is out of the acceptable range. The daily functionality of the remote system must be documented.

1.14 Daily Embryology Laboratory QC



Notes:

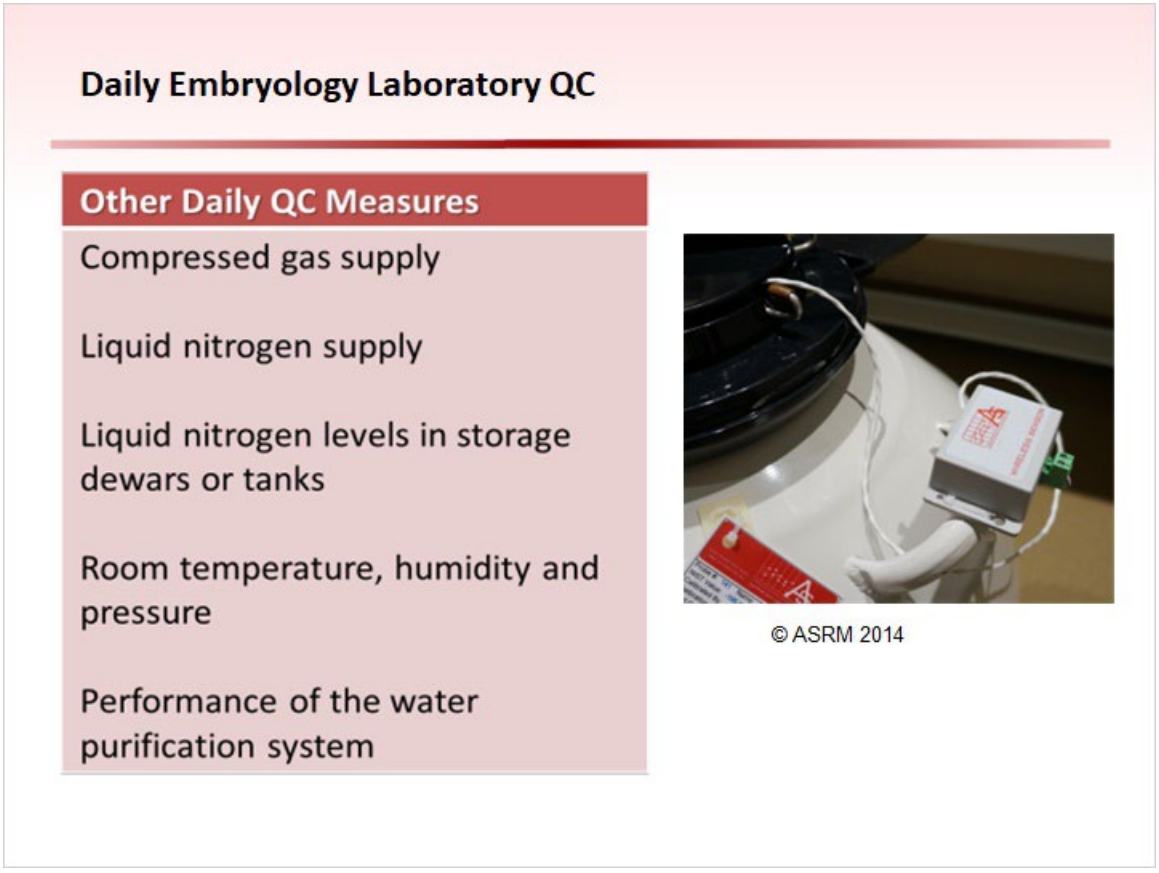
In addition to temperature, the culture conditions in incubators must be checked daily, or at a minimum, each day of use. The culture conditions are dependent on gas concentration and, if employed by the laboratory, relative humidity.

As with temperature, there are a variety of approaches the lab may use to measure the gas concentration in an incubator including the following: 1) using an independent measuring device; 2) performing daily checks of culture media pH; or 3) laboratories using a premixed gas for their embryo culture may retain the manufacturer's certificate of analysis as documentation of acceptable QC records instead of performing independent measures. Ideally, the laboratory will use a multifaceted approach to measuring the gas concentration in the incubator by calibrating the LED gas

concentration display with an independent gas analyzer and measuring the media pH concurrently. If the laboratory director adopts this approach, the frequency of each activity must be defined and should be performed in a timely manner to detect drifts associated with gas tank changes or culture media lot changes.

Laboratories that humidify their incubators do so to avoid media evaporation and an increase in culture media osmolality that could be detrimental to the embryo culture. Use of an oil overlay system may alleviate humidity control needs in the incubator. If the laboratory choses to incubate gametes in a humidified environment, they must determine acceptable limits and the method by which they measure humidity.

1.15 Daily Embryology Laboratory QC



Notes:

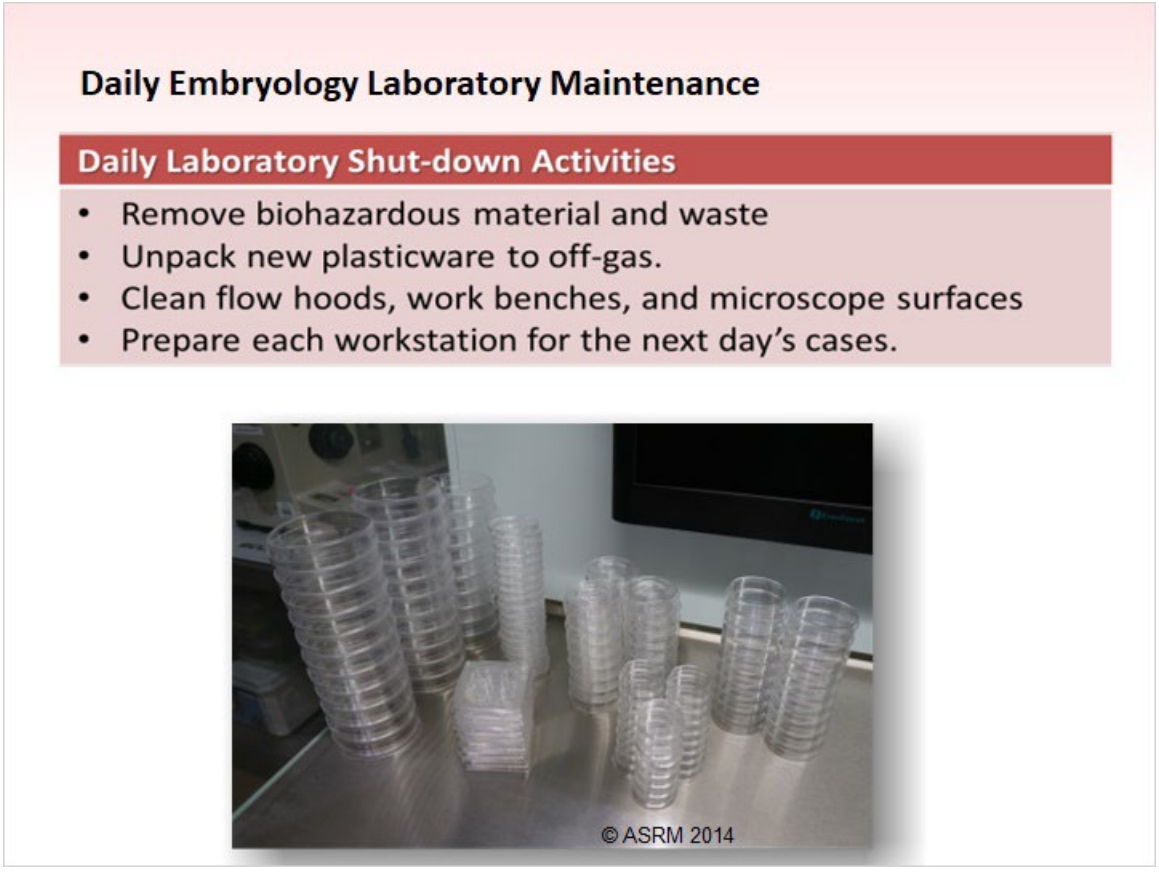
Additional daily QC measures should include documentation of compressed gas and liquid nitrogen supply. Measurement of daily consumption and establishment of generous, minimum levels that trigger a call to the supplier will assure that a sufficient amount of these supplies are on hand.

Liquid nitrogen levels in dewars or cryotanks should be monitored with a low alarm probe that has a relay to an alarm system. The probe and, if is not wireless, the wires leading to the alarm should be inspected daily. Probes that have accumulated ice should be thawed and cleaned.

Room temperature, humidity, and if the lab is under positive pressure, the room pressure, should be monitored and recorded daily. Low relative humidity (below 30%) can make static electricity an issue in the laboratory and excessive humidity (above 60%) can promote the development of bacteria and mold.

If water is purified onsite, the purification system's performance should be checked daily. If the water that supplies the system must be softened prior to cycling through the purification process, the hardness of the feed water should be tested and softener salt level monitored.

1.16 Daily Embryology Laboratory Maintenance



Notes:

At the end of the day the final maintenance steps must be taken before the lab is locked. All waste should be removed from the lab.

Plasticware should be unpackaged after all embryos have been put away. Some laboratories routinely off-gas their plasticware before use. While off-gassing times will vary, this practice should reduce the level of volatile organic compounds (VOCs) emitted from the plastic by the time it comes in contact with gametes or embryos.

The flow hoods, work benches, and microscope stages should have a final cleaning at the end of the day.

Finally, each workstation must be set up for the next day's cases so the embryologists opening the lab can tend to their laboratory duties in a timely fashion.

1.17 Other Embryology QC / Maintenance Activities

Other Embryology QC / Maintenance Activities

Activity/equipment	Recommended Minimum Frequency
Cryotanks – manually measure and refill	Weekly
Laboratory cleaning (floors and other non-bench areas)	Weekly
Check/refill water levels in circulating water baths and self-contained portable incubators	Weekly
Water (purchased or produced) for media preparation must be tested for microbial content, resistivity, total organic carbon, endotoxin testing	Weekly and each media preparation
Sterilizing device testing with a biological indicator	Each sterilization cycle
Test alarm system	Monthly or quarterly
Supply inventory	Weekly or monthly
Eyewash station test	Weekly

Notes:

Other frequent QC activities should include the following:

Measuring cryotank levels and refilling the tank to the established maximum level if the tanks are monitored with a low level probe. If the liquid nitrogen level is not monitored with a low level probe equipped with a relay to an alarm system, the level should be measured manually on a daily basis.

The laboratory surfaces that are not cleaned between cases or at the end of each week day should be cleaned weekly. This includes non-specimen benchtops, floors, and other surfaces that may collect lint or dust.

Water levels in circulating water baths that heat the surface of a laminar flow hood and water levels in self-contained portable incubators should be checked each week.

The microbial content, resistivity, particulate matter, or total organic carbon of water that is used for media preparation should be tested weekly and at the time of media production. Additionally, endotoxin testing must be performed at the time of media production.

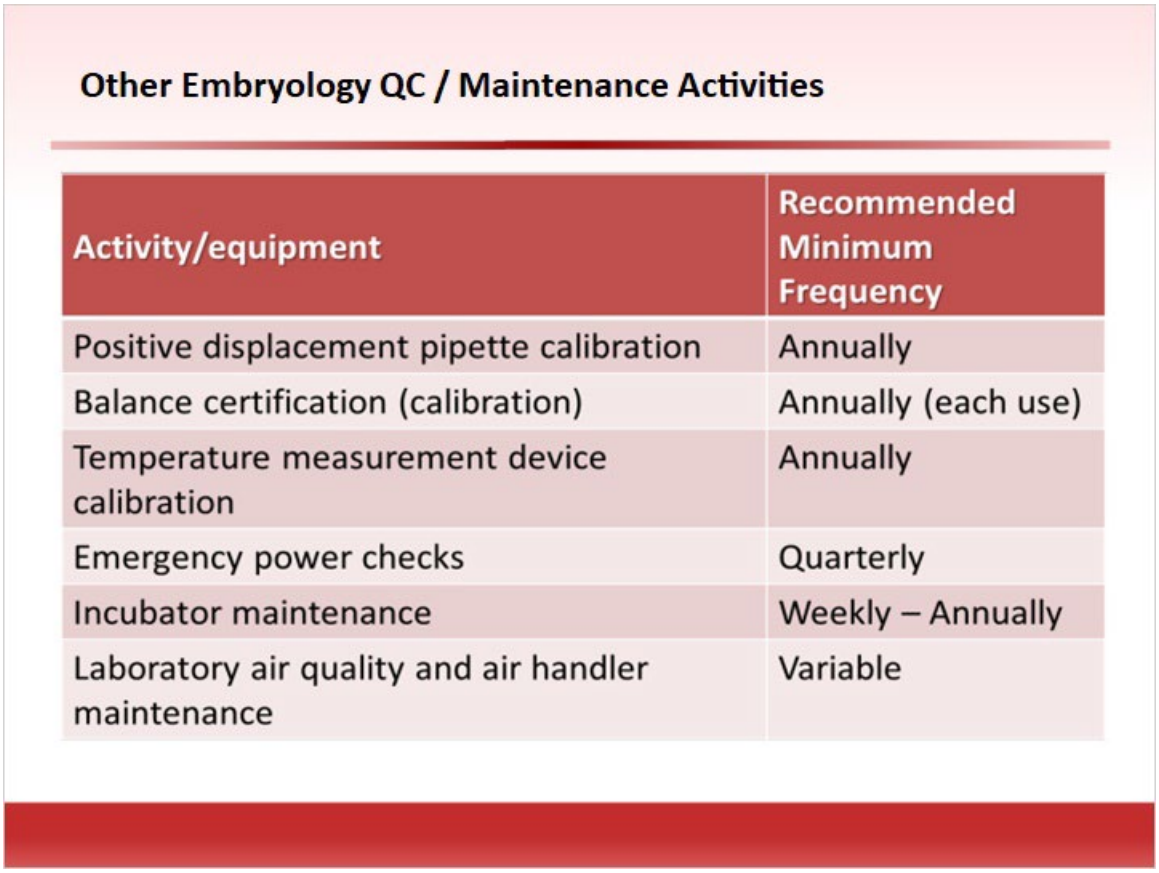
If the laboratory uses heat sterilization, a biological indicator should be included with each run to ensure that the heat sterilization oven achieves the temperature for the required duration.

The alarm system employed by the laboratory to detect equipment failure when the laboratory is not staffed should be tested at least quarterly. All alarms should be tested and the alarm call-out functionality confirmed.

An inventory of laboratory supplies including plasticware, micromanipulation tools, reagents, and culture media should be performed at a frequency relative to the needs and storage capacity of the laboratory.

Each eyewash station must be tested and flushed weekly.

1.18 Other Embryology QC / Maintenance Activities



Notes:

On an annual basis, positive displacement pipettes should be cleaned and calibrated. Additional periodic checks of pipette performance may be performed by weighing the amount of water displaced by the pipette.

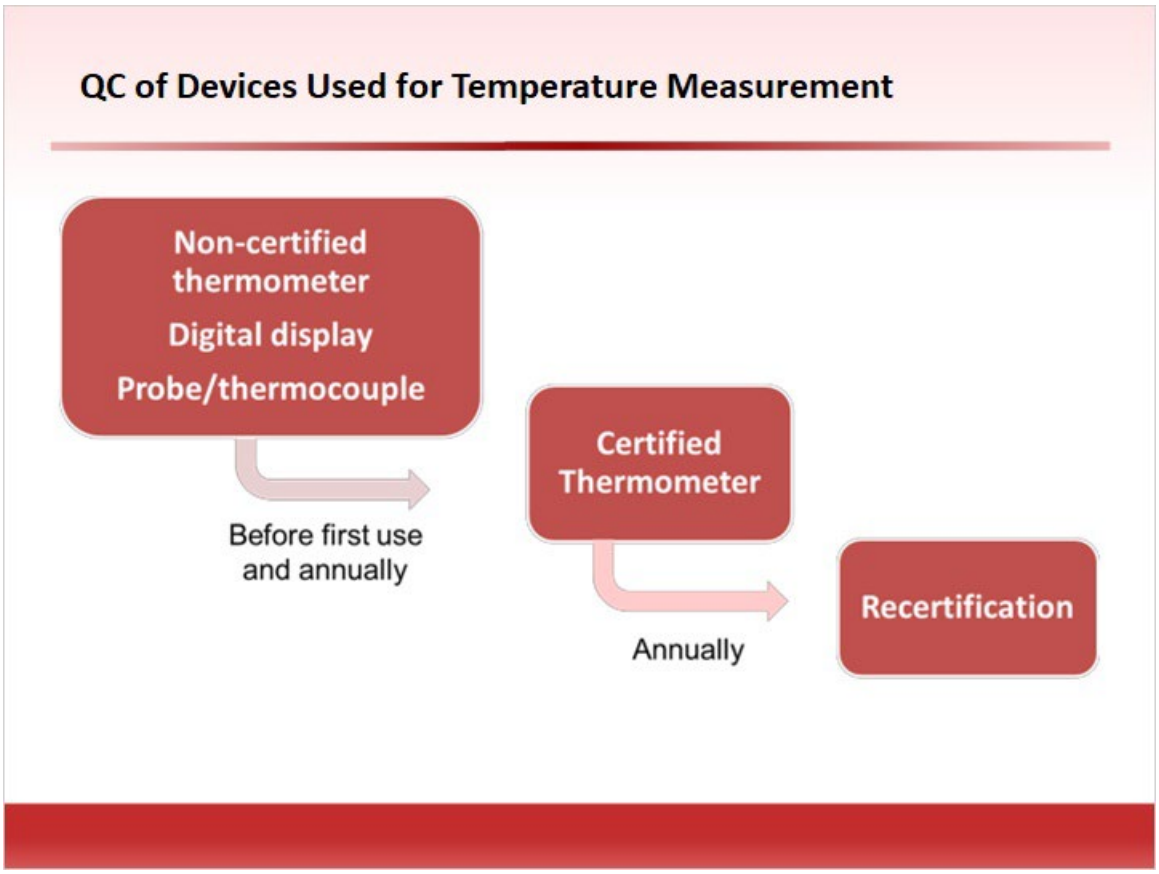
If the laboratory has a balance scale, annual certification is required. If media is produced in the laboratory, a calibrated set of balance weights should be used to confirm balance performance before measuring media ingredients.

Devices used for temperature measurement should be calibrated annually.

Testing of the emergency power back-up system should be performed on a periodic basis. Quarterly frequency is recommended, but the actual frequency is up to the laboratory director.

Details of incubator maintenance, assessment of the laboratory air quality, and air handler maintenance is next.

1.19 QC of Devices Used for Temperature Measurement



Notes:

The device used to measure temperature such as a noncertified thermometer, digital display, probe, or thermocouple should be checked against a thermometric device of known accuracy which is traceable to National Institute of Standards and Technology (NIST) standards, often referred to as a "certified thermometer," prior to initial use and at least annually.

The certified thermometer must be recalibrated or recertified prior to the date of expiration of the guarantee of calibration, typically annually.

1.20 Incubator Cleaning and Preventive Maintenance

Incubator Cleaning and Preventive Maintenance

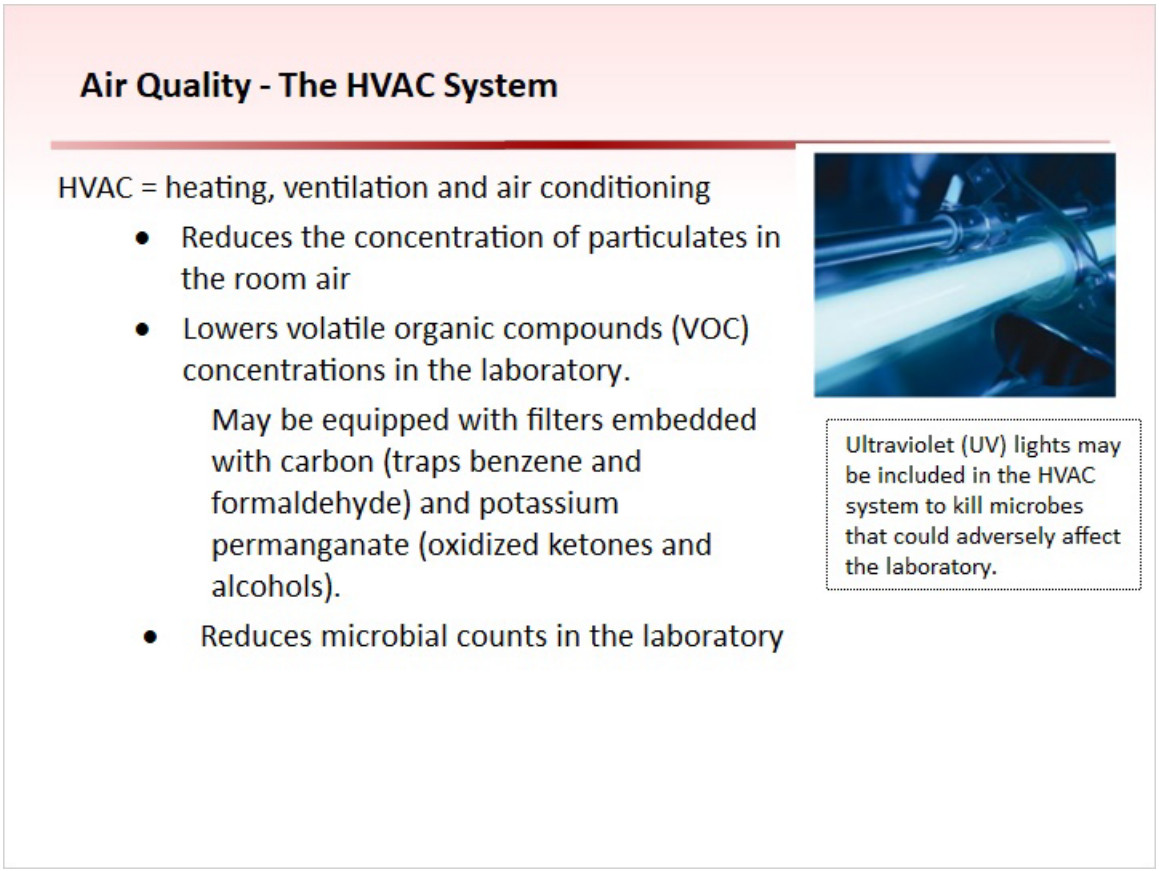
Preventive Maintenance	Minimum Frequency
Clean water pan or clean/replace reservoir and replenish sterile water supply	Weekly
Clean incubator shelves	Monthly
Replace HEPA filter and tubing	Annually
Replace gas line or incubator carbon filters if applicable	Manufacturer's recommendation
Clean and sterilize the incubator	2-4 times per year
If using a water-jacketed incubator, replenish rust inhibitor in water jacket	Biannually

Notes:

Incubator maintenance is multifaceted with varying degrees of frequency.
At least weekly, the water pan or reservoir should be cleaned and the sterile water replenished if the laboratory cultures embryos in a humidified environment.
Incubator shelves should be removed and cleaned monthly.
Incubators equipped with a HEPA filter need to be scheduled for filter replacement. The filter should be changed at least annually, but may need to be changed more frequently if the particulate count in the laboratory air is high.
Laboratories that have inline or incubator carbon filters need to schedule the filter replacement based on the filter manufacturer's recommendations or more frequently if desired.
The incubator is designed to support the growth of embryos as well as organisms that should not be nurtured in the incubator. Therefore, a cleaning and sterilization schedule must be determined for each incubator. The frequency of cleaning should be based on the level of humidity laboratory, the frequency of use, type of incubator, and the air quality of the lab. An incubator that is used for a few cases per week and is housed in a clean room may not need to be cleaned and sterilized as frequently as an incubator that has 12 openings per day and is housed in a room that is supplied with

unfiltered air.

1.21 Air Quality – The HVAC System



Notes:

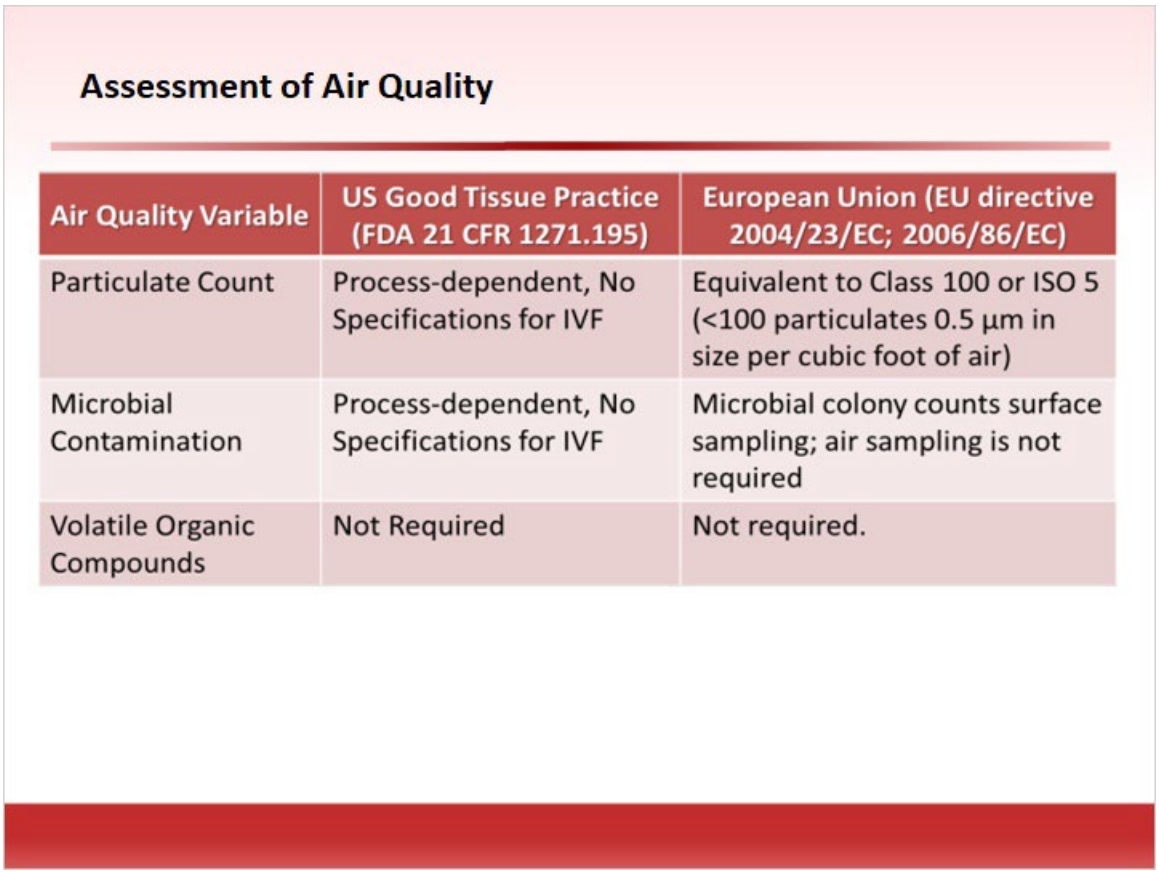
The heating, ventilation, and air-conditioning system, known as an HVAC system, is critical component of an embryology laboratory.

A properly designed HVAC system should support sufficient air exchanges to reduce the particulate count to the laboratory's goal. Some laboratories aim for a Class 100 or ISO 5 rating, which requires detection of fewer than 100 particulates >5µm per cubic foot of air.

The HVAC system may be equipped with filters embedded with carbon (traps benzene and formaldehyde) and potassium permanganate (oxidized ketones and alcohols) that remove volatile organic compounds before the air enters the laboratory. VOC levels have been shown to be inversely related to human embryo development.

Ultraviolet lights may be included in the HVAC system to kill microbes that could adversely affect the laboratory, especially if they enter the incubators!

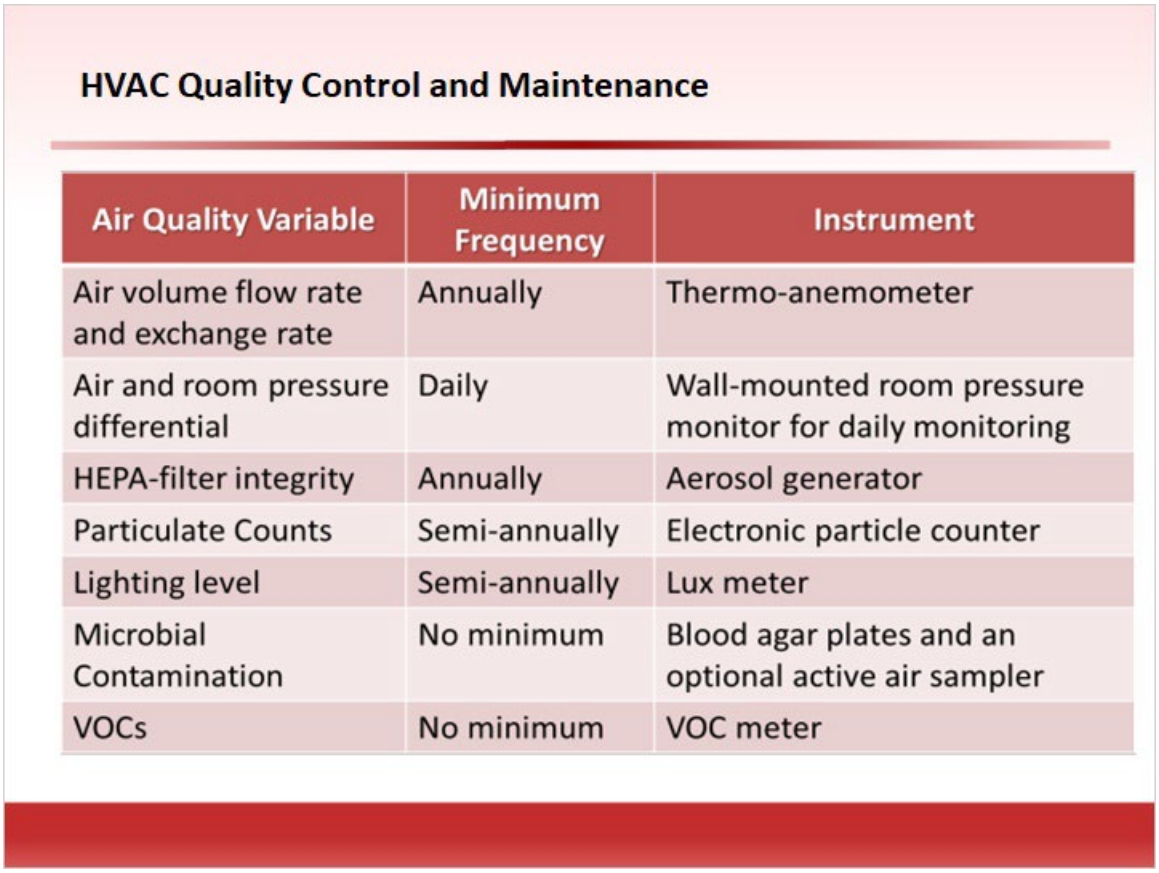
1.22 Assessment of Air Quality



Notes:

There are no current standards for air quality in embryology laboratories throughout the United States. Current Good Tissue Practice for Human Cells, Tissue and Cellular and Tissue-based products (also known as HDT/Ps) enforced by the US Food and Drug Administration excludes reproductive HCT/Ps. European counterparts have standards that are mandatory.

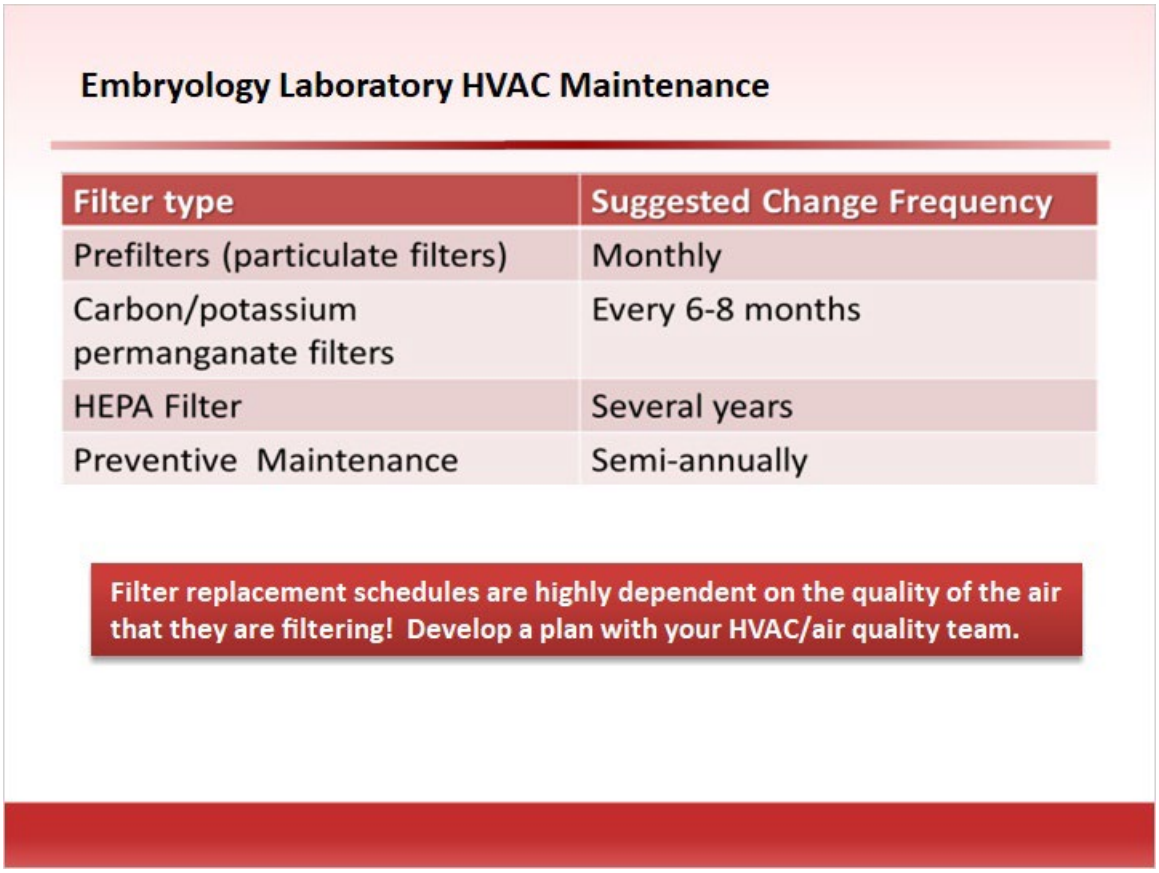
1.23 HVAC Quality Control and Maintenance



Notes:

Here is a list of some of the testing that may be included in an HVAC QC program for an embryology laboratory. Specific methodologies for each of these measures will not be discussed, but, a laboratory may choose to incorporate measurement of some or all of these air quality variables into their quality control program. Depending on the frequency that the laboratory chooses to perform air quality testing, they may opt to outsource at least some of the air quality testing to a firm that specializes in air quality assessment rather than purchasing and maintaining the equipment for air sampling.

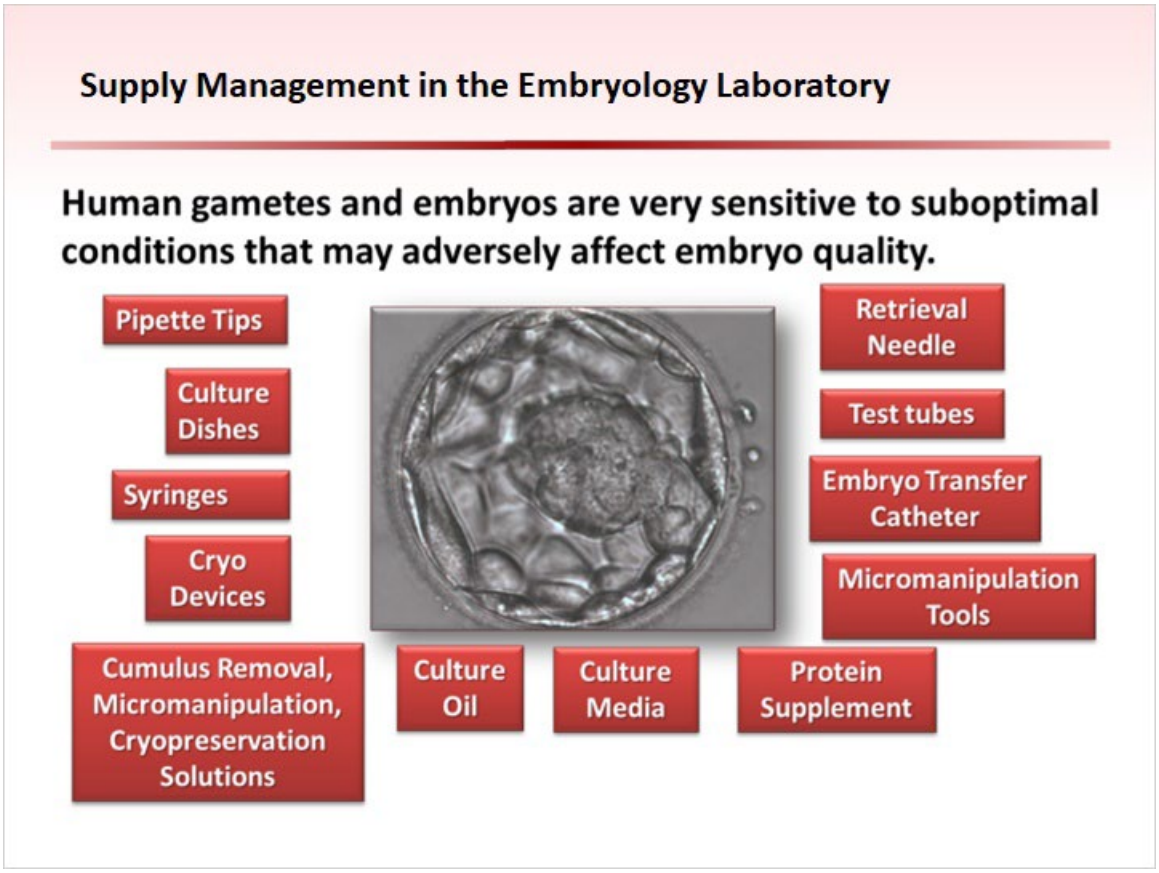
1.24 Embryology Laboratory HVAC Maintenance



Notes:

The HVAC system's maintenance plan depends on the outside air quality and the VOC load that is being added to the laboratory each time a bag of plasticware is opened. The plan needs to adapt to changing environments. For example, if there is an active construction site near the air handler intake, it may warrant changing the particulate pre-filters more frequently to assure that adequate intake air is maintained. If VOC levels in intake air have been elevated (for example, smoke from a nearby long-burning fire or increased traffic congestion that led to higher concentrations of automobile exhaust for several weeks), the carbon/potassium permanganate filters may need to be changed sooner than the schedule dictates.

1.25 Supply Management in the Embryology Laboratory

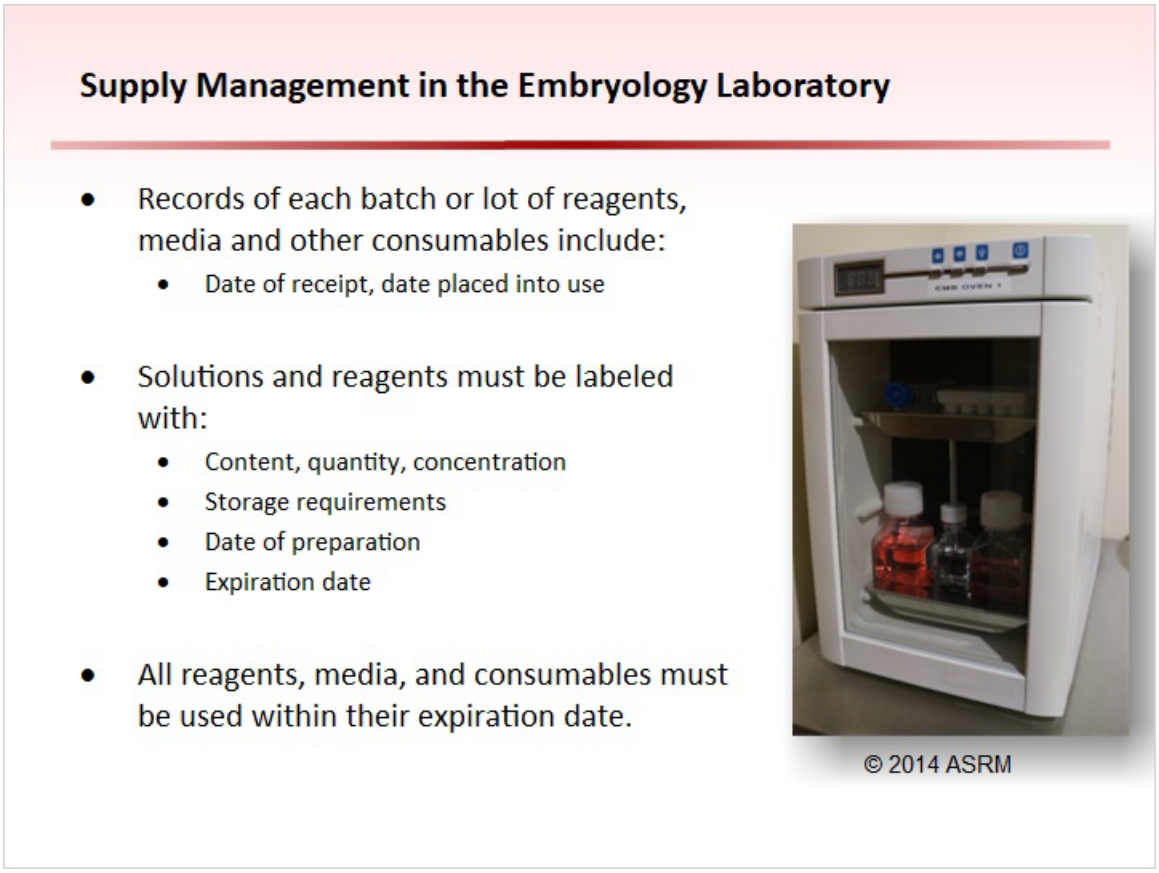


Notes:

The quality of consumables used in embryology procedures can impact the outcome. Human gametes and embryos are very sensitive to suboptimal conditions that may adversely affect embryo quality. Unfortunately, some consumables have been shown to have toxic effects on gametes and embryos. Therefore, in order to avoid subjecting gametes and embryos to toxic materials, all culture media, reagents, and other consumables that come in contact with gametes and embryos must be validated before use.

Once the lot of the product is cleared for use, the product number and lot number must be linked to each patient for continuous monitoring of product performance. This also will permit swift identification of cases that may have been affected by a recalled product or identify products that are associated with an adverse event.

1.26 Supply Management in the Embryology Laboratory



Notes:

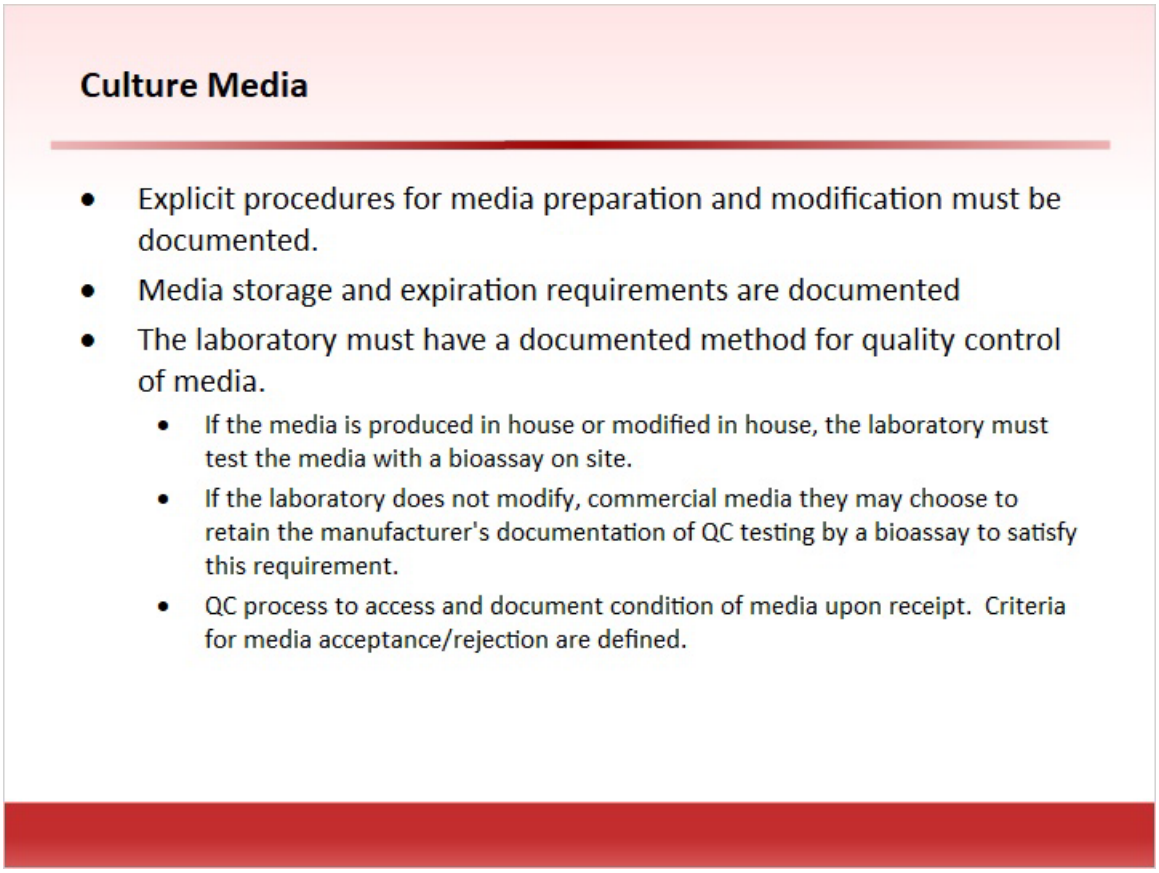
First is a review of the standards for supply management in the embryology lab as defined by the CAP/ASRM RLAP program to comply with the Fertility Success Rate and Certification Act of 1992 model program. The standards are specific for reagents and media, but the laboratory should include consumables under this umbrella.

Records of each batch or lot of reagents, media and consumables include date of receipt and the date placed into use.
Solutions and reagents must be labeled with:

Content, quality, and concentration of titer
Storage requirements
Date of preparation
Expiration date

All reagents must be used within their expiration date.

1.27 Culture Media



Notes:

For culture media, explicit procedures for media preparation and modification must be documented.
Media storage and expiration requirements must be documented.
The laboratory must have a documented method for quality control of media.

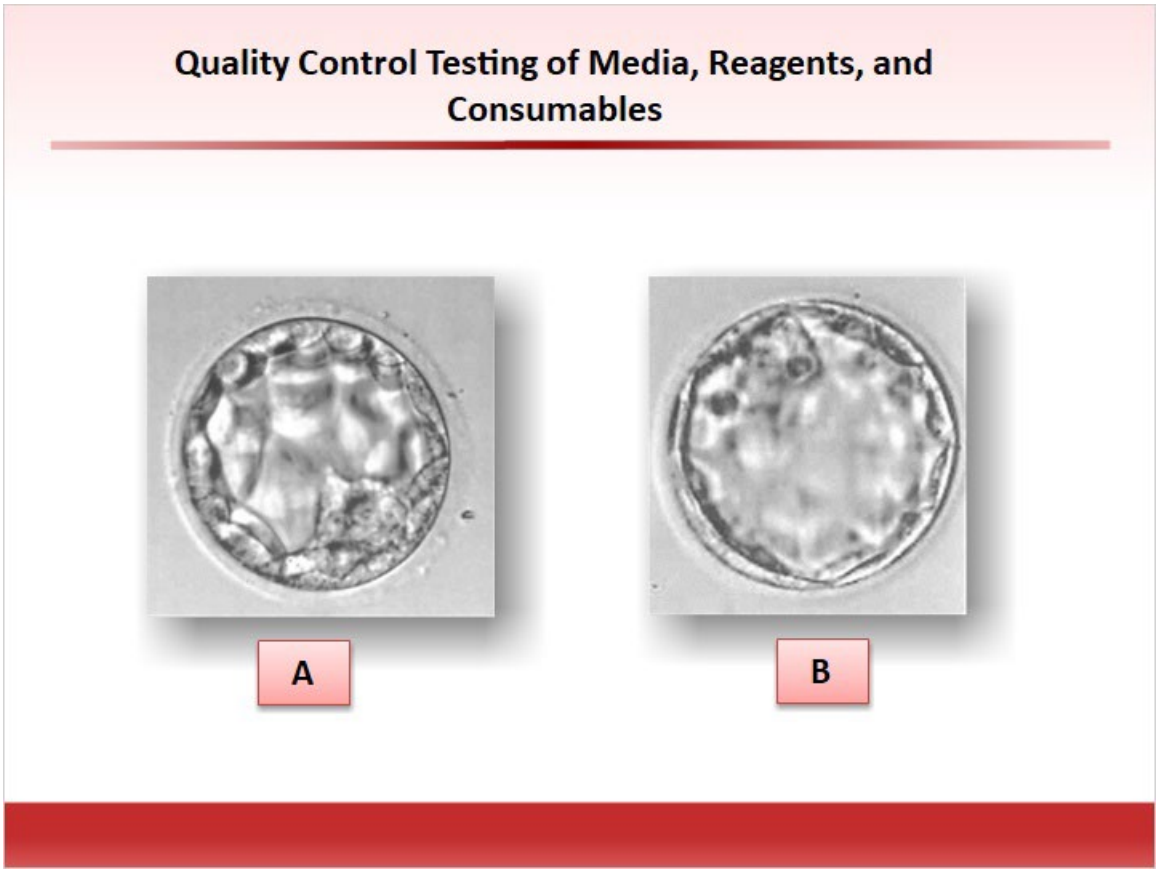
If media is produced or modified in-house, the laboratory must test the media on-site.

If the laboratory uses and does not modify commercial media they may choose to retain the manufacturer's documentation of QC testing by a bioassay to satisfy this requirement. That said, the laboratory director must determine how much to rely on the manufacturer's bioassay.

The laboratory must have a QC process to document the condition of media upon receipt. This includes media temperature and clarity, and condition of the shipping box of the media. This may catch a shipment that was overpacked with cold packs causing the media to freeze. This would not pick up on other post-production/post-release handling events that could adversely affect the culture media's ability to support embryo development. For example, a lot culture media was acceptable for release by the manufacturer but performed extremely

poorly in a bioassay and was rejected after it was received by the laboratory. The manufacturer discovered a handling error at the time of packing that led to the deterioration of the culture media. If the lab had not retested the media onsite and accepted the manufacturer's results, this inferior media would have been used for clinical cases.

1.28 Quality Control Testing of Media, Reagents, and Consumables



Notes:

Is there a way to assure that the products used in the embryology lab will support good embryo development and will provide more embryos that look like the embryo in picture A than B? The next section of this course will delve into testing options.

1.29 QC Testing Standards for Media and Reagents used in the Embryology Laboratory

QC Testing Standards for Media and Reagents used in the Embryology Laboratory

Culture Media and Micromanipulation Solutions	Cryopreservation/ Thawing Solutions	Protein Supplements
pH (post CO2 equilibration)	pH (post CO2 equilibration)	pH (post CO2 equilibration)
Osmolality	Osmolality	Osmolality
Limulus amebocyte lysate (LAL) endotoxin	LAL endotoxin	LAL endotoxin
Sterility (bacterial and fungal screen)	Sterility (bacterial and fungal screen)	Sterility (bacterial and fungal screen)
Bioassay	Bioassay after cryo-preservation and thawing	Bioassay
		Donors contributing to plasma protein fraction negative for HCV, HIV-1, HIV-2 Abs and HbsAG and screened for CJD

Notes:

Regardless of whether media and reagents are prepared in-house or purchased from a commercial manufacturer, the laboratory must have records of testing for each lot before they are released for use in the laboratory. As already noted, the product and lot numbers must be linked to each procedure so the laboratory may identify cases that may have been affected by a recalled product or identify products and lot numbers that are associated with an adverse event.

pH should be measured in a sample that has been equilibrated overnight at the same CO_2 concentration that is used for culture. The sample should be 37° C or measured with a pH meter with temperature correction. Several samples should be used for each analysis because the pH will vary with the duration of time between sample removal from the incubator and pH measurement. When possible, the same technologist should measure the pH so the samples are measured in the same manner.

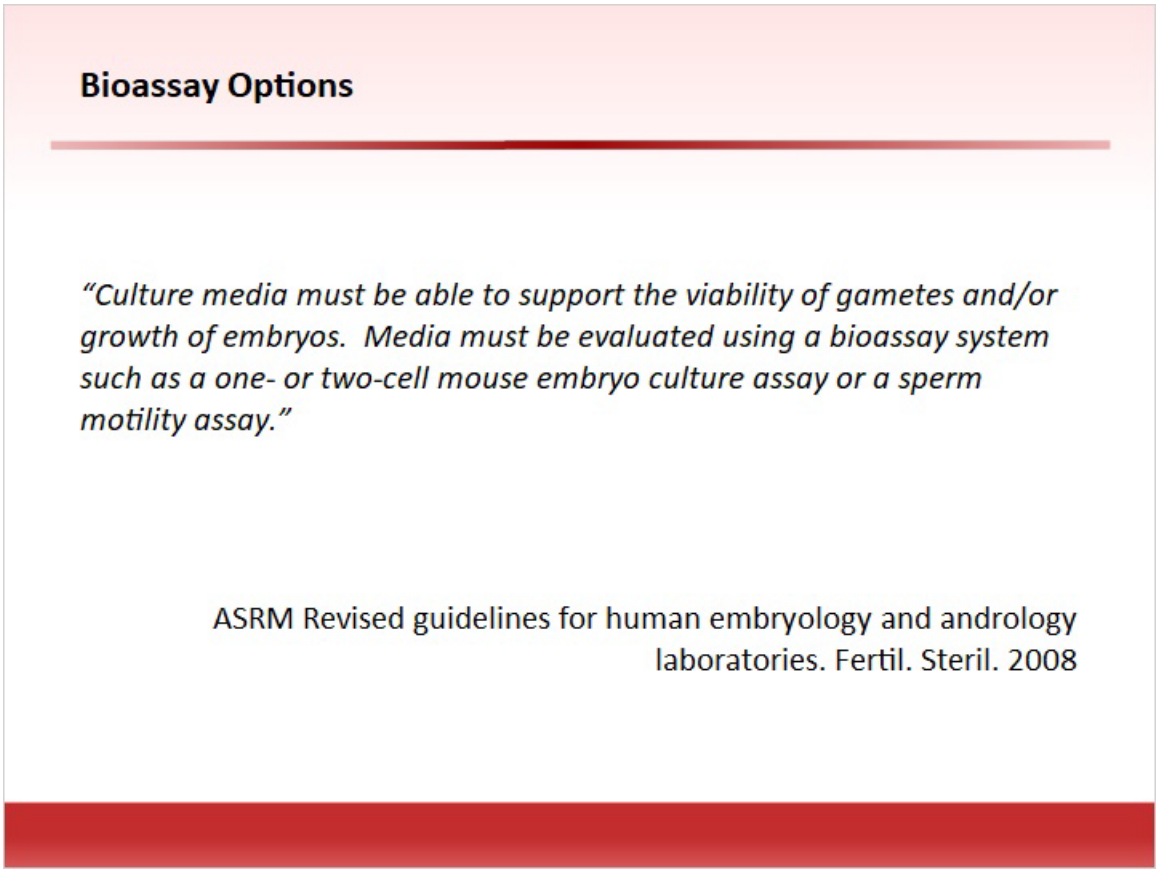
Multiple osmolality measurements should be measured from a single sample to obtain

an accurate value.

The limulus amebocyte lysate or LAL test is commonly used to test endotoxins. This assay relies on an enzymatic reaction and is typically outsourced to certified testing facilities as it is beyond the scope of most IVF laboratories. Water is a common source of endotoxins so if media is being prepared in-house, it is essential that the water is tested on a routine basis.

The methods for donor screening for plasma protein fractions in protein supplements are standardized and again, beyond the scope of most IVF laboratories. Laboratories are advised to find a reputable commercial source of protein for their embryo culture and to retest the protein with a bioassay in house before releasing it for use.

1.30 Bioassay Options



Notes:

Despite being required, there are no standards in the US for the bioassay that is used to

test media, reagents, or consumables. This is an excerpt from the ASRM Revised guidelines for human embryology and andrology laboratories:

> "Culture media must be able to support the viability of gametes and/or growth of embryos. Media must be evaluated using a bioassay system such as a one- or two-cell mouse embryo culture assay or a sperm motility assay."

1.31 Bioassay Options - Logistics

Bioassay Options - Logistics

Bioassay	Cell Type/State	Cell Source Considerations
Mouse embryo assay (MEA)	• Fresh one-cell • Fresh two-cell • Mouse IVF • Zona-free mouse embryo • Frozen one-cell • Frozen two-cell	Fresh embryos and mouse IVF require mouse maintenance. Animal care protocol required. Frozen embryos may be purchased from a vendor – expensive, but no animal housing required on site.
Human sperm motility assay	• Fresh Sperm	Fertile sperm donors. Consent and donor compensation may be required.
Hamster sperm motility assay	• Fresh Sperm	Requires hamster maintenance. 3 hamsters are typically used for each assay, but multiple items may be tested at once. Animal care protocol required.

Notes:

Several factors must be considered when determining which bioassay or bioassays should be used to test media, reagents, and contact material. The strength of the assay is dependent on its sensitivity and ability to reject the products that may not be optimal for gamete or embryo culture or handling. A bioassay that passes every item that enters the laboratory may be too robust and may not be able to detect toxins. An assay that frequently rejects items may be too sensitive. The laboratory must determine which bioassay is "just right" for its needs.

The 3 bioassays shown here are listed in order of frequency of reported use for

proficiency testing. Details about the variety of protocols and the assays' strengths and weakness will follow. When selecting a bioassay it is important to consider the logistics of obtaining the test material.

Animal housing, maintenance, and protocol approval by the animal care committee will be required if the laboratory opts to use fresh mouse embryos, mouse IVF, or the hamster sperm motility assay for their bioassay. The majority of the IVF laboratories in the United States do not have access to animal care facilities so their options are narrowed to a mouse embryo assay using frozen embryos or the human sperm motility assay. The mouse embryo assay and human sperm motility assay will be covered in detail.

1.32 Mouse Embryo Assay (MEA)

Mouse Embryo Assay (MEA)

There are several variations of the MEA that influence the assay's sensitivity, duration of the assay and total time required for the assay. The sensitivity of the assay has been shown to be affected by the following factors:

Start of the Assay	Assay Midpoints	Assay Endpoints
Age of the embryo	Division kinetics through the 3rd cell cycle[1]	96-hour expanded or hatching blastocyst formation
Embryos from IVF	% blastocysts at 78 hours of culture of 1-cell embryos	Blastocyst cell count
Zona-free embryos		ICM and trophectoderm cell counts
Mouse strain		Live pups following embryo transfer

[1] Wolff et al., Human Reproduction (2013) 28:1776-1782

Notes:

The mouse embryo assay is the only mammalian embryo assay that has been used for testing human IVF culture media and consumables. There are several variations on MEA the influence the assay's sensitivity, the duration of the assay, and the total time

required for the assay.

The sensitivity of the assay has been shown to be affected by the following factors:

- The sensitivity of the MEA is inversely related to the age of the embryo at the initiation of the culture. Thus in vivo-produced, one-cell embryos are more sensitive to adverse culture conditions than are two-cell stage embryos.
- Sensitivity has been reported to be further increased by using embryos derived from IVF. Removal of the zona pellucida has been shown to increase the sensitivity of mouse zygotes to culture conditions and endotoxins.
- The strain of the mouse can affect the sensitivity of the MEA. Embryos from inbred strains and their F1 hybrids are more tolerant of adverse culture conditions than are those from outbred strains.

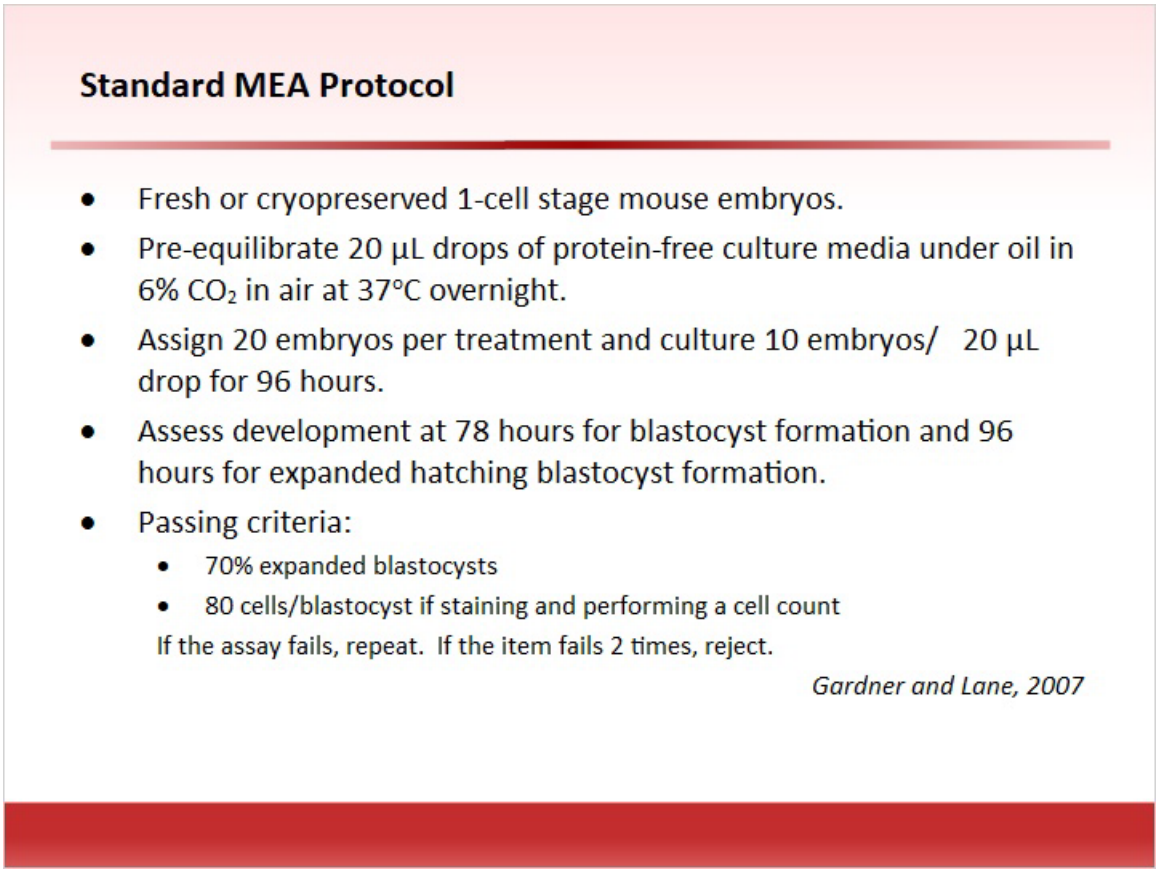
Wolff recently reported on the use of time-lapse imaging during the MEA. Cell cycle events occurring during the first 72 hours of culture were more sensitive to embryo toxicity than blastocyst formation.

Labs that do not have time lapse in-house should include a 78-hour observation of embryos and require at least 60% of the 1-cell stage embryos to have reached the early blastocyst stage by 78 hours of culture in the passing criteria.

The endpoint that is chosen for the MEA also can influence the test's ability to detect embryo toxins. Using morphology alone, one cannot distinguish between the expanded or hatching blastocysts with 30 cells versus 80 cells. Addition of cell number as an endpoint has been shown to improve assay sensitivity over using 96-hour embryo morphology. Performing embryo transfers at the conclusion of the culture period is the ultimate means of assessing a test item's ability to support embryo development, but most laboratories do not have the means *or time* to carry the MEA out to this extent.

Most manufacturers opt to use the MEA to satisfy the bioassay requirement. Laboratories that choose to rely on the manufacturers' bioassay need to be aware of these different approaches and that the most sensitive assay may not be required by the manufacturer. Items that arrive at their laboratory may have toxins that may adversely affect the embryos, but were not detected by the MEA.

1.33 Standard MEA Protocol



Notes:

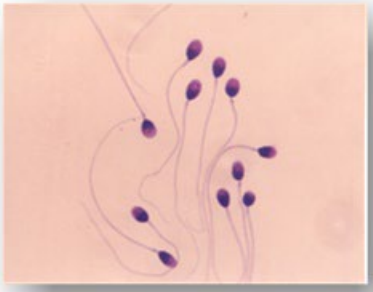
The following is a summary of a mouse embryo assay protocol described by Gardner and Lane in 2007. The protocol was written for fresh embryos, but cryopreserved and thawed embryos may be used.

- Prepare 20 microliter drops of the culture media to be tested. It may be a direct test of the culture media or an indirect test of an item that was exposed to the culture media. Do not add protein to the media unless testing the protein, as albumin can chelate toxins and reduce the sensitivity of the assay.
- Cover the drops with culture oil and pre-equilibrate the dish overnight in 6% CO_2 in air at 37°C.
- 20 embryos are required per treatment or test.
- Assess development at 78 hours for blastocyst formation and 96 hours for expanded hatching blastocyst formation.
- The criteria for an item to pass the assay includes: 70% expanded blastocysts and, if performing a cell count to enhance the sensitivity of the assay, 80 cells/blastocyst
- If the assay fails, repeat. If the item fails two times, reject the item.

1.34 Human Sperm Motility Assay

Human Sperm Motility Assay

Benefits	Disadvantages
Low cost	The sperm is not an embryo
Many items may be tested at once	May not detect toxins that could interfere with fertilization or embryo development.
Quick – 48 hours	



The 1-cell MEA was 20-times more sensitive to the culture oil supplemented with a peroxide than the human sperm motility assay.

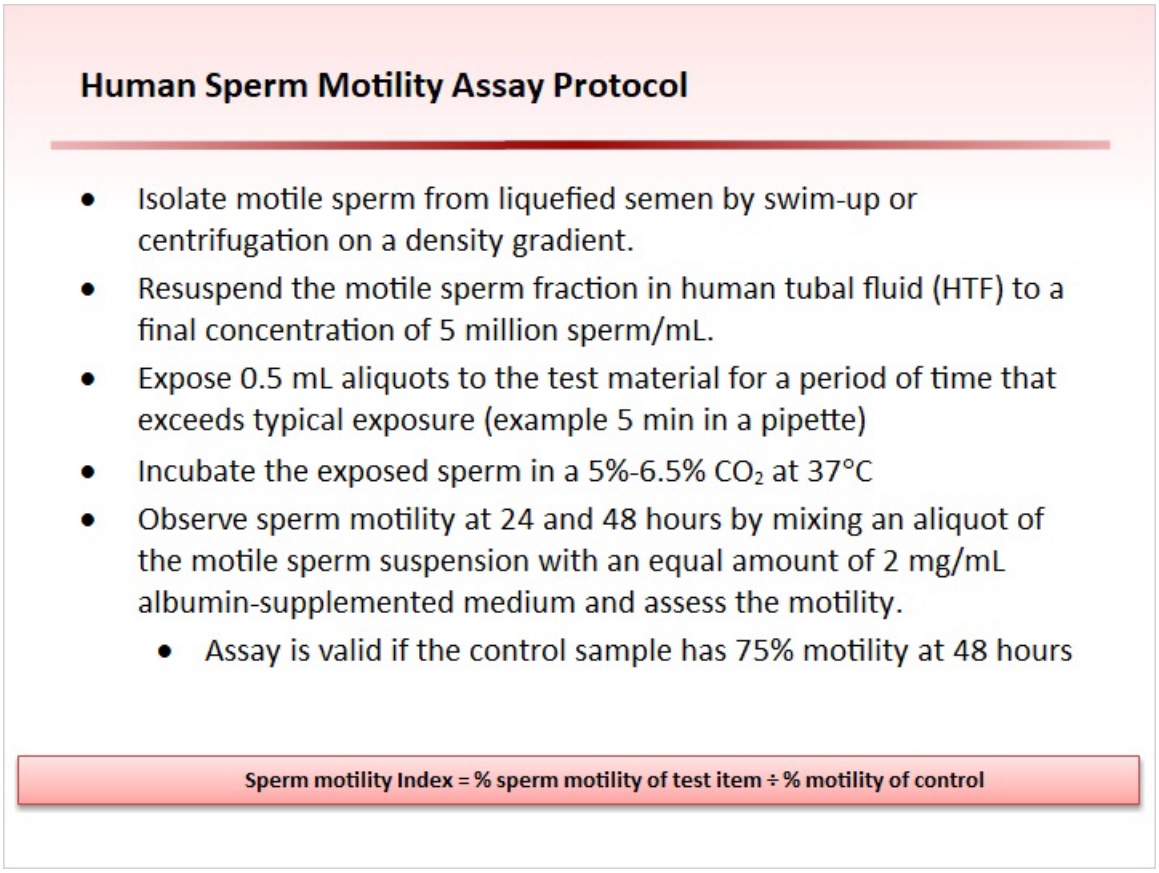
Hughes et al., Fertil. Steril. 2010

Notes:

The human sperm motility assay has many attributes that appeal to embryology laboratories including: low cost, the availability of multiple aliquots of motile sperm to potentially test several items in one assay, and the relatively short time that the laboratory is able to arrive at a conclusion.

Despite these advantages, the sperm is not an embryo and therefore, the test cannot detect all factors that may be toxic to embryos. For example, Hughes and coworkers compared the ability of the 1- and 2-cell MEA and the human sperm motility assay to detect peroxide in culture oil (a common embryo toxin that may develop during oil transport and storage). They found the 1-cell MEA to be twice as sensitive as the 2-cell MEA and 20 times more sensitive than the human sperm motility assay.

1.35 Human Sperm Motility Assay Protocol



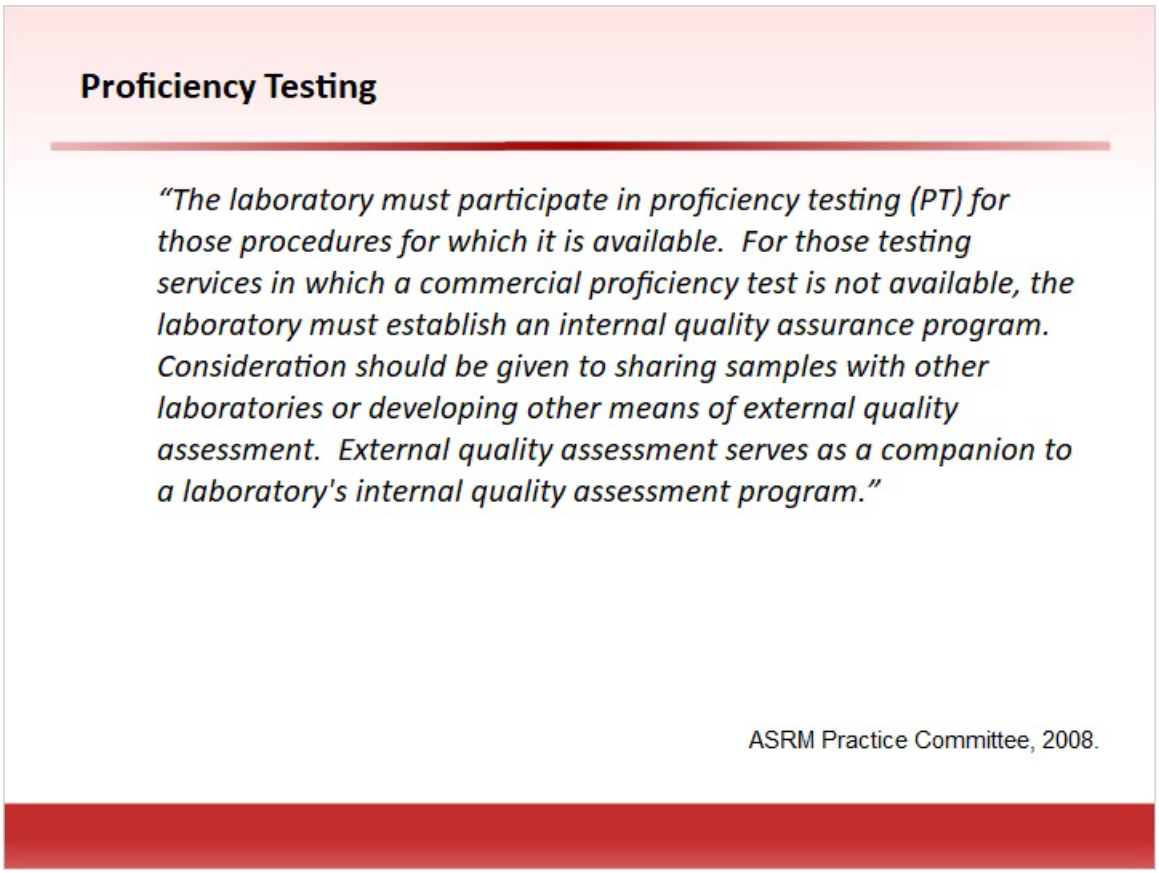
Notes:

Despite the lack of sensitivity to embryo toxins, the human sperm motility assay may be suitable for testing items that come in contact with the sperm prior to IVF.

Start by isolating motile sperm from liquefied semen by swim up or centrifugation on a density gradient.
Resuspend the motile sperm fraction in human tubal fluid (HTF) to a final concentration of 5 million sperm/mL.
Expose 0.5 mL aliquots to the test material for a period of time that exceeds typical exposure (for example 5 minutes of exposure to a graduate pipette exceeds the typical seconds that sperm would be exposed to this test item during semen processing.)
Incubate the exposed sperm in an environment of 5%-6.5% CO_2 in air at 37° C.
Observe sperm motility at 24 and 48 hours by mixing an aliquot of the motile sperm suspension with an equal amount of 2 mg/mL albumin-supplemented medium and assess sperm motility. The albumin helps prevent the sperm from sticking to the slide and coverslip.
The assay is valid if the control sample has 75% motility at 48 hours. The sperm motility index (SMI) should be calculated by dividing the percent sperm motility of the test item

by the percent motility of the control. The laboratory will need to determine thresholds for test item acceptance based on the sperm motility index.

1.36 Proficiency Testing

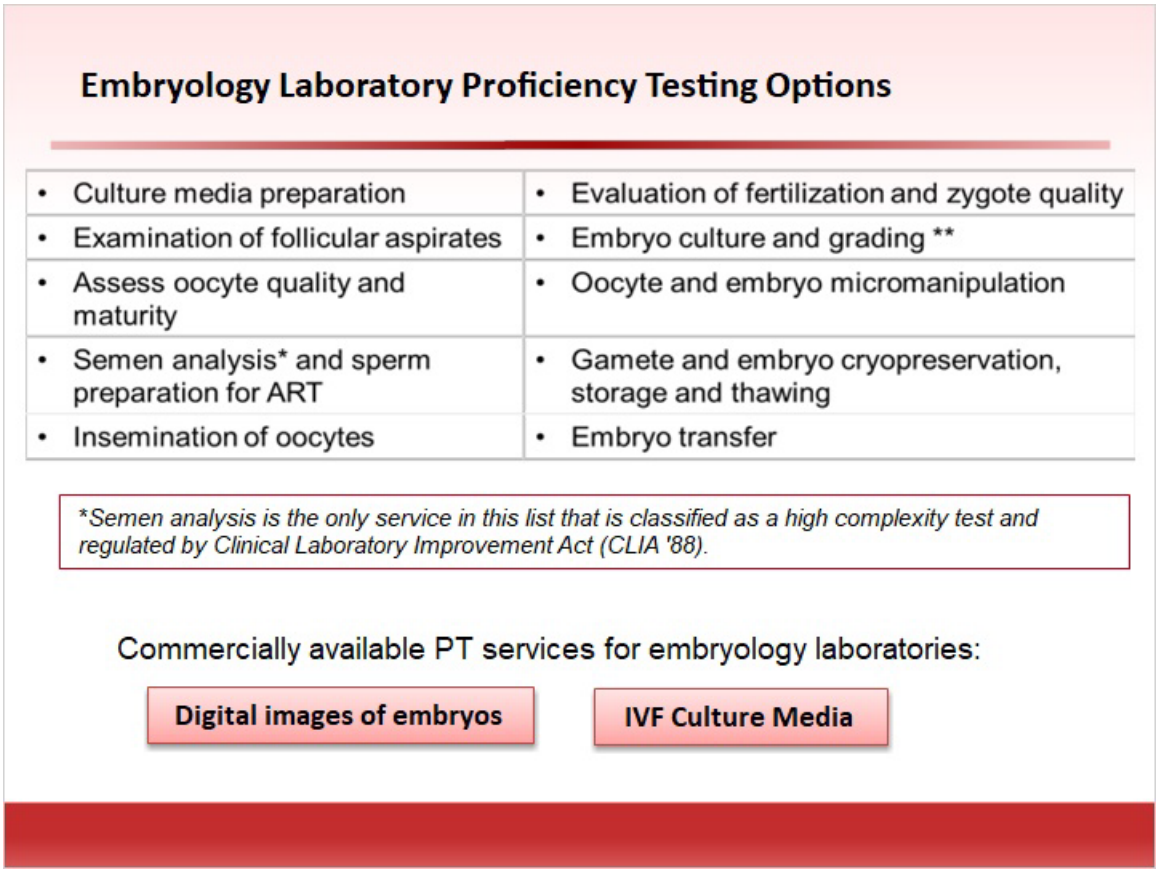


Notes:

The ASRM Practice Committee states:

> *"The laboratory must participate in proficiency testing (PT) for those procedures for which it is available. For those testing services in which a commercial proficiency test is not available, the laboratory must establish an internal quality assurance program. Consideration should be given to sharing samples with other laboratories or developing other means of external quality assessment. External quality assessment serves as a companion to a laboratory's internal quality assessment program."*

1.37 Embryology Laboratory Proficiency Testing Options



Notes:

In revisiting the list of activities performed in the embryology lab, the challenges of developing proficiency testing for each service must be considered. Clearly, proficiency testing for examination of follicular aspirates and oocyte or embryo micromanipulation are difficult to imagine. Proficiency testing programs for semen analysis and any other andrology services performed in the embryology laboratory are readily available and typically involve two testing events per year. Likewise, digital images of embryos are available from the College of American Pathologists and American Association of Bioanalysts proficiency testing services and embryology laboratories may subscribe to the American Association of Bioanalysts' IVF embryo culture media proficiency test for the laboratory's bioassay, measurement of media pH, and osmolarity.

1.38 Proficiency Testing – CAP All Common Checklist

Proficiency Testing - CAP All Common Checklist

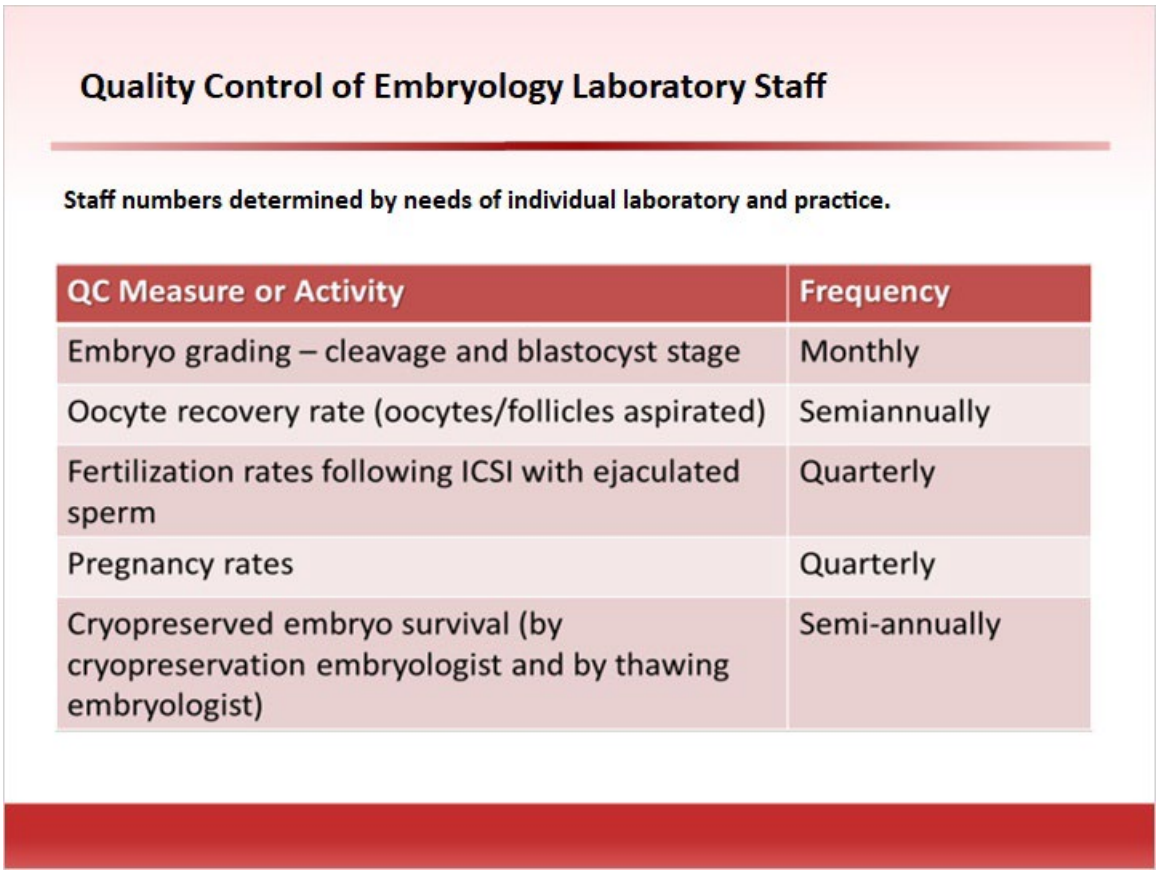
1. The laboratory must have written procedures for proficiency testing sufficient for the extent and complexity of testing performed in the laboratory.
2. The laboratory integrates all PT samples within the routine laboratory workload, and those samples are analyzed by personnel who routinely test patient/client samples, using the same primary method system as for patient/client/donor samples.
3. There is ongoing evaluation of PT and alternative assessment results, with prompt corrective action taken for unacceptable results.
4. There is a policy that prohibits inter-laboratory communication about PT samples until after the deadline for submission of the data to the PT provider.
5. There is a policy that prohibits referral of PT specimens to another laboratory or acceptance from another laboratory.

Notes:

These are highlights of proficiency testing requirements from the College of American Pathologists' All Common Checklist required for laboratory accreditation.

- The laboratory must have written procedures for PT sufficient for the extent and complexity of testing performed in the laboratory.
- The laboratory must integrate all PT samples within the routine laboratory workload, and those samples are analyzed by personnel who routinely test patient/client samples, using the same primary method system as for patient/client/donor samples.
- There is ongoing evaluation of proficiency testing and alternative assessment results, with prompt corrective action taken for unacceptable results.
- There is a policy that prohibits inter-laboratory communication about PT samples until after the deadline for submission of the data to the PT provider. For example, if the laboratory wishes to use the samples to assess inter-embryologist variation, they may certainly do so, but not until the laboratory's results have been reported to the PT provider and the deadline for data submission has passed.
- The laboratory must have a policy that prohibits referral of PT specimens to another laboratory or acceptance from another laboratory.

1.39 Quality Control of Embryology Laboratory Staff



Notes:

The number of staff required by a laboratory will vary based on each staff member's efficiency and the way the cases are distributed throughout the year. Even in the smallest program, more than one person needs to be available to perform embryology procedures in the laboratory. This may involve arranging for an off-site "back-up" embryologist who could drive or fly in from another laboratory to step in should the primary embryologist not be available.

Regardless of how few or how many embryologists are in the laboratory, the laboratory director must recognize that performance of staff is an integral part of the quality control or quality management program.

The laboratory director must ensure that all personnel receive appropriate training and demonstrate continued competency for embryology laboratory procedures performed. All activities associated with these endeavors must be documented and the documents must be organized so they may be readily reviewed.

Here is an example of typical QC or Quality Assurance activities related to staff performance. The frequencies are merely suggestions and need to be adjusted based on the number of cases performed in the laboratory. The director must establish acceptable ranges and have an action plan when corrective action is required.

The laboratory director must assure that all embryologists are grading the embryos' quality and assigning development scores in a similar fashion.

Individual performance measures may include:

- Oocyte recovery rate
- Fertilization rate following ICSI, including the abnormal fertilization rate and the percentage of oocytes degenerating after ICSI
- Pregnancy rates, ideally limited to good prognosis patients such as women <35 years undergoing elective single embryo transfer
- Cryopreserved embryo survival by both the embryologist performing cryopreservation as well the staff member performing thawing or warming.

Again, this is only a brief list of key indicators that may be incorporated into a quality management program to assure that all staff are delivering the same high quality service and patient care.

1.40 Summary

Notes:

This course has reviewed the ASRM recommendations for the embryology laboratory and provided a fairly detailed discussion about the elements of a quality control program that are required for laboratory certification.

While the development of a quality control program may seem overwhelming, once in place, the rewards clearly outweigh the effort that is required for program establishment and maintenance.

1.41 Thank you!

Notes:

Thank you for participating in this educational activity.