Cryopreservation of Client Depositor Semen

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1 Introduction

The primary purpose of the Andrology Center and Reproductive Tissue Bank is to provide quality sperm cryopreservation services to those patients who have been diagnosed with cancer and will undergo chemotherapy or radiation therapy. This service is also provided to pre-vasectomy patients, patients undergoing vasectomy reversals, as well as those needing sperm for IVF backup or general fertility preservation purposes.

Chemotherapy and radiation therapy may cause a patient to become temporarily or permanently sterile. Cryopreservation of sperm prior to treatment will allow these patients the opportunity to father their own children in the future. Future pregnancies would be achieved by insemination or other assisted reproductive techniques available at the time of use [1–3].

Therapeutic bank patients, referred to as "client depositors," can address the risks of infertility inevitable in certain medical treatments and surgeries by taking advantage of the controlled practice of cryopreservation.

2 Principle

Biological time ceases at the liquid nitrogen temperature of −196 °C. Thus, liquid nitrogen is used for the long-term preservation of sperm. Noiles and Kleinhans et al. have determined that human spermatozoa have morphological properties which allow them to swell to approximately five times their isoosmotic volume before lysing [3]. The controlled rate addition of TEST-yolk buffer and the removal during thawing by a slow thaw and wash procedure results in preservation of sperm membrane integrity.

3 Specimen Requirements

Client depositor semen should be collected by masturbation in the donor room located adjacent to the Andrology Laboratory. The specimen must be collected in a sterile plastic container. The client depositor should abstain from ejaculation between 48 and 72 h prior to specimen collection. The collected semen specimen is allowed to liquefy in a 37 °C incubator for a minimum of 20 min.

A. Unacceptable specimens: Any produced specimen is considered acceptable. While the semen specimen of a client depositor may not meet the minimal "normal criteria" of a banked specimen from a donor who is not compromised by a disease process, present and future techniques used for in vitro fertilization (IVF) may enhance a subnormal specimen.
B. Client depositor confers with the Andrology Director and/or his referring physician as to the acceptability of the banked specimen and its future use.
C. Specimens of moderate or high viscosity that do not liquefy after incubation can be manipulated by pipetting up and down with a sterile pipette.
D. Due to extenuating patient/client depositor circumstances, a specimen may need to be collected outside the donor room, i.e., the patient's bedroom. In this case, the technologist may provide a sterile collection cup to the patient and have him sign the "Therapeutic Sperm

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Banking Off-Site Collection" form when he drops off the specimen. A technologist should also sign the form as a witness to the patient's signature.

E. Only one client depositor should be processed at any time to avoid a mix-up or possible contamination. If more than one banker is scheduled for a given time slot, then another technologist should do the processing.

4 Equipment

A. Computer-assisted semen analyzer
B. Sperm counting chamber
C. Automatic pipettor
D. Vortex
E. −20 °C freezer
F. LN_2 Dewar with 11″ canisters
G. Sterile specimen container
H. Sterile 15 mL centrifuge tubes with screw caps
I. Sterile serological pipettes (2 mL capacity)
J. Sterile cryovials (1.8 mL capacity)
K. Colored cryomarkers
L. Test tube racks (for 15 mL test tubes)
M. Cryovial racks
N. Cryocanes
O. Plastic cryosleeves
P. Cryogloves
Q. Protective goggles
R. Vinyl/nitrile gloves
S. 37 °C incubator
T. LN_2
U. Viscosity treatment system (VTS)
V. Test tube rocker

5 Reagents

Freezing medium (TEST-yolk buffer with gentamicin sulfate; TYB). Each bottle is sterile and for one-time use. Parameters for acceptable reagent performance:

A. Quality control consists of a freeze and post-thaw performed on the new lot number of TEST-yolk buffer using a semen specimen of a normal donor meeting the following criteria:
   50 % survival, calculated by the following formula:

$$\frac{\%\,\text{postthaw motility}}{\%\,\text{prefreeze motility}}$$

B. Results are recorded in the appropriate Quality control book. Any unacceptable results are addressed in a quality assurance report with supervisory review.
C. Storage requirements: Stored frozen at −196 °C until time of use.
D. The lot number and vial expiration date are checked and recorded on the Cryopreservation Worksheet.

6 Calibration

No calibration standards exist for this procedure.

7 Quality Control/Quality Assurance

There are no prepared semen controls for routine use. Quality assurance is maintained by the quality control of the freeze media and testing equipment which is documented in the Quality control book.

8 Procedure

Note: A sterile technique should be used throughout specimen processing. Gloves are mandatory for all procedures dealing with body fluids. Latex, however, can be toxic to sperm. Therefore, care should be taken to prevent contamination of the specimen with latex or talc, or preferably, not used at all. Instead, vinyl or nitrile gloves are recommended.

A. The client depositor is registered with the Clinic, if not already a Clinic patient. A clinic number is assigned during registration. The clinic number is necessary for client vial identification and billing purposes. The following information is needed for registration:
   1. Full patient/client depositor name
   2. Patient/client depositor address
   3. Home phone number
   4. Patient/client depositor social security number
   5. Patient/client depositor date of birth
B. A bank file should be made for the client depositor. This consists of the following forms:
   1. The Collection and Storage Agreement. Ask the client to read through the entire agreement, including the fee schedule and initial/sign where indicated. This should be done on two separate agreements so that one can be kept in the client's file and one can be sent home with/to the client. The technologist should also sign as a witness and confirm that the patient's clinic number is accurate.

2. Sperm Bank Questionnaire. The demographics can be filled out by the technologist or by the client himself.

C. During the initial visit, the client depositor should be scheduled to meet with the Andrology Laboratory Director to be given information on the future usefulness of his frozen specimen. The exact quantification of the individual specimen banked can be provided to the client the following day by the Director over the phone or at the time of a subsequent visit.

D. The patient/client depositor is then provided a sterile collection cup and asked to label the container with his name and collect by masturbation in the donor room adjacent to the laboratory.

E. After collection, the patient/client depositor is asked data about his abstinence time, method of collection, whether any specimen was lost during collection, and time of collection. The information should be recorded on the Cryopreservation Worksheet. The specimen is then accepted, labeled by the technologist, and placed in the 37 °C incubator for at least 20 min (Fig. 16.1). At this time, a bottle of TEST-yolk buffer with gentamicin is thawed in the 37 °C incubator for at least 20 min.

F. The patient/client depositor then signs the semen ID form in the presence of the technologist after checking the labeling of his vials (see step "N" for proper labeling procedure). The lab technologist labeling the vials and witnessing the signature also signs this form. The cryovial label may not be altered, removed, or obscured after the patient checks the labeling of his vials. If the labeling is not acceptable to the patient, then the technologist should relabel the vials.

G. A final step for positive identification is made by taking the client depositor's picture with a digital camera or scanning the patient's driver's license. If a picture is taken, it should be saved on the share drive, printed, and placed in the patient's cryobank file. The picture should have with it the client's name, clinic number, and freeze number written at the bottom.

H. If the patient decides to have his blood drawn, it should be done as follows:

1. The blood tests performed by the Red Cross are anti-HIV I/II, HBV NAT, HIV NAT, RPR, HCV NAT, WNV, HBCAg, anti-HTLV I/II, anti-HCV, anti-HBc, anti-CMV, and Chagas.
   a. If any of the test results are positive, the Director and Supervisor of the Andrology Laboratory are contacted by Blood Bank.
   b. Positive test results are confirmed by retesting (done by Blood Bank).
   c. The Director or Supervisor should then contact the patient's ordering physician either by phone or letter when any of the tests are positive.
   d. If any of the blood results are positive, refer to the Storage Agreement as to the proper procedure. The cryopreserved specimens remain in a quarantined area and designated cryotank.
   e. All blood reports on client depositors are reviewed and initialed by the Director or Supervisor.
   f. The remaining 6 mL red top tube should be centrifuged and the serum removed. Aliquot the serum into a 1.8 mL cryovial. The cryovial should be labeled with the patient name, clinic number, freeze number, date, and word "serum" and then stored at −80 °C.

Note: The client depositor serum bank can be used for retesting or for future testing. The serum specimen should be archived for at least 10 years.

Fig. 16.1 Incubator set at 37 °C and depiction of sample undergoing liquefaction [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

I. After liquefaction, draw the semen specimen into a 2 mL sterile pipette, leaving at least 50 µL in the specimen container. Measure and record the volume and note any unusual consistency on the pink patient worksheet as well as the Cryopreservation Worksheet. Deliver the specimen into a labeled, sterile 15 mL conical tube that has been checked for cracks or defects. A moderate to highly viscous specimen can be further liquefied by pipetting up and down in the sterile pipette or adding viscosity treatment (VTS) when necessary.

J. Analyze the remaining aliquot in the specimen container per the "Semen Analysis" protocol using the computer-assisted semen analyzer for concentration, motility, curvilinear velocity, linearity, and amplitude of lateral head movement and record on the pink patient worksheet per protocol (this information should be duplicated on the Cryopreservation Worksheet). Presence of round cells in the specimen is quantified and an Endtz test conducted if ≥0.20 M/mL round cells are seen under wet preparation. At this point, any bacteria or foreign cells should be noted.

K. Within 1 h of specimen collection, add an aliquot of freezing medium equal to 25 % of the original specimen volume to the centrifuge tube with a sterile pipette.
Note: This volume should not exceed 1 mL.

L. Gently rock the specimen with the freezing media for 5 min on a test tube rocker (Fig. 16.2).

M. Repeat steps J and K three times or until the volume of freezing media added is equal to the original specimen volume (Fig. 16.3).

N. During the mixing steps above, use appropriately colored cryomarkers to label the cryocanes (the cryovials should be labeled ahead of time so the patient may examine them). The appropriate color of cryomarker can be found on a chart hanging in the cryobanking area (Appendix 1). This chart also indicates the appropriate color of the index card to be filled with the following information: the patient's name, clinic number, freeze number, bank color, and number of vials frozen and for future use the final thaw date, number of vials thawed, number of vials remaining, and tech initials.

For example, the 1st banked specimen and cryocane is labeled with a red cryomarker and recorded on a red index card.

Note: Examine each cryovial while labeling for any evidence of defects. Discard if any defect is found or there is any doubt as to the integrity of the cryovial before using.

1. Label cryovials as follows (with orange cap facing the left):
   a. Client depositor name
   b. Clinic number
   c. Freeze number, i.e., F13-001-A
   d. Date
   e. Word "SPERM"
   f. Clinic number
2. Three to six vials should be labeled with the following considerations in mind:
   a. The volume added to each vial should not exceed 1.8 mL/vial.
   b. Specimens from patients with very low prefreeze sperm counts can be frozen in even smaller quantities of as low as 0.2 mL of semen.
3. Label the top of the cryocanes with the client depositor's last name and current freeze number using the appropriate colored cryomarker.

Example:
SMITH
F13-001-A

O. Label an additional 1.8 mL cryovial. This will contain a leftover aliquot of the cryodiluted specimen to be assessed for cryosurvival 24 h after freezing. The top of the vial should be heavily marked with a black cryomarker to indicate that it is the post-thaw specimen vial.

P. A visual inspection should now be performed for the cryodiluted specimen for motility. A manual motility should be done using a sperm counting chamber and phase microscope. The percent motility should be documented as "pre-cryomotility %" on the Cryopreservation Worksheet.

Q. Evenly distribute the well-mixed, cryodiluted semen into the pre-labeled vials using a 2 mL sterile serological pipette (Fig. 16.4). Add at least 0.2 mL to the post-thaw cryovial.

R. Place labeled vials into the labeled cryocane(s). Place a maximum of two cryovials into top slots of canes while

Fig. 16.2 Sample rocked on a test tube rocker for 5 min after the addition of TEST-yolk buffer [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

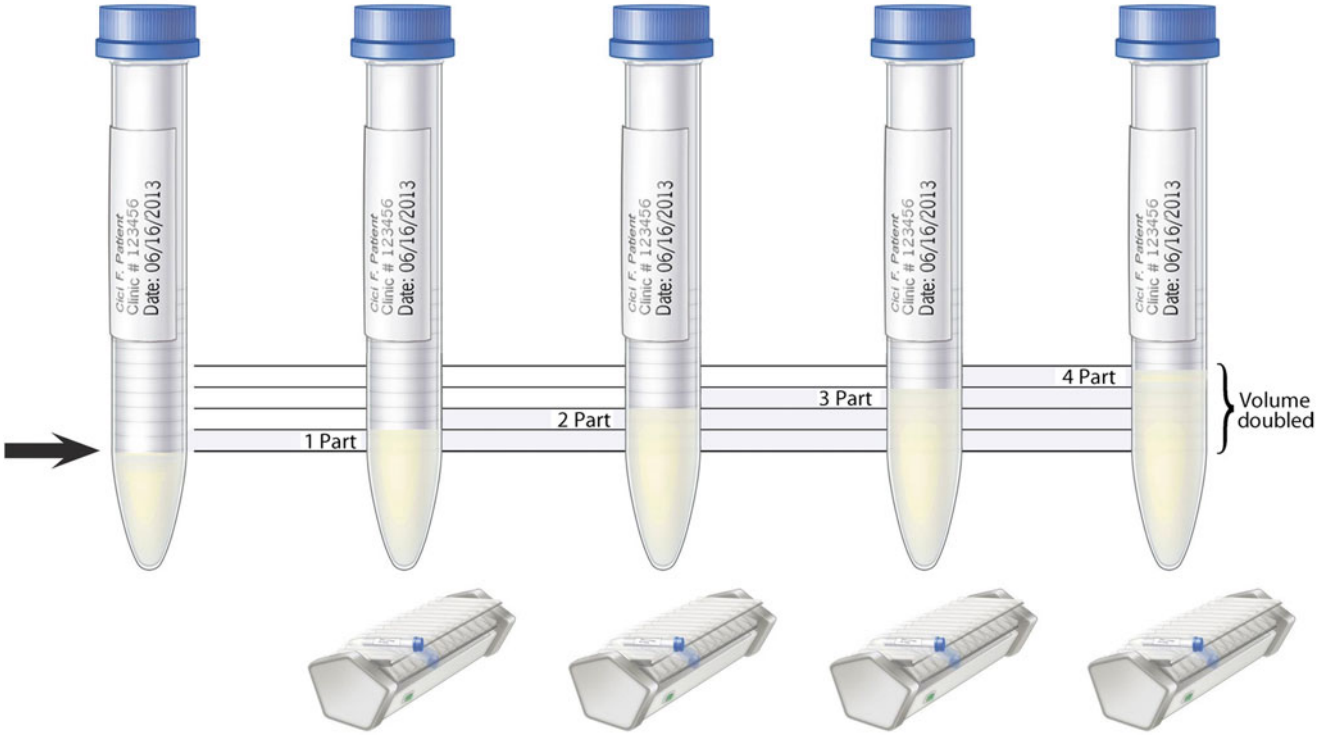

Fig. 16.3 Depiction of the stepwise addition of TEST-yolk buffer to patient sample. Volume of TEST-yolk buffer equal to ¼ volume of patient sample—added four times or until total volume in test tube has doubled [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

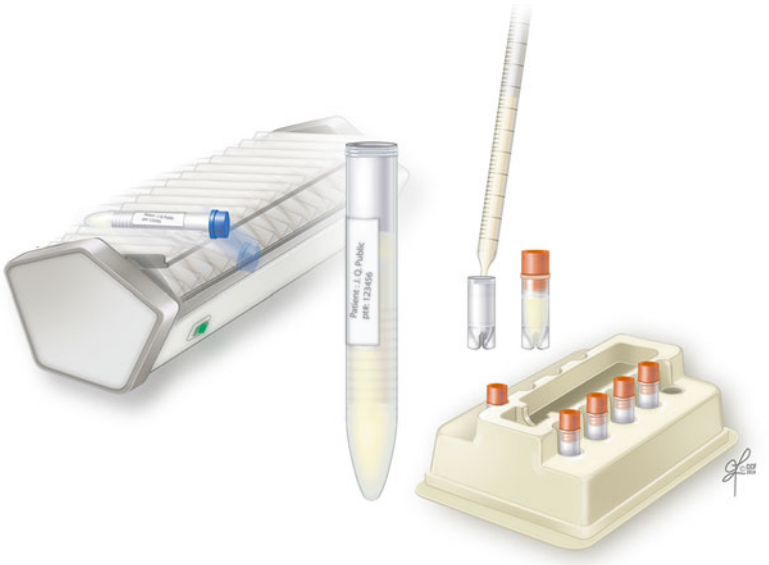

Fig. 16.4 Even distribution of cryodiluted patient sample into cryovials using a sterile serological pipette [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

holding the canes upside down (Fig. 16.5) (i.e., labeled portion of cryocanes should be facing the ground while placing the cryovials upright, with the orange top facing up). Cover cryocane(s) with cryosleeve(s) and place in the −20 °C freezer for 8 min (Fig. 16.6). Do not open the freezer during this incubation.

Note: Exposure to freezing conditions should occur within 1.5 h of specimen collection.

S. After the 8 min incubation, remove the cryocane(s) from the −20 °C freezer. Place into an appropriate LN_2 vapor tank for a minimum of 2 h (Figs. 16.7 and 16.8). After 2 h, the vials are frozen by the liquid nitrogen vapors (−80 °C).

Note: Be sure that no less than 12 cm of LN_2 liquid fills the canister.

Caution: Wear cryogloves and protective goggles.

Fig. 16.5 Proper placement of cryovials into cryocanes [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

Fig. 16.6 Cryovials, with cryocanes and cryosleeves, placed upright in −20 °C freezer for 8 min [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

T. After a minimum of 2 h incubation in the liquid nitrogen vapors (−80 °C), turn the canes upside down, immersing them into liquid nitrogen (−196 °C).
U. After a minimum of 24 h in liquid nitrogen, thaw the aliquot in the 1.8 mL post-thaw cryovial as follows:
   1. Using cryogloves and protective goggles, remove cane containing the cryovial and snap it out. Loosen the cap and place in the 37 °C incubator for 20 min.

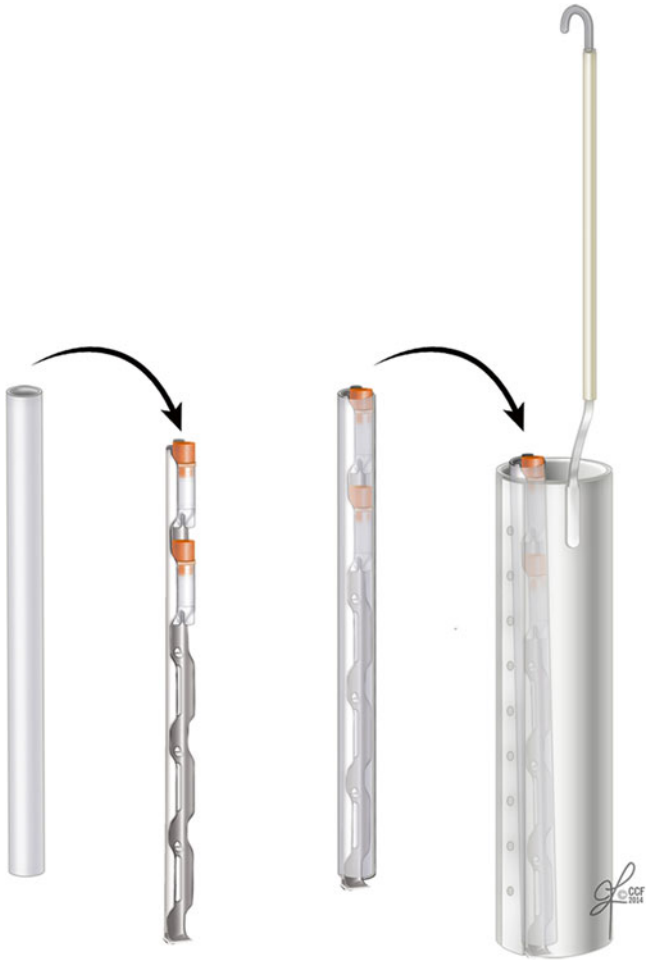

Fig. 16.7 Loading cryocanes and cryovials upright into the LN_2 cryotank canister [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

Fig. 16.8 Cryotank canister containing cryocanes and cryovials added slowly, upright into cryotank [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

2. Mix the vial well and analyze using the computer-assisted semen analyzer for count, motility, curvilinear velocity, linearity, and amplitude of lateral head movement.
3. Record results in the cryosurvival area of the Cryopreservation Worksheet. Assess cryosurvival using the following formula:

$$\frac{\%\,\text{motility of pos-thaw specimen}}{\%\,\text{motility of pre-freeze specimen}}$$

4. Calculate the number of inseminations based on the following recommended total motile sperm counts.
   1 Insemination = 15–20 M
   ½ Insemination = 7.5–14.9 M
   ¼ Insemination = 3.75–7.49 M
   0 Insemination = <3.75 M

V. All information from the Cryopreservation Worksheet should be entered into the clinic number of the andrology computerized database. The information on the Cryopreservation Worksheet is duplicated line for line when entered into the andrology computerized database. A copy is made of the cryoworksheet and put into the appropriately dated "Semen Cryopreservation" workbook.

W. A summary of the patient/client depositor information can be obtained by entering the patient's clinic number into the sperm bank computerized database "View Semen Cryopreservation Report." On subsequent visits, or over the phone, the client can be presented with this summary by the Director or the Supervisor in the Director's absence. From the client depositor's second bank and onward, he need only provide information of his abstinence and collection time and sign the semen ID form after first examining the vials for the day's bank.

X. Once the client depositor has determined that he is done banking, the "Therapeutic Post Bank Checklist" is attached to the inside of the client depositor's file folder and all points are to be completed. As time and resources permit, the banked specimens are taken out of short-term storage, or quarantine, and transferred to the secured 17 K or 24 K freezer for long-term storage at −196 °C in liquid nitrogen (Figs. 16.9 and 16.10). The client depositor specimens are stored indefinitely or until authorization is received to transfer or dispose of the specimens. Upon disposal, transfer or the client depositor's death, the patient charts should be stored in a secure area and are kept for at least 10 years.

Y. The chart is reviewed by the Medical Consultant prior to the release of the tissue. The review form (Appendix 2) should be placed in the client depositor's charts and signed by the Medical Consultant.

Z. Emergency transfer of samples: Sequester samples in the transfer tanks.

Fig. 16.9 Cryoboxes and cryotower [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

Fig. 16.10 17K long-term storage tank [Reprinted with permission, Cleveland Clinic Center for Medical Art & Photography © 2015. All Rights Reserved]

Addendum

Cryopreservation of the "Traveling Husband" Specimen

Introduction

A client depositor identified by the Gynecology Department as a "traveling husband" can make use of the time delay factor in the cryopreservation procedures, i.e., when the wife ovulates the husband may of necessity be out of town. Due to the fact that the husband is not present when the specimen is used for insemination, three forms must be signed and filled out with the other paperwork to allow for future use of the specimen.

Procedure

Note: Follow the procedure steps as detailed in the "Cryopreservation of Client Depositor Semen" protocol along with the steps below:

A. The traveling husband (client depositor) must also fill out the "thawed specimen ID" form (Appendix 10). He should print his name and clinic number on the top line of the form. He should sign his name on the line after "Client Depositor" and put the date of cryopreservation of the specimen after "Date." The wife's signature and lower portion of the form will be filled out on the date of specimen use.
B. Also, at the time of cryopreservation, the "Authorization to Transport Semen Specimens" form should be filled out on page 2 (Appendix 11). The client depositor should sign the form on the top line after the "Client Depositor" signature, as should his partner. Then he should print his name, address, telephone number, and date on the indicated lines. The technologist processing the specimen for cryopreservation should then sign after the "Witness Signature" and provide the date of processing.
C. Complete the "Release of Positive Blood/Semen Test Notification" (Appendix 9).

Appendix 1: Sperm Bank Patient List

Bank number	Color of marker	Letter code	Index card color
1st	Red	A	Red
2nd	Blue	B	Blue
3rd	Green	C	Green
4th	Black	D	Yellow
5th	Red	E	Orange
6th	Blue	F	White
7th	Green	G	White
8th	Black	H	White
9th	**Start rotation from top**		

Appendix 2

Client Depositor Name: _____

Clinic number: _____

Freeze Number: _____

I have reviewed the client-depositor's sperm bank results, blood testing results, and available medical information.

Date of Review: _____ _____

Medical Consultant
Andrology Lab & Reproductive Tissue Bank

Date of Release: _____

Comments:

_____
_____
_____
_____
_____

Appendix 3: Sperm Bank Questionnaire

SPERM BANK QUESTIONNAIRE

DATE: _____

RECORDED BY: _____

FREEZE NO.: _____

DEMOGRAPHICS

PATIENT NAME:_____Clinic number: _____

(Please Print) (First) (MI) (Last)

AGE: _____ DATE OF BIRTH: _____ SS # : _____

ADDRESS: _____ HOME PHONE: _____

_____ WORK PHONE: _____

IN CASE OF EMERGENCY CONTACT

NAME: _____ ADDRESS: _____

_____

RELATIONSHIP: _____ PHONE: _____

DIAGNOSIS

REASON FOR STORAGE:

_____

SYMPTOMS: _____

DATE OF DIAGNOSIS: _____

SURGERY OR CHEMOTHERAPY DATE(S): _____
(Previous Dates)

SURGERY/CHEMO TO BEGIN: _____
(Date)

TREATMENT PLAN: _____

(PLEASE TURN OVER)

HISTORY

MARITAL STATUS: ☐SINGLE ☐MARRIED SPOUSES NAME: _____

CHILDREN (age & sex) _____

SEXUALLY ACTIVE: ☐YES ☐NO FREQUENCY (weekly):☐1-2 ☐3-4 ☐5 or more

CAFFEINE USE (weekly): ☐1-5 (least)☐5-10 (most) ALCOHOL USE (weekly):☐1-2 ☐3-4 ☐5 or more

DRUG USE (weekly):☐1-2 ☐3-4 ☐none SMOKING HISTORY:☐1-2 ☐3-4 ☐5-6 ☐none (packs per week)

SEXUALLY TRANSMITTED DISEASES: _____ CHEMICAL EXPOSURE: _____

RADIATION EXPOSURE: _____ HEPATITIS EXPOSURE: _____

OTHER (please explain): _____

STORAGE PLAN: ☐LONG-TERM ☐SHORT-TERM ☐UNKNOWN

REFERRING DOCTOR

Clinic DOCTOR: ☐ YES ☐ NO

DOCTORS NAME: _____
(Current Doctor)

ADDRESS: _____

_____

_____

PHONE #: _____

AUTHORIZATION TO CRYOPRESERVE SPECIMENS

I, _____, Clinic number :_____,

(print full name)

have given my semen specimen to _____ of the

(technologist)

Clinic Sperm Bank. I have seen the freezing vials correctly labeled with my name and clinic number.

Date: _____

Client Depositor: _____

Technologist: _____

Freeze Number: _____

Appendix 4: Authorization to Cryopreserve Specimens

CRYOPRESERVATION WORKSHEET

NAME: ______________________________ FREEZE #: ____________
MRN #: ______________________________ COLOR: ______________

****

SPECIMEN LOCATION: ________________________________ SHIP DATE: ____________________

SPECIMEN LOCATION: ________________________________
SPECIMEN LOCATION: ________________________________ DISPOSAL DATE: _______________

- -

SERUM ACCESSION # __________________ SERUM ALIQUOT LOCATION: ___________________

****

PRE CRYOPRESERVATION

FILE # ____________________________

SPECIMEN DATE: ________________ SPECIMEN TYPE: _________________________
(i.e., fresh, epi aspiration, tissue)

COLLECTION TIME: ______________ MOTILE SPERM M/mL: ___________________________

RECEIVED TIME: _________________ CRYO MEDIA: _____ Lot #__________ Exp Date: _____

ABSTINENCE TIME: ______________ AMOUNT TYB ADDED mL: ________________________

SPERM COUNT M/mL: _____________ NUMBER OF VIALS ALIQUOTED: _______________

VOLUME mL: _____________________ VOLUME mL/VIAL _________________________

TOTAL COUNT M: ________________ MOTILE SPERM M/VIAL: ______________________

PERCENT MOTILE: _______________ COMMENTS: _______________________________________

VELOCITY μ/sec: _____________________________________ LINEARITY %: ____________________

ALH μ: ____

ROUND CELLS M/mL: ____________________________
PRE-CYRO MOTILITY %: __________________________
PRE EN %: ____
ENDTZ M/mL: ______________________________________
TECH: ____

TIME: INTO VAPORS: ______________ INTO LN_2: ___________ LOCATION: _________________

Appendix 5: Cryopreservation Worksheet

CRYOSURVIVAL

FILE # _____
THAW DATE: _____
THAW TIME: _____
% MOTILE: _____
VELOCITY μ/sec: _____

LINEARITY %: _____
ALH μ: _____
MOTILE SPERM M/VIAL: _____

% SURVIVAL: _____
TOTAL MOTILE M: _____
NO. OF INSEMS: _____
IVF ATTEMPTS: _____

COMMENTS: _____
MOTILE SPERM M/mL: _____

POST EN %: _____
TECH: _____

CRYOPRESERVATION WORKSHEET
PAGE 2

NAME: _____ FREEZE #: _____

CCF #: _____ COLOR: _____

INSEMINATION RECORD

DATE THAW	# VIALS USED	# VIALS REMAIN	TECH	BILLED

Appendix 6

I, _____, Clinic number _____,

Social Security #_____, have collected my semen specimen outside of the Clinic Andrology Laboratory specimen collection room.

The specimen was collected by masturbation **YES** or **NO** (circle one) or by other methods _____, the specimen was produced at _____, (given exact time) at my residence.

The Andrology Laboratory and the Clinic will not be responsible for incorrect collection method, contamination during specimen collection or changes in sperm due to delay in specimen processing: ____min (write age of specimen).

****

The sample for sperm cryopreservation belongs to me and I have labeled it correctly.

_____ _____

Witness Signature and Date Patient's Signature and Date

_____ _____

Witness Signature and Date Patient's Name

Appendix 7: Cryopreservation Report

Name:	**Clinic number**	**Bank ID number**
Specimen Type: Semen		
	Specimen A	**Specimen B**
Pre-cryopreservation		
Date:		
Time (24 h):		
Length of abstinence (days):		
Semen volume (mL):		
Viscosity[a]:		
pH[a]:		
Color[a]:		
Round cells (10^6/mL)[a]:		
Motility (%):		
Velocity (μ/s):		
Linearity (%):		
ALH (μ):		
Concentration (10^6/mL):		
Total count (10^6):		
Morphology (% normal sperm forms by Kruger method)[a]:		
Endtz (10^6/mL)[a]:		
Comments:		
Post-cryopreservation		
Motility (%):		
Velocity (μ/s):		
Linearity (%):		
ALH (μ):		
Total motile sperm (10^6):		
Motile sperm/vial (10^6):		
No. of vials:		
No. of possible insemination (IUI) attempts:		
No. of possible IVF attempts:		
Volume/vial (mL):		
Comments:		

[a]Data Entered for Specimen A Only

Appendix 8: Release of Reproductive Cells and/or Tissues Not Tested for Infectious Diseases

RELEASE OF REPRODUCTIVE CELLS AND/OR TISSUES NOT TESTED FOR INFECTIOUS DISEASES

Client Depositor Name: _____ Clinic number _____

Specimen cryopreserved:_____

In connection with the deposit of semen specimens for storage, Clinic performs tests on the depositor's blood sample for infectious diseases including Human Immunodeficiency Virus and Hepatitis B surface antigen (HIV ½, HIV Ag, HCV, HIVNAT, HbcAb, RPR, HVCNAT, HTLV ½, HbsAg and WNV).

I, the undersigned, understand that the Reproductive cells and/or tissues from the client depositor identified above **have not been so tested** or screened for infectious diseases at the request of the Client Depositor, who, by his signature acknowledges his demand to not test the specimens.

I, the undersigned, accept the responsibility for the use of the semen specimens for insemination.

I, the undersigned, understand that the semen specimens will not be released until this form is signed by all parties listed below and returned to:

Andrology Laboratory & Reproductive Tissue Bank

Insemination Physician: _____ Date: _____

Address: _____

Phone: _____

Client Depositor: _____ Date: _____

Address: _____

Phone: _____

Sexual Partner: _____ Date: _____

Address: _____

Phone: _____

Appendix 9: Release of Reproductive Cells and/or Tissues Positive Test Notification

RELEASE OF REPRODUCTIVE CELLS AND/OR TISSUES
POSITIVE TEST NOTIFICATION

Client Depositor Name: _____ Clinic number: _____
Date of Blood Draw: _____
Specimen cryopreserved:_____

☐ The following blood tests were reported to be reactive according to the American Red Cross:

___ anti-HIV-1 ___ anti-HIV 2 ___ HBsAg ___ anti-HCV

___ any test for sexually transmitted diseases, excluding CMV:_____

I, the undersigned, understand that the information checked above applies to blood and/or semen of the Client Depositor. I, the undersigned, accept the responsibility of the use of the semen specimens for insemination.

I, the undersigned, understand that the semen specimens will not be released until this form is signed by all parties listed below and returned to:

Reproductive Tissue Bank

Insemination Physician: _____ Date: _____

Address: _____

Phone: _____

Client Depositor: _____ Date: _____

Address: _____

Phone: _____

Sexual Partner: _____ Date: _____

Address: _____

Phone: _____

Appendix 10: Thawed Specimen ID Form

THAWED SPECIMEN I.D. FORM

I, _____, _____,
(Name)(Client Depositor) (Clinic No.)

certify that the vial(s) being used for artificial insemination/*in vitro* fertilization is labeled with my name and clinic number, as shown above.

Client Depositor Signature: _____ Date: _____

Intimate Partner Signature: _____ Date: _____

****

I, _____, of the Clinic Sperm Bank, have
(Technologist)

released the above semen specimen to _____, for the purpose
(Physician)

of artificial insemination/in vitro fertilization of _____
(Intimate Partner)

Date: _____

Technologist Signature: _____

Physician Signature: _____

Number of vials thawed: _____

Number of vials remaining: _____

Specimen I.D. Number: _____

Appendix 11: Authorization to Transport Semen Specimens

AUTHORIZATION TO TRANSPORT SEMEN SPECIMENS

I hereby authorize THE CLINIC to allow transport to my physician, whose name and address appear below, _____ vials from the _____ semen specimens which the Foundation is presently
(number) (number)
storing for me. I intend that these transported semen specimens will be used only for the purpose of artificial insemination by a physician. I agree that the clinic will have no responsibility for the ultimate use of any portion of the transported specimens or for the method of artificial insemination used. I further agree that the Foundation will have no responsibility for any damage to the semen incurred during transit or thereafter.

I request that the specimens to be sent to:

Physician:

Address:

AUTHORIZATION TO TRANSPORT SEMEN SPECIMENS

Patient Name (Please Print): Clinic number

Address:

Telephone:

_____ _____
Client Depositor Signature Intimate Partner (SIP) Signature
Date: _____ _____

Date: _____

TO BE COMPLETED BY THE PHYSICIAN

I am the physician whose name appears on this Authorization to Transport Semen Specimens and I am licensed to practice medicine in my state. I hereby request the transport of the semen specimens to my address as shown. It is my intention that the transported semen will be used only for the purpose of artificial insemination of the patient whose signature appears above.

Signature of Physician: _____ Date: _____

RETURN THIS FORM TO: The Clinic
Andrology Laboratory

Appendix 12: Positive Blood Test Notification

POSITIVE BLOOD TEST NOTIFICATION

Client-Depositor Name: _____

Clinic number: _____

Date of Blood Draw: _____

Ordering Physician: _____

The following blood tests were reported positive by the American Red Cross:

_____ Anti-HIV I/II	_____ HBV NAT	_____ HIV NAT
_____ RPR	_____ HCV NAT	_____ WNV
_____ HBcAg	_____ Anti-HTLV I/II	_____ Anti-CMV
_____ Anti-HCV	_____ Anti-HBc	_____ Chagas

Date Ordering Physician Notified: _____

Date Client-Depositor Notified: _____

Notified By: _____
Director, Reproductive Tissue Bank

Comments:
_____
_____
_____
_____
_____
_____
_____

References

1. World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 5th ed. Geneva, World Health Organization, Switzerland; 2010.
2. Standards for tissue banking. 13th Edition published by the American Association of Tissue Banks.
3. Noiles EE, Mazur P, Watson PF, Kleinhans FW, Critser JK. Determination of water permeability coefficient for human spermatozoa and its activation energy. Biol Reprod. 1993;48(1):99–109.