14. DOUBLY-LABELED WATER (DLW) PROCEDURES

14.1Procedures for Receiving Urine Samples

- As soon as the e-mail message is received by Lucinda Clarke at the Central DLW Laboratory (Laboratory), she will notify the security guard and the receiving dock staff at the USDA/ARS Children's Nutrition Research Center of the pending shipment.
- When the shipment arrives at the USDA/ARS Children's Nutrition Research Center, the receiving dock personnel or the security guard at the Center will inform Lucinda Clarke or the research assistant in the Laboratory so that the samples can be picked up immediately.
- At the Laboratory, Lucinda Clarke or the research assistant will check the samples in the box against the **Sample Shipping Spreadsheet**. If there is any discrepancy, the study site will be notified immediately by phone and by email. Lucinda Clarke or the research assistant will note the discrepancy and the resolution on the **Sample Shipping Spreadsheet**.
- Once all discrepancies, if any, are resolved, Lucinda Clarke or the research assistant will initiate and date the **Sample Shipping Spreadsheet** and then notify the Study Manager at the study site by email of the receipt of the samples.
- Lucinda Clarke or the research assistant will remove the samples from the shipping container, put them in a -80^{o}C freezer, and write down the location of the samples on the **Sample Shipping Spreadsheet**.
- The research assistant will scan the **Sample Shipping Spreadsheet**, convert it into a PDF document, and identify it by the study site and the date the samples were received.
- The hard copy of the **Sample Shipping Spreadsheet** will be stored in a "Sample Shipping Spreadsheet" ring binder identified by Study Site.

14.2Procedures for Logging in Urine Samples

- Once all discrepancies, if any, are resolved, Lucinda Clarke will upload the data from the electronic **Sample Shipping Spreadsheet** into the Gas-Isotope-Ratio Mass Spectrometry Access Database (Database). The electronic **Sample Shipping Spreadsheet** will be stored in a central server at Baylor College of Medicine and identified by the study site and the date the spreadsheet was received.
- In the Database, each set of urine samples as well as each urine sample as defined under section 10.2.1 (Label Sheets for Baseline DLW Studies) and section 10.2.3 (DLW ID Numbers for Post-Randomization Studies) will be identified.
- The research assistant at the Laboratory will verify the accuracy of the entry against the information on the **Sample Shipping Spreadsheet**.
- The date when each set of urine samples is uploaded into the Database will be automatically generated in the Database.

- The date generated by the Database and the date when the corresponding isotopic data are uploaded into the Database will be used to calculate the turnaround time for each set of urine samples received.

14.3Description of DLW Methods and Activities

Lucinda Clarke and the research assistants who are trained in gas-isotope-ratio mass spectrometry methods and under the supervision of Dr. William Wong will be responsible to carry out all the DLW methods and activities in the Central DLW Laboratory. Both sets of urine samples from the two baseline DLW studies for each study participant will be processed and analyzed at the same time. Urine samples from each complete set of post-randomization DLW study also will be processed and analyzed at the same time. Therefore, the same reference materials, the same dose dilution samples, and the same mass spectrometers will be used to analyze these urine samples.

14.3.1 $^{2}H/^{1}H$ Isotope Ratio Measurements

- Each set of urine samples scheduled for analyses will be retrieved from the freezer and thawed to room temperature.
- An aliquot of 10 μl of each urine sample without further processing will be transferred into a sample reduction tube containing 250 mg of zinc reagent under an atmosphere of dry nitrogen.
- A duplicate sample will be prepared, using another sample reduction tube, for each urine sample.
- After freezing the urine sample at liquid nitrogen temperature, the nitrogen gas is evacuated and the stopcock of the reduction tube is closed.
- The water in the urine sample is reduced to hydrogen gas by heating the zinc reagent in the sample reduction tube at 500^{o}C for 45 minutes according to the following reaction:

$$H_2O + Zn \rightarrow ZnO + H_2$$

- Upon cooling to room temperature, the tube containing the hydrogen gas is attached to a multi-port inlet system of a Finnigan Delta-E gas-isotope-ratio mass spectrometer in the Laboratory.
- The hydrogen gas is analyzed four times automatically against a laboratory reference H_2 gas in the following sequence: reference/sample/reference……sample/reference.
- The result is expressed as δ^2H units after correction for H_3^+ contribution.
- The δ^2H is defined as δ^2H (^{o}/oo) = (R_{sample} / $R_{standard}$ - 1) x 10^3 where R_{sample} and $R_{standard}$ are the $^{2}H/^{1}H$ ratio of the sample and the working standard, respectively.
- The result is written electronically onto a floppy disk.
- The instrument also provides a print out of the result that identifies the date and time of the analysis, the inlet port number, the sample ID, the $^{2}H/^{1}H$ isotope ratios of the reference H_2 and the sample H_2,

the calculated δ^2H value, and the precision of the isotope ratio measurements.

- The entire process of sample analysis, H_3^+ correction, and data collection is controlled automatically by the operating software of the gas-isotope-ratio mass spectrometer.

14.3.2 $^{18}O/^{16}O$ Isotope Ratio Measurements

- An aliquot of 100 μl of each urine sample without further processing will be transferred into an equilibration vessel of a VG ISOPREP-18 H_2O-CO_2 equilibration system.
- A duplicate sample will be prepared, using another equilibration vessel, for each urine sample.
- After evacuation of the atmospheric gases in the equilibration vessel through a capillary that allows only inert gases such as oxygen, nitrogen, carbon dioxide, and argon to pass through, the vessel is filled with CO_2 of known ^{18}O content to 300 mbar.
- The urine-CO_2 mixture is allowed to equilibrate for 10 hours at 25°C with constant shaking.
- At the end of the equilibration, the CO_2 in the vessel is allowed to expand through the capillary into the sample inlet system of a VG SIRA-12 gas-isotope-ratio mass spectrometer for $^{18}O/^{16}O$ isotope ratio measurements against a laboratory working reference CO_2 gas.
- The CO_2 is analyzed four times automatically against the laboratory reference CO_2 gas in the following sequence: reference/sample/reference……sample/reference.
- The result is expressed as $\delta^{18}O$ units.
- The $\delta^{18}O$ is defined as $\delta^{18}O$ (°/oo) = $(R_{sample} / R_{standard} - 1) \times 10^3$ where R_{sample} and $R_{standard}$ are the $^{18}O/^{16}O$ ratio of the sample and the working standard, respectively.
- The result is written electronically onto a floppy disk.
- The instrument also provides a print out of the result that identifies the date and time of the analysis, the equilibration system port number, the sample ID, the $^{18}O/^{16}O$ isotope ratios of the reference and the sample, the calculated $\delta^{18}O$ value, and the precision of the isotope ratio measurements.
- The entire process of evacuation, CO_2 injection, equilibration, CO_2 extraction, and data collection is controlled automatically by the operating software of the gas-isotope-ratio mass spectrometer.

14.3.3 Dose Dilutions

- For each batch of DLW dose mixtures, two dose dilutions (~1:400 and ~1:1,500) that simulate the expected isotopic enrichments of the post dose samples to be collected on day 0 and day 14 of the 14-day DLW study will be prepared.
- The dose dilutions will be analyzed along with the urine samples.

- The isotopic results of the dose dilutions will be used to monitor the performance of the mass spectrometers and to calculate the isotope dilution spaces of the study participant in each DLW study.

14.3.4 Isotope Dilution Space and TEE Calculations

- Once the isotopic data from the Finnigan Delta E and VG SIRA-12 gas-isotope-ratio mass spectrometers are uploaded into the Database, the isotope dilution spaces for ^{2}H (N_H) and ^{18}O (N_O) will be calculated as follows:

$$N_H \text{ or } N_O \text{ (mol)} = \frac{d \, x \, A \, x \, E_\alpha}{\alpha \; x \, E_d \; x \, 18.02}$$

where "d" is the dose of DLW mixture in grams, "A" is the amount of laboratory water in grams used in the dose dilution, "α" is the amount of DLW mixture in grams added to the laboratory water to prepare the ~1:400 dose dilution, "E_α" is the rise in ^{2}H or ^{18}O abundance in the laboratory water after the addition of the isotopic water, and "E_d" is the rise in ^{2}H or ^{18}O abundance in the urine samples at time zero obtained from the zero-time intercepts of the ^{2}H and ^{18}O decay curves in the urine samples.

- The use of dose dilution in the calculation of isotope dilution spaces was recommended by the International Dietary Energy Consultancy Groups to assure accuracy of the isotope dilution calculations.
- The fractional turnover rates of ^{2}H (k_H) and ^{18}O (k_O) will be calculated as the slopes of the regression lines for each isotope following the natural logarithmetic transformation of the isotopic enrichment over baseline value over time.
- Carbon dioxide production rate (rCO_2) will be calculated from the fractional turnover rates as follows:

$$rCO_2 \text{ (mol/d)} = 0.4812 \times [(k_O \times N_O) - (k_H \times N_H)] - 0.0246 \text{ x } r_g$$

where r_g is the fractionated water loss which is calculated as 1.05 x (N_O x k_O – N_H x k_H).

- The rCO_2 is converted to EE based on an energy equivalent of a liter of CO_2 to be 3.815/RQ + 1.2321 where RQ is the respiratory quotient.
- The RQ for the CALERIE phase 2 clinical trial is estimated to have a value of 0.86.

14.3.5 Total Body Water Calculations

- The isotope dilution spaces will be converted to total body water using the following relationships:

$$TBW_H\,(kg) = \left(\frac{N_H \times 18.02}{1.04}\right) \times 10^{-3}$$

$$TBW_O\,(kg) = (\frac{N_O \times 18.02}{1.01}) \times 10^{-3}$$

14.4 Procedures for On-going Quality Control in the Lab

Daily, weekly, and monthly quality control procedures are carried out in the Laboratory by Lucinda Clarke and the research assistants who are trained in gas-isotope-ratio mass spectrometry and under the supervision of Dr. William Wong. Semi-annual quality control procedures are carried out by the engineers from Pro-Vac Services Limited.

14.4.1 Daily Quality Control Procedures

14.4.1.1 Visual Inspection

- Pressure reading of the central cooling water for the oil diffusion pumps of the VG SIRA-12 mass spectrometer.
- Pressure readings of the compressed air that operates the pneumatic valves of the VG SIRA-12 and Finnigan Delta E mass spectrometers.
- Vacuum gauge readings of the mass spectrometers.
- If any of these readings are off by more than 10%, the regulators of the compressed air will be adjusted to obtain the desired pressure readings or the cause of the problem will be investigated and resolved prior to any sample analysis.
- The tracing on the chart recorder of the Finnigan Delta E mass spectrometer will be inspected because it provides an independent evaluation of the inlet system, the pneumatic valves, the gas reservoirs, and the viscous leaks. For example, any leakage in the inlet system will result in a spike in the tracing during the switching between the reference gas and the sample gas. Noisy tracing also is an indicator that the filament in the ion source is about to burn out. A low voltage tracing on the sample side will suggest potential leakage in the inlet port or incomplete reduction of the sample to H_2.
- The print out from the VG SIRA-12 mass spectrometer also will be inspected for the same reasons.

14.4.1.2 Peak Center and Peak Shape

- Prior to any sample analysis, the appropriate laboratory working reference gas will be admitted into the mass spectrometer.
- After adjusting the reference gas to the operating voltage, the mass spectrometer will be tuned for proper peak shape and optimal sensitivity.
- If the peak shape is not symmetrical and flat on top, the mass spectrometer will be retuned until the desired peak shape is obtained.

- Once the proper peak shape is obtained, the peak center program will be activated to make sure the minor peak of interest (mass 3 for $^{2}H^{1}H^{+}$ and mass 46 for $^{12}C^{16}O^{18}O+$) is at the center of the major peak (mass 2 for $^{1}H^{1}H^{+}$ and mass 44 for $^{12}C^{16}O^{16}O+$).
- If the center of the minor peak does not fall within the center portion of the major peak, the mass spectrometer will be retuned until the desired peak center is achieved.

14.4.1.3 Viscous Leak Adjustment

- Once proper peak center and peak shape have been achieved, the laboratory working reference gas is allowed to expand into the sample side of the mass spectrometer inlet system.
- With both the reference and sample inlet systems opened to each other, the reference gas or sample gas reservoirs are either compressed or uncompressed to achieve the normal operating voltage.
- With equal pressure on both inlet systems, the viscous leaks will be adjusted so that both viscous leaks will have similar flow rates into the ion source of the mass spectrometer.
- The procedure is to make sure the reference gas and the sample gas are entering the ion source of the mass spectrometer at the same rate and without isotope fractionation.

14.4.1.4 Zero Enrichment Measurement

- After the viscous leak adjustment, a zero enrichment measurement will be performed with the sample and reference gas inlet systems opened to each other, to make sure there is no difference in stable isotopic abundance between the sample and the reference gases.
- Since the same gas is put into the sample reservoir and the reference reservoir, the ^{2}H or ^{18}O content of the sample gas should be identical to the ^{2}H or ^{18}O content of the reference gas resulting in a zero ^{2}H or ^{18}O enrichment value.
- A second zero enrichment run will be performed with the sample and reference inlet systems isolated from each other.
- A zero enrichment value from the second run will confirm there is no difference in the viscous flows between the sample and reference capillaries.

14.4.1.5 H_3 Factor Determination

This procedure applies only to the $^{2}H/^{1}H$ isotope ratio measurements using the Finnigan Delta E gas-isotope-ratio mass spectrometer.

- If both zero enrichment measurements produce zero ^{2}H enrichment values, a $^{1}H_{3}^{+}$ correction run will be performed at five peak intensities, two at one and two units below the normal operating peak intensity, one at the normal operating peak intensity, and two at two and three units above the normal operating peak intensity.
- Although the source of the Finnigan Delta E gas-isotope-ratio mass spectrometers in the Laboratory are optimized for stable hydrogen isotope ratio measurements, $^{1}H_{3}^{+}$ ions are generated by impact between $^{1}H_{2}^{+}$ ions and H_2 molecules at the ion source.
- Since $^{1}H_{3}^{+}$ has the same mass number as the $^{1}H^{2}H^{+}$ ions, correction must be made or the ^{2}H enrichment value of the sample will be artificially inflated.
- The $^{1}H_{3}^{+}$ correction factor is calculated from the five measurements and all sample analyses will be adjusted using this correction factor.
- The $^{1}H_{3}^{+}$ adjustment is done automatically by the operating software of the mass spectrometer.
- The outcome of this procedure is recorded in an operation log book.

14.4.2 Providing %CR Prescription to Sites

1. The two consecutive DLW studies are performed at the CALERIE site. The corresponding urine samples are sent to the DLW lab together in a single shipment for analysis.
2. As soon as the samples from the two consecutive DLW studies have been analyzed, the DLW Lab sends an e-mail with an Excel spreadsheet attachment to the primary and back-up DLW person at the site
3. The Excel spreadsheet provides the following information:
   - Study site ID, CALERIE ID number, the sample collection date and time
   - the amount of dose given, the dosing time
   - the ^{2}H and ^{18}O content of the samples
   - the body weight and height of the study participant
   - the provisional RQ
   - the isotope dilution spaces (N_O, N_H), the calculated percentage of N_O and N_H relative to body weight, the N_H/N_O ratio, the fractional turnover rates (k_O, k_H)
   - the correlation coefficients of the fractional turnover rates
   - the calculated rCO_2
   - the calculated TEE

4. The spreadsheet calculates the average TEE from the two consecutive baseline DLW studies and their difference, as well as the calorie prescription (i.e., the average TEE of the two baseline DLW studies × 0.75).
5. The primary and back-up DLW at the study site reviews the information and either agrees to the CR prescription or raises concerns about the calculations.
6. If concerns are raised, a conference call among the DLW person at the study site, the DLW lab personnel and the CC is held and the results reviewed. An action plan is developed and implemented.
7. If no concerns are raised, the energy restriction is applied for that participant. The DLW person notifies the Study Manager that the prescription is appropriate. S/He then notifies the nutritionist, Intervention Leader and whomever else may need to know at the CALERIE site.
8. The prescribed calorie level is entered into the CTS tracking database by the Intervention Leader.

14.4.3 Weekly Quality Control Procedures

- Temperature of the heating blocks for the preparation of urine samples for $^{2}H/^{1}H$ isotope ratio measurements will be checked and recorded.
- Accuracy of the transfer pipets will be checked gravimetrically and recorded.

14.4.4 Monthly Quality Control Procedures

- Accuracy of the analytical balance will be checked and recorded.
- Pressure of the CO_2 reservoir in the VG ISOPREP-18 H_2O-CO_2 equilibration system will be checked and refill as necessary.

14.4.5 Semi-annual Quality Control Procedures

- The operation and specifications of the mass spectrometers are checked by the service engineer from Pro-Vac Services Limited (Pro-Vac).
- The vacuum pumps of the mass spectrometers and the sample preparation vacuum lines are checked and oil replaced by the service engineer from Pro-Vac.
- The turbo molecular pumps of the Finnigan Delta E mass spectrometers will be checked and oiled by the service engineer from Pro-Vac.

14.4.6 Reference Materials

- Reference materials of known ^{2}H and ^{18}O content such as the International Atomic Energy Agency's (IAEA) Standard Mean Ocean Water (SMOW), Standard Light Antarctic Precipitation (SLAP), Greenland Ice Sheet Precipitation (GISP), IAEA 302A, IAEA 302B, IAEA 304A, and IAEA 304B are analyzed annually on the mass spectrometers to make sure the results are within the acceptable ranges of the expected values.
- If there is a major tune up of the ion source of the mass spectrometer or the filament is replaced, the reference materials will be analyzed to document the accuracy and precision of the mass spectrometers prior to any samples are being analyzed.
- For the $^{2}H/^{1}H$ isotope ratio measurements, if a new batch of zinc is used, the laboratory working standard, the IAEA SMOW, and the IAEA SLAP will be analyzed. Unless the laboratory working standard and the IAEA reference materials (GISP, 302A, 302B, 304A and 304B) are within the acceptable ranges of their expected values, no samples will be analyzed.

14.4.7 Quality Control Based on Dose Dilutions

This quality control procedure is specifically designed for the CALERIE phase 2 clinical trial in order to monitor the variability of the isotope ratio measurements over time. These dose dilutions are not the same as the laboratory working standard. However, sufficient amount of the dose dilutions and the water used in the preparation of the dose dilutions will be saved so that the same dose dilutions and water can be used during the duration of the CALERIE phase 2 clinical trial.

- The dose dilutions (~1:400 and ~1:1,500) and the water used in preparing the dose dilutions will be analyzed daily in duplicate over a period of 10 days.
- The logarithmic transformed isotopic enrichments of these measurements will be used to generate the hypothetical constants for the conversion of these enrichment values to produce theoretical fractional turnover rates of ^{2}H (k_H, 0.1) and ^{18}O (k_O, 0.13) or a difference in fractional turnover rates ($k_O - k_H$) of 0.03.
- Subsequent measurements of these same dose dilutions and water will be converted to k_H and k_O using the hypothetical constants.
- The differences in the fractional turnover rates ($k_O - k_H$) generated from the subsequent dose dilution measurements will be compared to the theoretical difference of 0.03.
- The comparison will be presented graphically with percentage difference from 0.03 on the y-axis and date of analysis on the x-axis.
- The percentage difference is anticipated to be within 5%.

14.4.8 Anticipated Precision of Isotope Ratio Measurements

- The precision for stable hydrogen isotope ratio measurements is anticipated to be within 4.7 ^{o}/oo.
- The precision for stable oxygen isotope ratio measurements is anticipated to be within 1.0 ^{o}/oo.

14.4.9 Nonconformity Results and Corrective Actions

Results are reviewed daily by Lucinda Clarke and Dr. William Wong to identify nonconformity of the results so that corrective actions can be taken.

- If the isotopic enrichment of ^{18}O is too close to the baseline value as defined as a $\delta^{18}O$ over-baseline-value of 10 ^{o}/oo or lower, the DLW outcome variables will be recalculated without the isotopic data collected on day 14. The reason for the recalculation will be documented in the report to the Coordinating Center.
- If the chart recorder tracing of the Finnigan Delta E mass spectrometer identifies a leak in one of the sample pneumatic valves, the result of the specific samples will be discarded and the sample will be repeated using a different pneumatic valve.
- Samples will be reanalyzed if there is a filament failure or malfunctioning of any of the changeover valves, pneumatic valves, and the gas reservoir system.
- Samples also will be reanalyzed if there is evidence of incomplete conversion of the water to hydrogen gas during the zinc reduction procedure.
- Samples will be reanalyzed if there is any suggestion of sample mixed up.
- The calculated fractional turnover rates, the correlation coefficients of the fractional turnover rates, the ratio of the isotope dilution spaces, the percentage of body water relative to body weight, and the energy expenditure values will be used to identify nonconformity. If any of these calculated values appear to be out of the normal ranges (e.g. fractional turnover rates lower than 0.08 or high than 0.15; correlation coefficients lower than 0.97; ratio of the isotope dilution space (N_H/N_O) lower than 0.9 or higher than 1.08; the percentage of body water relative to body weight lower than 40% or higher than 60%; energy expenditure values lower than 1,500 kcal/d or higher than 3,500 kcal/d), the isotopic data will be scrutinized to identify potential outliers.
- If outliers are not identified in the isotopic data, the dose information and the time of sample collection will be checked for accuracy in the Database.
- If all the information is correct, the Study Manager at the study site will be notified.

- If necessary, the entire set of samples will be reanalyzed.
- All nonconformity results and the corrective actions taken will be recorded.

14.4.10 Data Storage and Security

14.4.10.1 Hard Copies

- Hard copies of all the results generated from the Laboratory are put in storage boxes and placed in the basement of the Children's Nutrition Research Center
- The boxes are clearly labeled by the Center's investigator, the instrument, and the duration in which the data were collected.
- These boxes are accessible only through the Building Manager.
- These boxes are kept for a minimal of three years.

14.4.10.2 Electronic Copies

- All the computers in the Laboratory, including the one in Dr. William Wong's office, are connected to the central server at Baylor College of Medicine.
- A full backup of all the files, directories and drives on these computers to tapes is performed every night.
- These tapes are kept either on-site in fire-proof vaults or offsite for 45 days.
- Archive clone sets of these tapes are made for the month of March, June, September and December and will be retained for one year.
- All the computers in the Laboratory are password protected and can only be accessed by authorized Laboratory personnel.

14.5 Transferring Results Electronically to the Coordinating Center

- The Database will be programmed to produce a cumulative pipe (|) delimited ascii file (DLW data file) containing the DLW sample ID (nn-XXXX-z-PDa or nn-XXXX-z-D14b where "nn" is the study site in numeric format; "XXXX" is the numeric CALERIE study number for the baseline DLW studies or the numeric randomly generated numbers for the post-randozimation DLW studies; "z" is a numeric study sequence number for the baseline DLW studies or a numeric placement number for the post-randomization DLW studies; "PDa" or "D14b" are the pre-defined sample collection sequence); the DLW dose mixture ID and dose bottle number (mixture of alphabets and numbers: CA-xxxx-nnn where "CA" stands for the CALERIE clinical trial, "xxxx" is the numeric batch number for the DLW dose mixture, and "nnn" is the numeric bottle number), the dose amount (numeric: xxx.dd); the dose administration and sample collection time (mm/dd/yyyy hh24:mi); the ^{2}H and ^{18}O isotopic data (numeric, xxxx.dd); the isotope dilution spaces: N_H and N_O (numeric: xx.dd), the N_H/N_O ratio (numeric: x.dddd); the total body water: TBW_H andTBW_O (numeric: xx.dd);

the percentage of total body water: $\%TBW_H$ and $\%TBW_O$ (numeric: xxx.dd); the fractional turnover rates: k_H and k_O (numeric: x.ddddd); the correlation coefficients of the fractional turnover rates: r_H and r_O (numeric: x.dddd); the carbon dioxide production rate: rCO_2 (numeric: xxx); total energy expenditure: TEE (numeric: xxxx); and date of analysis (mm/dd/yyyy).

- The updated DLW data file will be posted to the coordinating center's FTP site on a bi-weekly basis for the baseline period of the study. Once the baseline visits for all participants have been completed, transfer frequency will be reduced to monthly.
- The turnaround time to report the results for the post-randomization DLW studies is six weeks.

14.6 *QC Reports for the QC Committee*

- As described under section ***14.4.6 Quality Control Based on Dose Dilutions***, subsequent measurements of the ~1:400 and ~1:1,500 dose dilutions will be converted to k_H and k_O using the hypothetical constants generated from the logarithmic transformed isotopic enrichments of these measurements over a 10-day period.
- The same dose dilutions will be used during the CALERIE phase 2 clinical trial.
- The differences in the fractional turnover rates ($k_O - k_H$) generated from the subsequent dose dilution measurements will be compared to the theoretical difference of 0.03.
- The comparison will be presented graphically to the QC Committee with percentage difference from 0.03 on the y-axis and date of analysis on the x-axis.
- The QC Reports will be transmitted electronically to the QC Committee via email on a quarterly interval.
- Dr. William Wong or Lucinda Clarke will be responsible for the QC Reports.

14.7 Monthly Activity Report to the Steering Committee

- The number of DLW studies analyzed, the turnaround time for each set of DLW study, any nonconformity results, and the corrective actions taken will be reported to the Steering Committee via email.
- The report will be in the format of a table consisting of the following columns: DLW Shipped, DLW Received, DLW Study Analyzed, Turnaround Time, Nonconformity, and Corrective Actions.
- Dr. William Wong or Lucinda Clarke will be responsible for this report.