PROCEDURE	**RNA Collection -** Placental Tissue	
PREPARED BY	GAPPS staff	
DATE ADOPTED		
REVIEWED BY	**SIGNATURE**	**REVIEWED DATE**

REVISED BY	**SIGNATURE**	**REVISED DATE**

SUMMARY OF CHANGES TO THIS SOP
Version 1.0

PURPOSE

This Standard Operating Procedure (**SOP**) describes a procedure for the extraction of high quality RNA from placental tissues and the determination of the quality of the collected specimen for storage and distribution.

SCOPE

This procedure covers the processing and storage of placental tissue to RNA.

Authority and Responsibility for SOP's

1. The GAPPS Medical Director (or his/her designee) and Laboratory Manager have the authority to establish this procedure.
2. The GAPPS Laboratory and the QA monitors are responsible for the implementation of SOP documentation at participating sites.

Supplies

1. Qiagen RNAeasy Mini Kit
2. Molecular Grade EtOH
3. Tissue Homogenizer
4. Molecular Grade H2O

Supply Preparation:

1) Add 4 volumes of ethanol (96-100%) as indicated on the bottle to obtain the working solution of RPE
2) Upon receipt of Agilent RNA kit, heat denature ladder at 70˚C for 2min and aliquot in 2µL volumes. Store at -80˚C.

Safety

All technicians are expected to be trained and follow universal precautions when handling biological or hazardous materials prior to completing this protocol.

Limitations of the Procedure

To avoid poor quality RNA specimens, care should be taken once RNA extraction protocol is begun to clean work surface and pipetmen with RNase zap. Also clean gloved hands frequently to avoid RNase contamination.

Tissue Homogenization and Cell Lysis:

1) β-Mercaptoethanol (β-ME) must be added to Buffer RLT before use. Add 100 µL β-ME to 9.9mL Buffer RLT (β-ME is added at a rate of 10 µL per 1mL of RLT).
    - Dispense in a fume hood and wear appropriate protective clothing. Buffer RLT containing β-ME can be stored at room temperature for up to 1 month.
2) Remove 12 samples from -20˚C staging area and place on ice.
3) Cut 15-30mg of tissue from each sample and place in respectively labeled tubes. Record weight for each sample.
    a. If using flash frozen tissue, use Cell Crusher kit (crucible, mallet, liquid nitrogen, small Styrofoam container).
    b. Fill Styrofoam container with liquid nitrogen to roughly midpoint of crucible.
    c. Take crucible out of Styrofoam box once sufficiently cooled.
    d. Place sample in crucible, (add liquid nitrogen to sample to freeze more if wanted) cover with pestle and gently strike with mallet.
    e. Retrieve appropriately sized pieces to use for run.

4) Weigh the remaining sample and record the 'Punch Weight'.
5) Add 600µL of the RLT with β-ME to each sample and leave on ice. Return completed samples to QC completed staging area in lab freezer.
6) Pour approx. 20mL of molecular grade EtOH and 30mL of molecular grade H2O 2x in to individual 50mL conicals.
7) Clean homogenizer briefly before use by running in ethanol and flaming. After flame is extinguished wash probe 2x in the respective water washes.
8) Dry with wipe.
9) Take first sample and process first at slow speed and then at a higher speed until sample is well homogenized and sample is no longer intact (10-20 seconds).
10) Wipe probe dry and discard wipe as biohazard.
11) Clean homogenizer by running in ethanol and flaming. After flame is extinguished wash probe 2x in the respective water washes.
12) Dry with wipe. Clean briefly with RNase Zap.
13) Repeat until all samples have been processed, cleaning the homogenizer thoroughly after each sample to prevent sample carryover.

RNA Extraction:

1) Transfer homogenized samples to appropriately labelled 1.5mL microcentrifuge tubes.
2) Centrifuge samples for 3min at full speed.
3) Pipet off of pellet to a fresh, labelled tube and add 1 volume of molecular grade 70% EtOH.
4) Mix tubes immediately by pipetting and transfer up to 700µL of sample to the appropriately labelled Qiagen spin column.
5) Centrifuge columns for 30sec at 10,000 x g. Discard flow through.
6) Pipet remaining sample to column and repeat the centrifugation. Discard flow through.
7) Pipet 350µL of buffer RW1 to each column. Spin 30sec at 10,000 x g. Discard flow through.
8) Remove prepared DNase I aliquot (120µL) from lab freezer. Combine with 840µL of RDD buffer in separate microcentrifuge tube. Mix gently by inversion.
9) Carefully pipet 80µL of the prepared DNase solution to the center of the filter on each column. Be careful to not puncture the filter membrane.
10) Incubate at room temperature for 15min. Record DNase in the 'Treatment' column.
11) Add 350µL of RW1 to each column and centrifuge for 30sec at 10,000 x g. Discard flow through.
12) Add 500µL of RPE to each column and centrifuge at 10,000 x g for 30sec. Discard flow through.
13) Repeat with another 500µL of RPE and centrifuge at 10,000 x g for 2 min. Discard flow through.
14) Transfer all columns to new collection tubes and spin at full speed for 1 min to remove any residual ethanol from sample.
15) Transfer column to appropriately labelled 1.5mL microcentrifuge tubes.
16) Pipet 30µL of RNase-free H2O to each sample and centrifuge at 10,000 x g for 1 min.
17) Repeat with an additional 30µL of RNase-free H2O.

Agilent Bioanalyzer for the determination of RNA quality:

1) Remove the agilent reagents from the lab fridge at 4˚C. Allow to warm to room temperature while protected from light (approx. 30min).
2) Remove prepared filtered gel matrix from lab -20˚C freezer.
3) Vortex well the blue dye concentrate and pipet 1µL into 65µL of the thawed gel matrix. Vortex well to mix.

4) Centrifuge 13,000 x g for 10min.
5) Pipet 9µL into the bolded 'G' well on the Agilent chip. Apply pressure from syringe for exactly 30sec.
6) Pipet an additional 9µL to the other two G wells.
7) Vortex green RNA marker. Pipet 5µL to all 12 sample wells and ladder well.
8) Pipet 1µL of sample to each well on the Bioanalyzer chip.
9) Remove RNA ladder from bottom shelf in -80˚C freezer. Pipet 1µL into the respective well.
10) Mix chip 1min on Agilent vortexer.
11) Clean Bioanalyzer with RNase zap and molecular grade H2O.
12) Place chip and run system.
13) Fill in the RIN values for analyzed samples in the *Specimen Form* sheet.

Concentration and Purity Determination

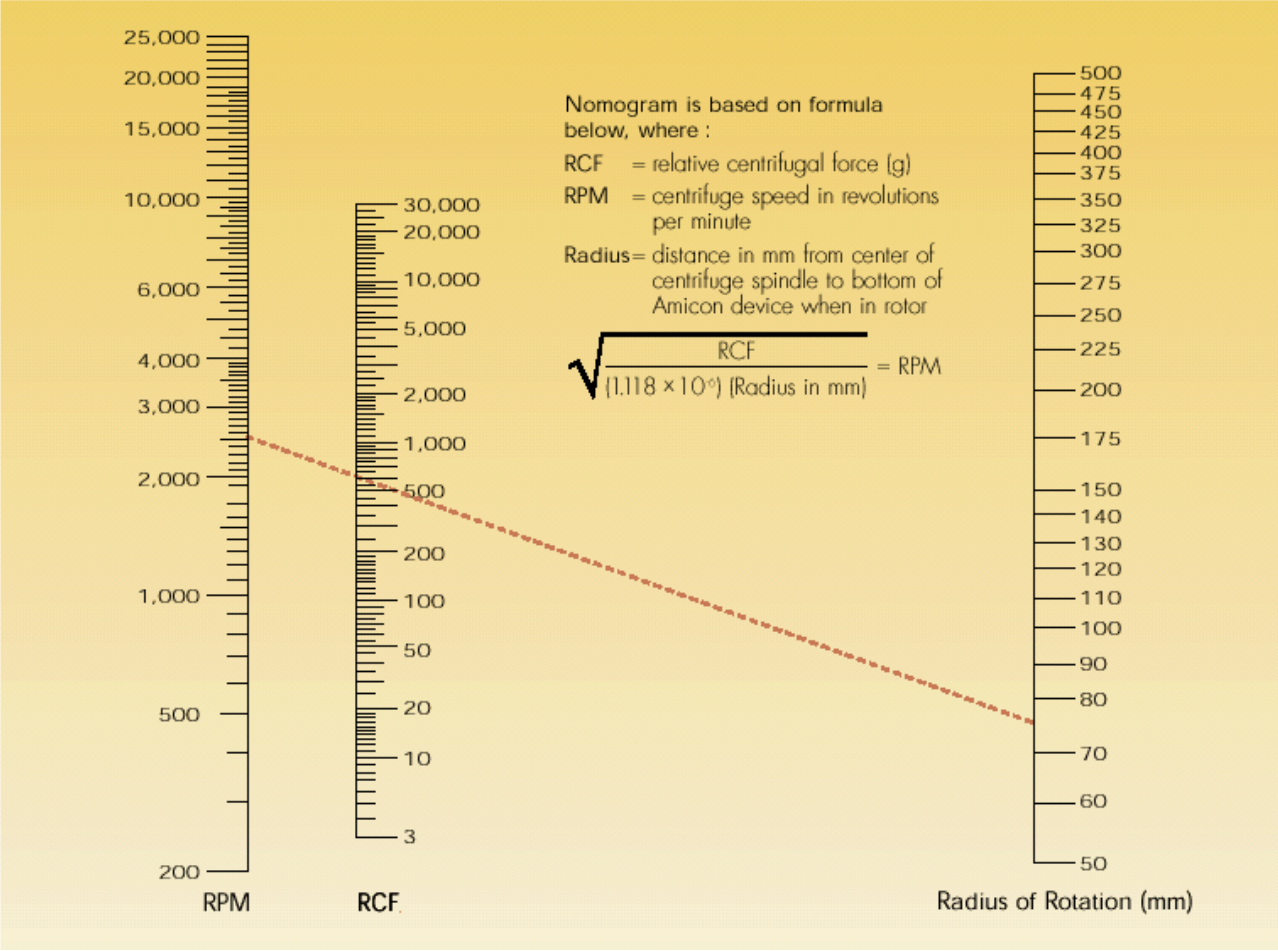
1) Gently mix all purified RNA samples.
2) With the sampling arm open, pipet 0.5-1µL of RNAse-free water onto the lower measurement pedestal.
3) Blank system. Clean with soft laboratory wipe.
4) With the sampling arm open, pipet 0.5-1µL of sample onto the lower measurement pedestal.
5) Close the sampling arm and initiate spectral measurement using Nanodrop software. Be sure to select RNA as the sample type.
6) When the measurement is complete, open the sampling arm and wipe the sample from the upper and lower pedestals using a soft laboratory wipe.
7) Repeat with remaining RNA samples.
8) When finished, clean upper and lower pedestals with moistened laboratory wipe.
9) Export data to excel.
10) Dilute RNA samples to a concentration of 100µg/mL.

Storage of purified RNA Samples

1) Once all samples are of a concentration of 100µg/mL ± 10%, aliquot units of 10µg each into pre-barcoded Nunc Bank-It vials. Final RNA amount as well as any vials with less than 10µg should be recorded.
2) Record the total volume of each sample.
3) Record freezer location of each aliquot.

Converting RCF (g) to RPM:

Nomogram for conversion of g to RPM



To convert maximum relative centrifugal force (RCF) to RPM: Determine centrifuge 's radius of rotation (in mm) by measuring distance from center of centrifuge spindle to bottom of device when inserted into rotor. Lay a ruler or draw a line from radius value in right-hand column value that corresponds to the device 's maximum rated g-force. Then read the maximum value from column at left.