

<p style="text-align: center;">SJÖGREN'S INTERNATIONAL COLLABORATIVE CLINICAL ALLIANCE (SICCA) APPENDIX B: QUALITY ASSURANCE AND QUALITY CONTROL OF SPECIMENS</p>
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I. INTRODUCTION

The purpose of this appendix is to describe the Quality Assurance (QA) and Quality Control (QC) program of the SICCA Biorepository.

Quality Assurance (QA) is an integrated system of management activities involving planning, implementation, documentation, assessment, and improvement to ensure that a process, or item, is of the type and quality needed for the project.

Quality Control (QC) is the system of technical activities that measures the attributes and performance of a process, or item, against defined standards, to verify that the stated requirements are fully met. For example, if a laboratory is responsible for performing a diagnostic assay, measures must be included with each assay to verify that the test is working properly. QA would be concerned with if the right specimen is selected for a particular test and that the right result and interpretation is delivered to the correct person.

SICCA Repository Quality Assurance Program

The Quality Assurance Program of the SICCA Repository is an ongoing process that requires daily attention by all staff. The following is a list of the components of our repository QA program:

- Equipment Maintenance and Repair Records
- Training Records of Staff
- Data Management
- Record Keeping
- Safety Plan
- Specimens –identification, labeling, processing, and transportation
- Facilities
- SOP manuals
- Quality Control

Description of QA Components

Equipment maintenance and repair records must be maintained in order to document calibration and repair of equipment that is used in the processing and storage of specimens. This includes: laminar flow hoods, refrigerators, ultra-low freezers, liquid nitrogen freezers, centrifuges, balances, ph meters, and coulter counters. Ultra-low freezer temperatures and liquid nitrogen freezers have

temperatures recorded by a wireless, web enabled computer alarm system. The repository staff also record liquid nitrogen levels and temperatures daily.

Training records of staff are maintained and updated. All staff are trained in processing procedures, identification of specimen or blood tube types, care of equipment, data entry and specimen tracking protocols, safety classes by Environmental Health & Safety (EH&S), shipping, specimen inventory, and emergency plans. This training must be documented and annually. All staff are reviewed annually.

Data management for a repository is crucial. All specimens coming into the lab must be verified against the accompanying paperwork to verify study name, specimen type, patient ID, date and time of collection, and any lab request for real-time testing. After verification of the specimens all information must be entered into the database. After processing, actual amount and location of specimen must be entered. Performing double entries and then running reports to check for data inconsistencies verify accuracy of data.

Record keeping is part of data management but involves keeping records of all correspondence, specimen requests, purchase orders, receipt of specimens (date and time), location of specimens, clinical lab requisitions, laboratory accidents, shipments, use of equipment, and personnel training.

Safety plans include evacuation procedures in the event of fire, earthquake, or chemical spill. Safety also includes infection control policies and procedures for the handling of specimens. Documentation must be made of all personnel's vaccinations, if required, and training in the handling of chemicals, specimens, and radioactive materials.

Specimens- The SICCA repository has a receiving and processing area set aside in the laboratory to monitor and record all incoming specimens and the request that accompany them. The documentation of specimen arrival time in the lab as well as other specific test request data is an important part of the quality assurance process. It is important that the specimen status can be determined at any time – that is, where in the laboratory processing system a given specimen can be found. Turnaround time is an important factor and processing time, analyses, and reporting of results within an acceptable time frame is a part of the quality assurance process as a whole. The repository does not perform clinical testing, but we are responsible for filling out lab requisitions and sending specimens out for clinical testing. It is our turn around time that is crucial for the reporting of clinical results.

Specimens must be inspected upon receipt in the lab to ensure they are suitable for either real-time testing or future research. Lipemic (serum or plasma has a milky appearance), hemolyzed (specimen will be red, usually clear red due to lysis of red blood cells) or contaminated samples may interfere with assay

performance. If a specimen appears lipemic or is hemolyzed, this must be noted on the paperwork to document the condition the specimen was received. The field site that collected the specimen will be contacted immediately; informing them of the poor condition of the blood sample. It is recommended that the patient have their blood re-drawn and submitted to the repository for processing. If a specimen is leaking, it will be discarded and the field site will be contacted and the repository will request a re-draw.

Study participant information must be on the accompanying paperwork and all specimens must be properly labeled and identified before data entry and specimen processing.

SICCA specimens from Argentina, China, Denmark, Japan, India, John Hopkins, U Penn, and England were shipped to UCSF. All specimens were received frozen (with the exception of slides and paraffin blocks). All vials were examined by the repository staff to ensure they were labeled, frozen, and the vials were not cracked or leaking. Specimens were examined to insure they were clearly identified and labeled. Paperwork accompanied the shipment in order to verify what was received. If there were unlabeled specimens, missing paperwork, or questionable specimens, the study coordinator was contacted immediately. The specimens will remain in the freezer until all questions are answered.

Specimens that could not be identified by the depositor were destroyed. The repository staff that handles the incoming specimens made a note of this.

Facilities – The SICCA repository lab has a receiving and processing area to monitor and record all incoming specimens and the requests that accompany them. The processing areas of the laboratory are separate from the main lab and do not share the same hoods. The processing areas are under negative pressure with respect to the rest of the laboratory area. All work surfaces are cleaned and decontaminated daily. All specimens are processed under a Class II laminar flow hood, which is certified annually. The department of Environmental Health & Safety (EH&S) at UCSF is responsible for disposing of all biological wastes generated by our laboratory.

Frozen biopsies and PBMCs are stored in liquid nitrogen freezers, which are programmed to alarm when liquid nitrogen levels are low. These freezers are located at the SICCA repository lab. Ultra-low freezers are housed on Parnassus and off site at Oyster Point, South San Francisco. The ultra-low and liquid nitrogen freezers are hard wired into a programmable wireless, web enabled alarm system that monitors temperature changes and power outages. As soon as a unit warms up or loses power, the bank staff is notified immediately by cell telephone or email of the situation.

SOP manuals – The laboratory procedure manuals or standard operations manuals (SOP) are updated to reflect changes in protocols, new techniques, and

new studies. The manuals contain study descriptions, processing protocols, and data entry procedures. Senior technicians are responsible for updating the manual and the Director of the repository must review the manual prior to release to the laboratory. All staff is responsible for reviewing the manuals for updates and protocol changes. SICCA specimens were processed according to SICCA's Manual of Procedures, which were prepared during the previous contract (see appendix A).

Quality control activities include monitoring and recording instrument performance, reagents, products, and equipment. In the process of quality assurance, it is important to document the performance of quality control measures. This will include any corrective actions taken if an instrument fails. Records such as preventative maintenance records and temperature charts can be used as quality control records.

Equipment that is monitored, checked, calibrated, and subject to routine preventative maintenance in the SICCA repository are:

- Class II laminar flow hoods – decontaminated daily and tested annually by a certified technician.
- Microscopes – cleaned after each use and checked quarterly by a trained technician.
- Freezers – checked daily by staff for temperature readings and checked quarterly for performance of compressors and refrigerant levels by trained technician.
- Centrifuge – daily decontamination, quarterly speed calibrations.
- Autoclave- monthly test for temperature and pressure
- Coulter Counter – daily calibration of cell counts, quarterly cleaning and maintenance by licensed technician, and annual certification.
- Pipetting devices – annual cleaning and calibration.

Other QC issues that the repository staff must monitor is the use of reagents in the processing of specimens and transport media. All chemicals are dated upon receipt in the lab. All media preparation is performed under sterile conditions. All expired chemicals are disposed of properly.

To ensure that specimens are stored properly, the temperature of ultra-low and liquid nitrogen freezers are monitored daily by the SICCA repository staff. The scanning alarm system prints daily readings of all ultra-low freezers temperatures, four times a day.

Specimen Quality Control

Specimen integrity – The QC procedures for testing the integrity of a specimen includes visible inspection of incoming specimens, both fresh and frozen. The repository staff looks for broken vials, leaking specimens, cloudy or hemolyzed

blood specimens. The staff also insures that all specimens received are accurately labeled. This is noted in the specimen bank database if there are problems with an incoming specimen.

All SICCA specimens were sent to a CLIA (Clinical Laboratory Improvement Amendments) certified laboratory, Quest Diagnostics. Quest Diagnostics as part of its quality control must run control samples along with the specimens it is testing. If a sample collected from SICCA is contaminated, provides poor or abnormal results, is poorly labeled, or a requisition is not filled out properly, a representative from Quest will contact us immediately.

The following descriptions are the QC processes that the SICCA Specimen Biorepository performed during the original contract with the exception of PBMCs. We currently submit freshly prepared PBMCs to the AIDS Clinical Trials Group (ACTG).

Peripheral Blood Mononuclear Cells (PBMCs) – The UCSF Research Group (RG) is the only SICCA site that collected and processed PBMCs. The Specimen Bank participates in the ACTG's PBMC Cryopreservation and Viability Panel every quarter, which test for viability and cell counts. A report is sent back every quarter reporting the cell count and viability of the samples sent for testing. All groups must have a cell viability of 80% or higher. For the past seven years the Specimen bank has passed the IQA.

DNA - DNA is extracted from whole blood; which was collected by all SICCA RGs. The DNA Bank sent a report of the amount of DNA extracted from each specimen. If there were poor samples and low yields the project coordinator was informed that an additional specimen will be needed during the participant's follow-up visit.

RNA conjunctival imprints. Each RG participating in this procedure collected an extra set of imprints on a participant and UCSF sent these samples to a lab to verify that there are cells present on the filters and RNA can be extracted from the cells. One round of testing was done and there were no problems with the RG that participated. RNA was extracted from the imprints and was of good quality.

Serum – There is currently a lack of sensitive biomarkers for the QC of cryopreservation conditions (Chaigneau C, *et al*, 2007). Yvonne De Souza is an active member of ISBER (International Society of Biological & Environmental Repositories). Yvonne queried key ISBER members as to QC practices in the management of their biorespositories. Those that responded stated that there is no standard in the field for QC of biological fluids in storage. Many of those that responded perform PBMC viability tests and the same visual inspection as Yvonne's staff on serum samples. Yvonne is a member of the ISBER Proficiency

Testing team and they are researching the literature for biomarkers in sera to perform some pilot testing in the near future.

The table below lists some of the comments made by Directors and Managers of Biorepositories.

Name and Title	Institute/Company	QC Protocols
Rebecca Barnes	BC Cancer Agency Tumour Tissue Repository	Researching QC procedures on sera and plasma – no appropriate methods.
Anne Carter, Head of Operations – Quality and Standards	onCore, UK – created to serve as national biospecimen & information resource for cancer research.	Investigating QC procedures and have found none for serum.
Warren Davis, PhD, Lab Director	Roswell Park Cancer Institute, Data Bank and Biorepository	PBMCs – cell count and viability. Still researching for QC methods to test for storage process and specimen integrity.
Scott Jewell, Director of Tissue Procurement	Human Tissue Resource Network at Ohio State University Medical Center	Visual inspection of plasma and sera upon receipt, PBMC viability, RNA or DNA quality.
Peter Kierulf, Professor	Ullevål University Hospital, Oslo, Norway	PBMCs- viability using flow cytometry
Kathi Shea, President, Biorespository Services	Precision Bioservices DAIDS Repository	Visual inspection of plasma and sera upon receipt, PBMC viability and cell count. Serum and plasma – still researching what to test for. Looking at study specific QC procedures for serum and plasma.
Sharan VedBrat, PhD, President	KamTek, Inc.	Still investigating biomarkers.

Some proteins degrade over time, such as matrix metalloproteinase-9 (MMP-9), a cardiovascular marker, whereas some coagulation proteins are stable after many years of storage. Ideally, in order to perform any QC measures on stored serum samples, a biomarker that has an ‘on-off’ response to temperature variation would be ideal but more research is needed in this area to identify this biomarker.

Tears – The SICCA repository has 4 half strips per participant in storage. Thus, to use one strip for QC purposes is not advisable.

Saliva – Whole and unstimulated saliva samples cannot be used for QC testing because we have so little on our participants. Many of them have just 1 or 2 (200 µl per vial) vials in storage.

Labial Salivary Glands (LSG) – SICCA LSG biopsies were collected during the previous SICCA contract. Once the glands were collected, some were set aside for formalin preservation and the remainder of the glands were snapped frozen in liquid nitrogen. The formalin preserved glands were embedded in paraffin and sectioned for hematoxylin and eosin staining. UCSF oral pathologists examined these sections for focus scoring and for quality of biopsy. If a biopsy was made too shallow or was of poor quality, a focus score could not be made and the tissue section would be of poor quality. If we received poor sections, the project coordinator and clinician was contacted by Dr. Troy Daniels. Dr. Daniels would review the biopsy procedure with the clinician.