

Section VII

Laboratory Guidelines for the Practice of Cell Based Medicine

Chapter 1-Introduction and Historical Context

The ICMS is an organization with the mandate to produce laboratory and clinical guidelines for minimal culture expansion of autologous, adult stem cells (A-ASC's). The ICMS maintains a laboratory best practices committee consisting of the following categories of experts:

- Physicians directly involved in the supervision of adult stem cell laboratories and who have direct clinical experience with adult stem cell therapy
- Doctorate level researchers involved in adult stem cell research
- Clinical laboratory directors involved in cGTP/cGLP processing of adult stem cells

The committee reviewed existing guidelines for laboratory and tissue practices and incorporated many concepts into the ICMS guidelines. However, since A-ASC therapy has very specific needs, many of the current guidelines needed updating or other items added. Most regulatory guidelines for processing human tissue are focused on preventing allogeneic disease transmission, whereas the use of A-ASCs involves no such disease transmission risk (other than cross contamination). Other examples include the differences between whole tissue processing and the processing of cellular material.

As historical context, the evolution of *in vitro* fertilization (IVF) illustrates the development of a minimal culture expansion process. The first IVF procedures were performed in the late 1970's. In the 1990's, fertility specialists had decided that extended cell culture of the fertilized eggs to the blastocyst stage resulted in improved implantation outcomes. Using human blastocysts marked the transition from IVF as a simple procedure for transplanting fertilized eggs to implanting human embryos derived from extended cell culture. Advances in genetic screening will likely require additional blastocysts to be cultivated and longer culture times may be needed for genetic testing.

Fertility specialists have maintained that Assistive Reproductive Technology (ART) cell culture techniques is the practice of medicine and the not the manufacturing of a biologic drug. While the FDA has proposed guidelines to regulate IVF clinics (21 CFR parts 50, 56, and 312-Oocyte cytoplasm transfer), the agency has failed to classify IVF culture as manufacturing a biologic drug. The CDC has published guidelines for Assistive Reproductive Technology (ART) clinics, intended to assist states in producing their own certification programs. However, federal oversight here is not mandated. As a result, the American Society for Reproductive Medicine and the Society for Assisted Reproductive Technology (SART) have put forth their own criteria for certification in order to maintain the standards required for obtaining

accreditation. The certification of most ART labs is either through the College of American Pathologists Reproductive Laboratory accreditation program or the Joint Commission on Accreditation of Healthcare Organizations. ICMS is following the lead of ART providers in producing its own guidelines, similar to the ART guidelines promulgated by ASRM and SART.

Chapter 2: Adult Stem Cell Types

There are multiple cell types present in adult tissues. These adult cells range from the most differentiated cell types (functioning parenchyma and stroma) to the least differentiated cell type (totipotent stem cells). Each cell type within this group has its own unique attributes and cell culture requirements.

- A-Differentiated Cells (DCs)
- B-Progenitor Cells (PCs)
- C-Germ Layer Lineage Stem Cells (GLSCs)
- D-Pluripotent Stem Cells (PSCs)
- E-Totipotent Stem Cells (TSCs)

A cell can be classified as a differentiated cell (DC) (i.e., parenchyma or stroma) if it is derived from a single cell, has a lifespan limited to less than 50 population doublings, and is a functioning portion of the tissue.

A cell can be classified as a progenitor cell (PC) if it is derived from a single cell, it has a defined biological clock of 50-70 population doublings, and is the immediate precursor for adult differentiated cell types.

A cell can be classified as a germ layer lineage stem cell (GLSC) if it is derived from a single cell, it has unlimited proliferation potential and forms all cell types within a single germ layer lineage, i.e., ectoderm, mesoderm, and endoderm.

A cell can be classified as a pluripotent stem cell (PSC) if it is derived from a single cell, it has unlimited proliferation potential, it can form multiple cell types from all three germ layer lineages, but cannot produce gametes (sperm or ova) or placenta cells.

A cell can be classified as a totipotent stem cell (TSC) if it is derived from a single cell, it has unlimited proliferation potential, it can form multiple cell types from all three germ layer lineages, and it can produce gametes (sperm & ova) and placental cells.

Chapter 3: Lab Practice Requirements

These requirements govern the methods used in, and the facilities and controls used for, the processing of biologic samples for culture of A-ASC's. The core requirements fall into the following categories:

- A-Procedures
- B-Lab Facilities and Environmental Control
- C-Equipment
- D-Supplies and Reagents Management and Control
- E-Receipt and Processing of Tissue
- F-Labeling Controls
- G-Storage Requirements
- H-Transfer of A-ASC's

Section A- Procedures

Standard operating procedures should describe in detail the activities performed in the laboratory so as to provide uniformity, consistency and reliability in each of the activities performed in the laboratory, reduce systematic errors, and provide training and guidance for new staff. The procedures should define the field of application and adequately and appropriately prevent the introduction, transmission, or spread of communicable diseases and contamination by microbiologicals. Each procedure should be written clearly and without ambiguity so that it can be understood by staff with and without experience. Each step of performing the procedure should be described in detail. New and revised standard operating procedures should be reviewed by staff prior to implementation. Review and associated training should be documented.

Patient record notebooks can be in paper or electronic formats. If electronic, adequate computer back up procedures should be in place to prevent data loss. This includes nightly off-site back-up of all data. If electronic records of notebooks are kept, the electronic software must support GLP and GTP compliance with electronic signature functions. Scanned copies of original paper form documents can be kept. These files are intended for the recording of data, observations, and non-conformance to the standard operating procedure in a usable and permanent manner. Data generated in the laboratory is recorded in notebooks containing patient information, original observations, calculations, and raw data. Enter all observation and data directly into the notebook in legible black or dark blue permanent ink or in suitable electronic format. Record all the materials and equipment used; include serial numbers, lot numbers, manufacturer's name, catalogue or reference number, dates of receipt and expiration date – where applicable. Initial and date each page (or electronic equivalent). Verify and date each page within a timely manner. Cross out errors with a single line. Identify errors to include date of correction, reason why a correction was made, and initials. Records will be kept for at least 10 years after the date of administration. If the administration date is unknown, records will be kept at least 10 years after the date of distribution. Retain records of A-ASC in cryostorage.

B- Lab Facilities and Environmental Control

Cell culture requires strict control of infection prophylaxis and contamination. Any facility used in the processing A-ASC's must be of suitable size, construction, and location to prevent contamination of A-ASC's with communicable disease agents, infectious contaminants and microorganisms. The facility should be in a good state of repair. Lighting, ventilation, plumbing, and drainage should be adequate to prevent the introduction, transmission, or spread of contaminants or communicable disease. The facility should be operated in a manner to minimize risks to the health and safety of both patients and employees.. Equipment used in the facility should be adequately maintained and calibrated. Laboratory staff training, continuing education and continued competency for the performance of all operations should be documented

The facility used in the processing of A-ASC's should operate under Good Laboratory Practice (GLP) procedures, i.e., be kept in a clean, sanitary, and orderly manner, to prevent the introduction, transmission, or spread of communicable disease or the introduction of infectious agents and microbiologicals. Potentially contaminated sewage, trash, and other refuse should be autoclaved, incubated in bleach for a minimum of 24 hours, or disposed through a service that documents safe disposal of medical waste. All other sewage, trash, and other refuse should be disposed in a timely, safe, and sanitary manner.

The facility used in the processing of A-ASC's should be divided into separate or defined areas of adequate size for each operation that takes place in the facility (for example cell culture, tissue processing, tissue handling, reagent preparation), or other control systems to prevent improper labeling, contamination, or cross-contamination.

Procedures for routine facility cleaning and sanitation should be established and maintained. These procedures should assign responsibility for sanitation and should describe in sufficient detail the cleaning methods to be used and the mandatory schedule for cleaning the facility. Records of all cleaning and sanitation activities performed to prevent contamination of should be maintained. Cleaning records should be retained after their creation and available upon request.

Proper environmental control systems must be utilized where circumstances could reasonably be expected to cause contamination or cross-contamination of A-ASC's. Proper conditions for operations should be environmentally controlled for equipment to prevent accidental exposure of A-ACS's to communicable disease agents.. The following systems should be provided:

- (1) Temperature and humidity controls. In humid areas, dehumidifiers should be used to control the spread of infectious agents in the lab.

(2) Ventilation and air filtration. Cell culture areas require a positive pressure environment. Human tissue and A-ASC's should only be handled in Biological Safety Cabinets with classifications of Class II or above designed with HEPA filtration.

(3) Cleaning and disinfecting of rooms and equipment should be undertaken to ensure aseptic processing operations. Disinfectant should be approved by the equipment manufacturer and OSHA for use of decontaminating blood borne pathogens and microbiologicals or by steam pressure autoclaving, hot air drying, or any other acceptable procedure.

(4) Only one patient sample should be handled in any defined work area at a time. This area should be sanitized using proper antiseptic technique between patient samples.

(5) Maintenance of equipment should be undertaken to control conditions necessary for aseptic processing operations by calibrating and validating equipment on a routine schedule.

Environmental control should include inspections of systems and equipment on at least a daily basis. You should inspect each environmental control system periodically to notationally verify that the system, including necessary equipment, is adequate and functioning properly. You should take appropriate corrective action as necessary.

The environment should be monitored. You should monitor environmental conditions where environmental conditions could reasonably be expected to cause contamination or cross-contamination of A-ASC's or equipment, or accidental exposure of A-ASC's to communicable disease agents. Where appropriate, you should provide environmental monitoring for microorganisms.

You should document and maintain records of, environmental control and monitoring activities. This is to be performed on a daily basis by lab personnel and a review of the daily records should be performed.

Section C-Equipment

Equipment should be of appropriate design for its use, with barrier functions, and should prevent the introduction of infectious agents or cross contamination between patient samples. Equipment should be suitably located and installed to facilitate operations, including cleaning and maintenance. Any automated, mechanical, electronic, or other equipment used for inspection, measuring or testing should be inspected regularly for its ability of producing valid results. You should clean, sanitize, and maintain equipment according to established schedules.

You should establish and maintain procedures for cleaning, sanitizing, and maintenance of equipment to prevent malfunctions, contamination or cross contamination, accidental exposure of A-ASC's by infectious agents or microorganisms.. Where appropriate, routine calibration of your instruments and equipment should be performed and documented according to established manufacturer's procedures.

Section D-Supplies and Reagents Management and Control

You should not use supplies and reagents until they have been verified to meet specifications designed to prevent circumstances that increase the risk of the introduction, transmission, or spread of infectious diseases. Verification may be accomplished by the establishment that uses the supply or reagent, or by the vendor of the supply or reagent.

Reagents used in processing and preservation of A-ASC's must be sterile, where appropriate. You should validate and/or verify the processes used for production of in-house reagents.

You should maintain the following records pertaining to supplies and reagents:

- Records of the receipt of each supply or reagent, including the type, quantity, optimal storage temperature, manufacturer, reference number (catalog number), lot number, date of receipt, and expiration date;
- Records of the verification of each supply or reagent, including test results or, in the case of vendor verification, a certificate of analysis from the vendor; and
- Records of the lot of supply or reagent used in the processing in each A-ASC
- MSD sheets of all reagents used in the laboratory

Fetal Calf/Bovine Serum (FBS) normally contains inductive agents (i.e., bioactive factors) that can alter/induce *in vitro* the differentiative state of uncommitted adult stems to commit to certain tissue lineages. As a result, the ICMS does not recommend the use of FBS, unless it is processed under the strictest procedural controls and purchased from a reliable source dedicated to that specific purpose.

As a result of the BSE transmission risk, ICMS recommends the use of autologous serum-based media or serum-free defined media containing human recombinant proteins and isolated reagents and compounds verified as safe for cell culture use and, wherever possible, using reagents which are generally recognized as safe ("GRAS").

Since minimal culture expansion is a medical procedure, only FDA approved drugs (off-label use permitted), pharmacy compounded medications, or GRAS products should be used in the portions of the cell culture process that involve

direct human contact. USP grade reagents can be used in the portions of the cell culture process that do not involve direct human contact. Any drugs used to promote stem cell growth, proliferation or differentiation in the culture process must be monitored closely for clinical adverse events. In addition, microarray analysis, differentiation assays, gene expression profiling (qtRT-PCR) or periodic flow cytometric analyses should be performed in order to quantitatively analyze the population(s) of cells generated in cell cultures by these drugs. At a minimum of several times a year, karyotyping of cells grown in cultures should be conducted as a quality control procedure to assure that the reagents are not mutagenic.

Records of patient allergies must be maintained to assure that the antibiotics being used in culture do not promote an allergic reaction in the patient. If allergies to the planned antibiotic, reagent or material (such as latex) are detected, than a suitable alternate should be sought. If antibiotic prophylaxis needs to be avoided, then all cell culture procedures need to be performed in a Class IV Biological clean room.

Any reagents used must be approved for human use (where practical). If specific reagents are not available for human use, they should be compounded by a pharmacist and appropriately tested for sterility and only used within their expiration dates.

The lab should periodically discard all reagents that have passed their expiration dates.

Section E-Receipt and Processing of Tissue

You should process A-ASC's in a way that does not cause contamination or cross-contamination during processing, and that prevents the introduction, transmission, or spread of infectious disease. Human cells or tissue from two or more donors must not be pooled (placed in physical contact or mixed in a single receptacle) during processing.

Any change to a process should be verified and monitored to ensure that the change does not create an adverse impact elsewhere in the operation. Additionally, before implementation of any process change, there must be written approval of the change by a responsible person, i.e., a supervisor, laboratory manager, or above, with appropriate knowledge and background.

You should validate and approve the A-ASC process according to established or published procedures. The validation activities and results must be documented, including the date and initials of the person performing the procedure and the signature of the individual(s) approving the validation.

Section F-Labeling Controls

All areas where A-ASC's will be exposed to the environment must be clearly labeled with the patient's name, DOB, or an automated code number while the workspace is occupied with that patient's sample.

All A-ASC samples sent from the clinic to lab should be labeled with:

- Patient name
- Date of Birth
- Harvest date and time
- Volume of the samples contained in the transport bag
- Date the laboratory received the samples

If clinic or lab personnel detect a break in the sterile transport bag or if the exterior of sample becomes contaminated, then an assessment of the sample integrity should be verified and documented. If actual sample contamination is suspected, the sample should be discarded.

All A-ASC samples sent from the lab to the clinical facility should be labeled with at a minimum:

- Patient Name
- Date of Birth
- Number and type of A-ASC's in the Sample
- Other Reagents in the A-ASC Sample
- Quality or Grade of the A-ASC's in the Sample
- Indicate time of when the A-ASC's were placed in the transport container. Include in the transport container additional controls such as a countdown timer and/or thermometer.
- Upon receiving the sample by the clinical facility, the above information should be inserted into the clinics database.

Section G-Storage Requirements

When freezing A-ASC's it is important to prevent formation of large crystals in the cell due to crystals causing disruption of organelles and cell structure, killing the cells. In an unprotected cell, the destructive effect of ice crystal formation occurs between 0°C and -20°C. To avoid possible damage to the A-ASC's, two precautions are to be taken. These are the use of cryoprotective agents and controlled rate freezing. The A-ASC's are suspended in a growth medium, serum or other agent containing a cryopreservative. The most commonly used cryopreservative are dimethylsulphoxide (DMSO) and glycerol. The ratio and type of cryopreservative should be optimized based on previous literature and in house testing. The cryopreservation process should be validated prior to implementation. Each cryo preserved A-ASC should be labeled at minimum with patient identification, cell type, number of cells, and date.

Records of the cryostorage log should have at minimum: location of the cryotube (cane/rack and box number), cell type, date of storage, number of cells stored, patient identification, and date removed from storage. Records of the current cryostorage stock should be updated as soon as possible. Acceptable temperature limits should be established. Each storage temperature must be maintained and recorded. Review temperature records periodically to ensure that temperatures have been within acceptable limits. Corrective actions should be documented whenever proper storage conditions have not been met. Records should be retained for 10 years after the date of distribution or expiration of the A-ASC's. Records should be retained of remaining A-ASC in cryostorage.

H-Transfer of A-ASC's from the Lab to the Clinic

A-ASC's should be packed under sterile conditions into an approved medical device or container (such as a syringe). Using sterile technique the A-ASC's are to be contained within a secondary transport container (such as a sterile sample bag). Maximum time in the container must be established based on either peer reviewed scientific literature or stability testing performed by the lab. This maximum container time must be documented and all lab and clinic staff should be properly trained on this concept. If A-ASC's exceed this maximum container time, the physician performing the transplant procedure must be immediately notified and a determination made if the A-ASC's will be transplanted or discarded.

All samples should be clearly labeled. A-ASC's should be transferred in a transport cooler with ice packs. A-ASC's should be located in the transport cooler as to prevent the accidental freezing of the biological sample. These coolers must not be used for any other purpose. The outside of the transport cooler should be labeled with the name; address; and phone number to the facility, biohazard symbol, and purpose of use statement (such as samples are for autologous use only).

If clinic or lab personnel detect a break in the sterile transport bag or if the exterior of sample becomes contaminated, then an assessment of the sample integrity should be verified and documented. If actual sample contamination is suspected, the sample should be discarded.

Chapter 4-Quality Control Program

The A-ASC lab should have a quality control program. Functions of the quality control program should include:

- Establishing and maintaining appropriate procedures relating to these guidelines.
- Ensuring that procedures exist for receiving, investigating, evaluating, and documenting information relating to these guidelines.

A-ASC continuous quality monitoring should also be a part of this program. This should include at a minimum:

- Periodic testing of A-ASC samples with microarray analysis, progression assays, differentiation assays, flow cytometry and/or transcriptional profiling to assure phenotype.
- Morphology grading should be used on each patient sample that is sent for clinical use.

Chapter 5: Personnel

Personnel should be sufficiently qualified to ensure compliance with these guidelines. They should have the necessary education, experience, and training to ensure competent performance of their assigned functions. Personnel should perform only those activities for which they are qualified and authorized. Any new personnel must be trained and retrained as necessary, to perform their assigned responsibilities adequately. Documentation of training should be recorded and maintained.

The minimum requirements for a staff cell biologist should be a B.S. or M.S. in Cell Biology, Immunology, Medical Engineering or related discipline with 1-3 years of direct cell culture experience. If the cell biologist does not have the requisite cell culture experience, a minimum of 1 year of stem cell culture training is required with a trained cell biologist working at an associate level position. Additional training may be required depending on the complexity of the cell culture process.

A lab supervisor should be named who has the expertise, appropriate skill levels, knowledge, and training for this position(s).

A Laboratory Director/Manager should be named who is qualified by training or experience for the scope of activities carried out in the facility. The Lab Director is responsible for all procedures and administrative operations of the facility, including compliance with these standards. The Laboratory Director should participate regularly in educational activities related to the field of cell and tissue processing. The Laboratory Director should report directly to the Medical Director. The lab director should hold regular meetings or regular communication with the lab supervisor(s).

A Medical Director should be a licensed physician. This individual is directly responsible for the medical aspects of the processing procedures. The Medical Director should participate regularly in education activities related to the field. The Medical Director may also serve as the Laboratory Director if appropriately qualified. The Medical Director should hold regular meetings or regularly communicate with the Laboratory Director and lab supervisors.

DRAFT

Abbreviation Definitions

°C – degree Celsius
A-ASC – Autologous, Adult Cell
ART – Assistive Reproductive Technology
ASRM – American Society for Reproductive Medicine
BM – Bone marrow
BP – Blood Product
BSE – Bovine Spongiform Encephalopathy
CDC – Center for Disease Control
DC – Differentiated Cells
DMSO – Dimethylsulphoxide
FBS – Fetal Calf/Bovine Serum
FDA – Food and Drug Administration
GLP – Good Laboratory Practices
GLSC – Germ Layer Lineage Stem Cells
GRAS-Generally recognized as safe
HEPA- High Efficiency Particulate Air
IVF – In Vitro Fertilization
PC – Progenitor Cells
PSC – Pluripotent Stem Cells
PSIS – Posterior Superior Iliac Spine
qtRT-PCR-quantitative reverse transcriptase polymerase chain reaction
OSHA – Occupations Safety and Health Administration
SART – Society for Assisted Reproductive Technology
TSC - Totipotent Stem Cells