

Appendix 5M

**Automated Dissociated Enhanced Lanthanide Fluoro
Immunoassay System (AutoDELFIA) for Prenatal Screening Using
Maternal Serum: 1st Trimester Markers are Pregnancy Associated
Plasma Protein-A (PAPPA) and Human Chorionic Gonadotropin
(HG1), 2nd Trimester Markers are Human Chorionic Gonadotropin
(hCG), Alphafetoprotein (AFP), and Unconjugated Estriol (uE3)**

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I. Title

Automated Dissociated Enhanced Lanthanide Fluoro Immunoassay System (AutoDELFIA) for Prenatal Screening Using Maternal Serum: 1st Trimester markers are Pregnancy Associated Plasma Protein-A (PAPPA) and Human Chorionic Gonadotropin (hCG), 2nd Trimester markers are Human Chorionic Gonadotropin (hCG), Alpha-fetoprotein (AFP) and Unconjugated Estriol (uE3)

II. Principle

The AutoDELFIA assays are solid phase, time-resolved fluoroimmunoassays developed by Perkin Elmer Wallac. The marker or label for the assays is a lanthanide metal, europium. Measurement of the marker is by time resolved fluorometry where the Eu marker is excited by an energy beam and the decaying energy is measured. Measurement consists of about 1000 excitation cycles, the duration of each being one millisecond. Time-resolved fluorometric assays are more sensitive than conventional fluorometric immunoassays primarily because background fluorescence from interfering substances is eliminated.

The hCG and AFP immunoassays use the "sandwich principle" in which two mouse monoclonal antibodies are directed against two separate antigenic determinants on the analyte molecule. The specific Eu-labeled antibody binds to the antigen (hCG or AFP) which is bound to the antibody coated onto the walls of the reaction well. A series of steps occurs where the coated antibody, antigen, Eu-labeled antibody are brought together for the reaction to occur. For hCG, 12 uL of maternal serum and 475 uL of diluent are delivered into the dilution strips, from which 25 uL are pipetted into the reaction well. For AFP, 25 uL of maternal serum are delivered into the reaction well. At the completion of the reaction, reaction wells are washed and aspirated to remove the unbound Eu-labeled antibody. An enhancement solution is used to dissociate the Eu ions and to form fluorescent chelates. The pulses counted from the decaying fluorescence are proportional to concentration of the analyte.

The uE3 is a competitive immunoassay where uE3 from the specimen competes with the Eu-labeled uE3 for sites on the polyclonal antibody, which in turn is specific for the antibody coated onto the reaction well. This assay is a two-step assay where the coated antibody is allowed to react with the polyclonal antibody. In the second step, 50 uL of maternal serum and the tracer are added such that the uE3 from the sample and the Eu-labeled uE3 compete for sites on the polyclonal antibody. At the completion of the reaction, the wells are washed and aspirated to remove the unbound Eu-labeled uE3. The same enhancement solution as above is added to dissociate the Eu ions and form fluorescent chelates. The pulses counted from the decaying fluorescence are inversely proportional to concentration of uE3.

The PAPPA immunoassay uses the "sandwich principle" in which monoclonal antibodies, derived from mice, are directed against two separate antigenic determinants on the analyte molecule. The biotin labeled antibody binds with the streptavidin, coated onto micortiter plates. The Eu-labeled antibody binds to the antigen (PAPPA) which is bound to the biotin label antibody which is bound to streptavidin, coated onto the walls of the reaction well. A series of steps, reagent addition, incubation, washing, occurs where the biotin labeled antibody, antigen, Eu-labeled antibody are brought together for the reaction to occur. Enhancement solution is used to dissociate the Eu ions and to form fluorescent chelates. The pulses counted from the decaying fluorescence are

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proportional to concentration of the analyte. For PAPP_A, maternal serum is diluted 1:5, 12 uL of maternal serum and 48 uL of diluent are delivered into the dilution strips from which 25 uL are pipetted into the reaction well.

The HG1 assay is identical to the 2nd trimester hCG assay with one exception. The maternal serum is diluted 1:100, 10 uL of maternal serum and 990 uL of diluent are delivered into the dilution strips from which 25 uL are pipetted into the reaction well.

The flow charts for each assay are shown in Appendix 6, 7, 8 and 9.

III. Specimen Collection and Type

Serum samples are collected from pregnant women in their 1st and 2nd trimester using the Becton Dickinson SST collection tubes, 3.5 mL draw (13 x 75 mm). Collection kits are supplied to the providers by the Genetic Disease Screening Program.

IV. Equipment and Supplies**A. Equipment**

1. Supplied by Perkin Elmer Wallac
 - a. AutoDELFIA System with PC
 - b. Review Workstation PC
 - c. Server PC
 - d. Barcode label printers with PC
 - e. External Modem
 - f. Laser Printer
 - g. Uninterruptible Power Supply (UPS) unit for each AutoDELFIA
 - h. UPS unit for the Server PC
2. Supplied by GDL
Centrifuge, Beckman TJ6, capable of attaining 1000 g centrifugal force, use with Model TH4 Rotor and with 2 sets of four racks
3. Supplied by NAPS Laboratory
Balance, 2 pan mechanical balance

B. Supplies

1. Supplied by Perkin Elmer Wallac
 - a. Sample rack tray, 2/AutoDELFIA
 - b. Sample racks, 2 sets of 36 racks/AutoDELFIA
 - c. Cap holder, 1/AutoDELFIA
 - d. Dilution racks, 1/AutoDELFIA
 - e. Dilution vessels
 - f. Diluent cups, square, for diluting samples for hCG assay
 - g. Dilution strips
 - h. Pipette tips
 - i. Computer paper

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- j. Printer toner/ink cartridge
- k. Barcode labels
- l. Barcode ribbons
- m. DAT data tape, 4 mm, 3M DDS-90 or equivalent
- 2. Supplied by NAPS Laboratories
 - a. Pipette, volumetric, class A, 20°C, TD, 5 mL
 - b. Pipette, fixed volume, 1500 uL
 - c. Pipette, adjustable, up to 250 uL
 - d. Tubes, plastic, with 4 different colors of caps, 12 x 75 mm
 - e. Tubes, glass or plastic, 16 x 125 mm
 - f. 250 mL beakers
 - g. Cotton swab
 - h. Pipette, disposable
 - i. Safety caps for collection tubes

V. Reagents

- A. Supplied by Perkin Elmer Wallac
 - 1. Specific for hCG/HG1
1st trimester HG1 and 2nd trimester hCG assays use the same reagent kits.

All reagents are stored refrigerated 2 - 8° C. Use until date of expiration. Each box of hCG reagents contains 3 units of reagents plus 3 barcode labels for the reagent cassette, encoded for analyte and lot number. Each unit contains reagents for 4 trays. Each unit contains:

- a. hCG Standards, prepared in a matrix of human male serum, with <0.1 % sodium azide as preservative.

<u>Name</u>	<u>Concentration*</u>	<u>Quantity**</u>
A	0.00 IU/mL	
B	0.01 IU/mL	
C	0.09 IU/mL	
D	0.90 IU/mL	
E	4.64 IU/mL	
F	9.21 IU/mL	

*Samples are diluted 1:40, 12 uL of serum to 475 uL of diluent. Based on concentrations listed, the effective range of the assay is 0.00 to 368.40 IU/mL. Actual concentrations may change slightly from lot to lot.

**2 sets of STDs, 1.1 mL per vial.

- b. Anti-hCG microtiter strips, 12 wells per strip, 8 strips per tray, 4 trays.
- c. Anti-hCG Eu tracer, 20 ug/mL, 2.2 mL/vial, 4 vials.
- d. DELFIA buffer, Tris-HCL buffered salt solution, pH 7.8, with bovine serum albumin, bovine globulin, mouse IgG, Tween 40, an inert dye, and <0.1 % sodium azide as preservative, 175 mL/bottle, 2 bottles.

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2. Specific for AFP

All reagents are stored refrigerated, 2 - 8° C. Use until date of expiration. Each box of AFP reagents contains 3 units of reagents plus 3 barcode labels for the reagent cassette, encoded for analyte and lot number. Each unit contains reagents for 4 trays. Each unit contains:

- a. AFP Standards, Tris-HCL buffered (pH 7.8) salt solution containing bovine serum albumin, with < 0.1 % sodium azide as preservative.

Name	Concentration*	Quantity**
A	0.00 ng/mL	
B	1.207 ng/mL	
C	12.16 ng/mL	
D	120.2 ng/mL	
E	603.2 ng/mL	
F	1213 ng/mL	

*Actual concentrations may change slightly from lot to lot.

**2 sets of STDS, 1.1 mL per vial.

- b. Anti-hAFP (mouse monoclonal) microtiter strips, 12 wells per strip, 8 strips per tray, 4 trays.
- c. Anti-hAFP-Eu tracer, stock solution, ~ 20 ug/mL, 1.7 mL/vial, 4 vials.
- d. MultiBuffer, Tris-HCL buffered salt solution (pH 7.8) with bovine serum albumin, bovine globulin, mouse IgG, diethylenetriaminepentaacetic acid, Tween 40, blockers, an inert dye, with <0.1 % sodium azide as preservative, 175mL/bottle, 2 bottles.

3. Specific for uE3

All reagents are stored refrigerated, 2 - 8° C. Use until date of expiration. Each box of uE3 reagents contains 3 units of reagents plus 3 barcode labels for the reagent cassette, encoded for analyte and lot number. Each unit contains reagents for 4 trays. Each unit contains:

- a. uE3 Standards, human serum matrix, lyophilized, with < 0.1 % sodium azide as preservative.

Name	Concentration*	Quantity**
A	0.00 ng/mL	
B	0.178 ng/mL	
C	0.379 ng/mL	
D	1.55 ng/mL	
E	5.19 ng/mL	
F	17.3 ng/mL	

*Actual concentrations may change slightly from lot to lot.

**2 sets of STDS, reconstitute to 1.5 mL per vial.

- b. Anti-rabbit IgG microtiter strips, 12 wells per strip, 8 strips per tray, 4 trays.
- c. uE3-Eu tracer, lyophilized, 40 nmol/L, reconstitute with 1.5 mL, 2 vials.

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- d. uE3 Antiserum, in Tris-HCL buffered salt solution, pH 7.4, with bovine serum albumin and Dextran T 10, < 0.1 % sodium azide as preservative, 2.0 mL/vial, 2 vials.
- e. uE3 assay buffer, Tris-HCL buffered salt solution, pH 7.8, with an inert dye, <0.1 % sodium azide as preservative, 120 mL/bottle, 2 bottles.

4. Specific for PAPP A

All reagents are stored refrigerated, 2 - 8° C. Use until date of expiration. Each box of PAPP A reagents contains 3 units of reagents plus 3 barcode labels for the reagent cassette, encoded for analyte and lot number. Each unit contains reagents for 4 trays. Each unit contains:

- a. PAPP A Standards, lyophilized, contains purified human-based PAPP A, Tris-HCL, and bovine serum albumin, < 1 % sodium azide as preservative.

<u>Name</u>	<u>Concentration*</u>	<u>Quantity**</u>
A	0.00 mU/L ***	
B	10 mU/L	
C	40 mU/L	
D	200 mU/L	
E	800 mU/L	
F	2000 mU/L	

*Actual concentrations may change slightly from lot to lot.

**2 sets of STDs, 1.5 mL per vial.

*** The assay standards use mU/L as the unit, but PAPP A results are reported by SIS as μ U/mL. mU/L is identical to μ U/mL.

- b. Streptavidin Microtitration strips, 12 wells per strip, 8 strips per tray, 4 trays
 - c. Anti-PAPP A Eu tracer, salt solution with bovine serum albumin, <0.1 % sodium azide as preservative, 2.5 mL/vial, 2 vials.
 - d. Anti-PAPP A Biotin antibody, mouse monoclonal, salt solution with bovine serum albumin, <0.1 % sodium azide as preservative, 2.5 mL/vial, 2 vials.
 - e. Multibuffer, Tris-HCL buffered salt solution, pH 7.8, with bovine serum albumin, bovine globulin, mouse IgG, diethylenetriaminepentaacetic acid, Tween 40, blockers, an inert dye, and <0.1 % sodium azide as preservative, 175 mL/bottle, 2 bottles.
- 5. Enhancement Solution, use for all analytes, with Triton X-100 (registered trademark of the Rohm and Haas Co.), acetic acid, and chelators, 250 mL/bottle, store protected from light at 2 - 8° C, use until expiration date, 8 bottles/box.
 - 6. Wash concentrate, concentrated Tris-HCL buffered salt solution, pH 7.8, with Tween 20 and Germall II (registered trademark of Sutton Laboratories Inc.) as preservative, 250 mL/bottle, store at 2 - 25° C, use until expiration date, 8 bottles/box.
 - 7. System Controls, universal control for TMS containing hCG, AFP, uE3, 3 levels [low(I), medium(II), high(III)], lyophilized, stable when refrigerated for 14 days after reconstitution, use until lot expiration date.
NOTE: System Control for the HG1 assay is the same SQC as for TMS screening.
 - 8. System Controls, contains only PAPP A, lyophilized, stable when stored frozen for 7 days after reconstitution, use until lot expiration date.

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9. Diluent II, buffer based, use to dilute samples for hCG testing, containing <0.1% sodium azide as a preservative. Store at 2-8° C, use until expiration date, 250 mL/bottle, 8 bottles/box.
10. Diluent I, human serum, use to dilute samples >STD; contains <0.1% sodium azide as preservative. Store at 2 to 8 °C. Use for 3 months once opened. 50 mL/bottle, 1 bottle/shipment.
11. Diluent 3, use to dilute samples for PAPP A testing

B. Supplied by GDLB

1. Tray Control, universal control for 4 markers, contains hCG, AFP, uE3, and Inh, 1 level, 5 mL/vial, store frozen, use until date of expiration.
2. Tray Control, single marker, PAPP A, 1 level, 5 mL/vial, store frozen, use until date of expiration.
NOTE: Tray Control for the HG1 assay is the same TQC for TMS and Inhibin screening.

C. Supplied by NAPS Laboratories

1. Distilled water or water equivalent to NCCLS Type I
2. Household bleach
3. Ethanol (do not use denatured ethanol), or isopropyl alcohol

VI. Calibration and Quality Control

A. Calibration

The calibration is performed daily using a specific set of standards for each assay. For each analyte the calibrators are run in duplicates on tray 1 and consist of six levels. Two levels of calibrators are also run in duplicate on each subsequent tray. The nominal concentrations for each level of each analyte are listed on Section V. A.1.a., 2.a., 3.a. and 4.a.

B. System Controls

A set of three system controls is provided by Perkin Elmer Wallac and is formulated to contain AFP, hCG, and uE3. A second set of three system controls is provided by Perkin Elmer Wallac and is formulated to contain PAPP A.

A single replicate of each of the system controls is run along with the calibrators on plate 1 and a single replicate of each as the last 3 samples of every analytical run.

C. Tray control

Two tray controls are provided by Genetic Disease Laboratory Branch. One contains hCG, AFP, and uE3. A second contains PAPP A. A single replicate of the tray control is run as the first sample on tray 1 after the calibrators and the three system controls, as the last sample on tray 1, as the first and last sample on each subsequent full tray, and as the first sample of the last partial tray with four replicates at the end. The tray control is not prediluted for hCG, HG1, and PAPP A and is run exactly as the maternal serum specimens.

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VII. Procedures

A. Barcoding

NOTE: A dedicated PC and a barcode generator/printer are provided for this use. This dedicated system prints barcode labels specifically for 1st and 2nd trimester prenatal screening specimens. Do not use the barcode generator for newborn screening.

1. Turn power on to monitor, barcode printer, and PC. The screen defaults to **TMS Barcode software** and opens the **California Screening Project Barcode Printing Program – Prenatal** window..

NOTE: Perkin Elmer Wallac recommends that power to the unit be shut down once a week to allow the system to reinitialize.

The power to the barcode generator/printer must be shut down to reinitialize whenever there is a change in the lot number for the tray quality controls (TQC) and system quality controls (SQC). This allows the system to pick up the new lot number of the TQC or the SQC. Turn off and on **prior** to printing the barcode of the new lot of TQC or SQC.

2. Click Exit.
3. Double click the **First Trimester Barcode** icon. There are seven icons representing the 7 types of barcode labels. Barcode labels can be printed for patient specimens, proficiency test samples (this function is not used at the screening laboratories), tray control lot # (for PAPP), low, medium, high SQC lot numbers (for PAPP), and reference samples.
4. Click **Patient**. Accession numbers for patient specimens consist of three digits for the Julian date, two digits for the check digit, three digits for the sequence number, a forward slash (/), a P (2nd trimester prenatal screening for hCG, APF, and uE3) or an A (1st trimester prenatal screening for PAPP and HG1), the year, and the lab site. The PC software has a calendar and clock, knows the Julian date, prints the time on the label, has an algorithm to calculate check digits, and knows the identification of your laboratory site.
5. Determine if there have been barcodes generated for the current date. Click **Enter start sequence number** and enter the first sequence number, 001.
6. Click **Number of labels** and enter 999. By entering 999, you will avoid making the error of entering a used sequence number for "start sequence number" resulting in the same barcode for two different specimens/test request forms. Also, by entering 999, you do not have to know the exact number specimens and can barcode specimens as they are received by your laboratory throughout the day. You must never enter as the Start sequence number, a sequence number which has been printed.
7. Use the drop down menu for **Label type** and select **P - Second Trimester**.
8. Click **Start**. The first accession number is printed on two labels. Two barcode labels are printed for each accession number.
9. Remove the barcode label on the right and put it on the test request form. Remove label on the left and put it on the tube so that the edge of the barcode label is just above the I number (The barcode label must not cover the I number and, if possible, serum should be visible to determine inadequacy due to hemolysis). The right label **MUST** be taken off the printer first. (This is necessary only if the printer is set to

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dispenser mode, prints one label at a time.) There is a sensor under the left label so that when the left label is taken off, the next accession number is printed on the next set of 2 labels. If the next 2 labels are printed, and the second label (of the prior accession number) is still on the printer, they will stick together. In the event there is a misstep, throw the labels away and use the next set of 2 labels with identical accession number. The screen will display the patient sequence number (the number of accession numbers printed) and the barcode being printed.

10. Continue until all 2nd trimester specimens and test request forms are barcoded.
11. Click **Stop** after the last specimen and test request form are barcoded.
12. Repeat for 1st trimester specimens by selecting **A – First Trimester** from **Label type**.
13. Give the Test Request Forms to the data entry staff.

B. Sample Handling

1. Centrifuge the samples.
Instructions provided to the collection sites are to centrifuge all 1st trimester specimens prior to sending. (If a 1st trimester specimen is not centrifuge when it arrives at the lab, it is an inadequate.) Centrifuge all 1st and 2nd trimester specimens received at your laboratory.
 - a. Place the collection tubes, labeled with a barcode, into the centrifuge racks. Keep all specimens for TMS screening (primarily P barcodes) together and all specimens for PAPP-A and HG1 (primarily A barcodes) together.
 - b. Arrange the collection tubes in opposite racks containing equivalent volumes.
 - c. Balance the centrifuge racks using a double pan balance. Opposite racks must be balanced within 10 grams. If not, use glass tubes, filled with water, and place in available wells.
 - d. Centrifuge for 10 minutes at 1000 to 1300 g.
 - e. Remove the racks from the centrifuge.
2. Transfer serum from nonstandard SST or red top tube.
 - a. Transfer the maternal serum from the collection tube to a sampling tube 1) when the collection tube cannot be sampled by the AUTODelfia, and 2) when the volume of maternal serum is low to protect the sample probe from contacting the gel. Minimum volume needed for screening is at least 500 µL.
 - b. Sort from the centrifuge rack specimens with low serum volume and specimens collected in a tube that cannot be sampled by the AUTODELFIA.
 - c. Process one sample at a time to avoid sample mix up. For each such sample, print two additional barcode labels with accession number identical to the accession number on the collection tube. Place one on the sampling tube and place the second on the TRF. The appearance of two barcode labels on the TRF is confirmation that an identical accession label was printed. Only the readable part of each barcode needs to be showing.
 - d. Transfer the serum into the barcoded tube. Place all specimens into a sample rack for testing. After transfer, it is not necessary for the analyst to determine whether a specimen with low volume has sufficient volume for TMS. The

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liquid handling system of the AutoDELFI A will perform the sampling step. If low volume is detected, result is flagged with an error message.

- e. Change the designation of the specimen to Inadequate if any result has a flag for low volume. Enter specimen in the Inadequate Specimens Form and enter "Q, quantity insufficient", as the reason for inadequacy. Give the form to data entry. Call the Prenatal Diagnostic Center coordinator and have another specimen collected.
3. Transfer the maternal serum specimens from the centrifuge racks (or DSX samples racks) into the AD sample racks. Transfer P type specimens follow by A type specimens. (You can transfer A type follow by P type as well.) Sample racks must be in numeric order. Certain positions on the sample racks are marked. These marked positions are for the TQC samples only. For reference to determine the total maternal sera that can be tested per run, see the following table.

# of Trays	Tube #	Total # of maternal specimen tested
1	1-78	73
2	1-170	163
3	1-262	253
4	1-354	343

Every position on the sample rack must be filled, leaving no gaps. Sample racks must be in numeric order. Certain positions on the sample racks are marked. These marked positions are for the TQC samples only. On a run with the maximum of one full tray, the positions for TQC and Maternal Sera (MS) are:

Rack #7	Rack #2-6	Rack #1	Position #
MS	MS	TQC	1
MS	MS	MS	2
TQC	MS	MS	3
TQC	MS	MS	4
TQC	MS	MS	5
TQC	MS	MS	6
Empty	MS	MS	7
Empty	MS	MS	8
Empty	MS	MS	9
Empty	MS	MS	10
Empty	MS	MS	11
Empty	MS	MS	12

On a run with a maximum of two full trays, the position for TQC and Maternal Sera are:

Rack #15	Rack #14	Rack #8-13	Rack #7	Rack #2-6	Rack #1	Position #
TQC	MS	MS	MS	MS	TQC	1
TQC	MS	MS	MS	MS	MS	2
Empty	MS	MS	MS	MS	MS	3
Empty	MS	MS	MS	MS	MS	4

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Empty	MS	MS	MS	MS	MS	5
Empty	MS	MS	MS	MS	MS	6
Empty	MS	MS	MS	MS	MS	7
Empty	MS	MS	MS	MS	MS	8
Empty	MS	MS	TQC	MS	MS	9
Empty	MS	MS	TQC	MS	MS	10
Empty	TQC	MS	MS	MS	MS	11
Empty	TQC	MS	MS	MS	MS	12

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One a Run with a maximum of three full trays, the position for TQC and Maternal Sera are:

Rack #22	Rack # 16-21	Rack #15	Rack #8-14	Rack #7	Rack #2-6	Rack #1	Position #
MS	MS	MS	MS	MS	MS	TQC	1
MS	MS	MS	MS	MS	MS	MS	2
MS	MS	MS	MS	MS	MS	MS	3
MS	MS	MS	MS	MS	MS	MS	4
MS	MS	TQC	MS	MS	MS	MS	5
MS	MS	TQC	MS	MS	MS	MS	6
TQC	MS	MS	MS	MS	MS	MS	7
TQC	MS	MS	MS	MS	MS	MS	8
TQC	MS	MS	MS	TQC	MS	MS	9
TQC	MS	MS	MS	TQC	MS	MS	10
Empty	MS	MS	MS	MS	MS	MS	11
Empty	MS	MS	MS	MS	MS	MS	12

On a run with the maximum four full trays, the positions for TQC and Maternal Sera are:

Rack #30	Rack #24-29	Rack #23	Rack#16-22	Rack #15	Rack #8-14	Rack #7	Rack #2-6	Rack #1	Position #
MS	MS	TQC	MS	MS	MS	MS	MS	TQC	1
MS	MS	TQC	MS	MS	MS	MS	MS	MS	2
TQC	MS	MS	MS	MS	MS	MS	MS	MS	3
TQC	MS	MS	MS	MS	MS	MS	MS	MS	4
TQC	MS	MS	MS	TQC	MS	MS	MS	MS	5
TQC	MS	MS	MS	TQC	MS	MS	MS	MS	6
Empty	MS	MS	MS	MS	MS	MS	MS	MS	7
Empty	MS	MS	MS	MS	MS	MS	MS	MS	8
Empty	MS	MS	MS	MS	MS	TQC	MS	MS	9
Empty	MS	MS	MS	MS	MS	TQC	MS	MS	10
Empty	MS	MS	MS	MS	MS	MS	MS	MS	11
Empty	MS	MS	MS	MS	MS	MS	MS	MS	12

4. Place a TQC into the marked positions. When all the maternal sera specimens have been placed into the sample racks, place a TQC into the next four positions after the last maternal sera specimen.
5. Determine inadequacy for testing due to hemolysis. A sample is inadequate due to hemolysis when the color of the serum portion is darker than the serum portion of the tube in the color standard. To determine whether maternal serum is darker, hold the sample upright against the white background and next to the tube in the color standard. There are two copies of the color standard. One is the working copy and the other copy is used for backup and reference purposes. Keep the reference copy in

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a plastic envelope in a safe, dark place. Refer to Section VII.S.4.f. , Monthly Maintenance, for use of the reference copy.

- a) Do not put the inadequate hemolyzed samples into the sample racks. Enter the information on the Record of Expanded AFP Inadequate Samples form (Appendix 1). Give the form to data entry to enter the information into the central computer. Follow usual procedures for inadequate and call the AFP coordinator to have another maternal serum specimen collected.
 - b) Write "H" on the tube and store in freezer.
6. Remove the caps and check specimens for air bubbles. Remove air bubbles using a disposable pipette.
 7. Add specimen(s) which need repeat testing for TMS at the end or beginning of the batch of specimens for TMS screening. Likewise for specimens that need repeat testing for PAPP_A or HG1. See VII.P.
 8. Add specimens that were reclassified from 1st to 2nd trimester or visa versa by SIS. See VII.Q.
NOTE: For any specimens which had been stored in refrigerator or freezer, mix, and re-centrifuge before testing.
 9. Place the sample racks into the sample rack tray. Each AutoDELFI_A system is supplied with 2 sets of 36 sample racks and 2 sample rack trays.

C. Warnings and Precautions for Users

1. The reagents are manufactured from human blood components. The source materials have been tested by immunoassay for hepatitis B surface antigen, anti-hepatitis C, and anti-HIV antibodies and found to be negative. Nevertheless, all blood derivatives, including patient specimens, should be considered potentially infectious and all recommended precautions for the handling of such should be observed. Refer to the U.S. Department of Health and Human Services (Bethesda, Md., USA) publication No. (CDC) 88-8395 on laboratory safety procedures.
2. Reagents contain sodium azide (NaN₃) as a preservative. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up.
3. Wear disposable gloves and safety goggles while handling reagents and maternal serum specimens. Thoroughly wash hands after removing gloves.
4. Dispose of all waste in accordance with your local, state, and federal regulations.

D. Preparation of Reagents

1. Reconstitute uE3 standards by adding 1.5 mL of distilled water to each vial. Use a 1500 uL fixed volume pipettor to deliver 1.5 mL. Allow to stand for at least 30 minutes. Mix gently before use. Once reconstituted, store refrigerated and use for 5 days. Write the expiration date on the box of the reconstituted standards
2. Reconstitute uE3-Eu tracer by adding 1.5 mL of distilled water to each vial. Use a 1500 uL fixed volume pipettor to deliver 1.5 mL. Allow to stand for at least 30 minutes. Mix gently before use. Once open use for 7 days.

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3. Reconstitute PAPP A standards by adding 1.5 mL of distilled water to each vial. Allow to stand for at least 30 minutes to dissolve fully. Mix gently before use. A vial of standard has sufficient volume for 2 standard curves. **NOTE:** After the initial use, store in refrigerator. Use a 2nd time only within two days. Write the expiration date on the box of the reconstituted standards.
4. Reconstitute low, medium, and high Universal TMS SQC.
 - a. Add 5 mL distilled water to each vial. Use a 5 mL volumetric pipette to deliver the 5 mL.
 - b. Allow to stand for at least 30 minutes. Mix gently by inverting.
 - c. Dispense 500 uL of each level into 10 plastic tubes and cap each level with a different colored cap.
 - d. Use one tube per level per run per AutoDELFIA. Store refrigerated. Use for 14 days after reconstitution when stored frozen.
 - e. Place a barcode on each plastic tube encoded for the lot number. Write the expiration date on each tube of the prepared SQC. To print the TMS system control barcode labels, open the **TMS Barcode software** icon and click the **Low System Ctrl**, **Medium System Ctrl**, and **High System Ctrl** icons and specify 5 (for 10 labels) as the number of labels. Prepare one level at a time to eliminate error of placing the wrong label on the tubes. The lot number is the current lot number and under the control of GDL. Make sure the lot number on the barcode label matches the lot number on the vial.
5. Reconstitute low, medium, and high PAPP A SQC.

NOTE: Vials of PAPP A SQC are label Sero 1, 2, and 3. Sero 1 is the high SQC, Sero 2 is the medium SQC, and Sero 3 is the low SQC.

 - a. Add 3 mL distilled water to each vial. Use a 3 mL volumetric pipette to deliver the 3 mL.
 - b. Allow to stand for at least 30 minutes for the solid to dissolve completely. Mix gently by inverting.
 - c. Dispense 400 uL of each level into 7 plastic tubes and cap each level with a different colored cap.
 - d. Use one tube per level per run per AutoDELFIA. Store refrigerated. Use for 7 days after reconstitution.
 - f. Place a barcode on each plastic tube encoded for the lot number. Write the expiration date on each tube. To print the PAPP A system control barcode labels, open the **First Trimester Barcode** icon and click the **Low System Ctrl**, **Medium System Ctrl**, and **High System Ctrl** icons and specify 4 (for 8 labels) as the number of labels. Prepare one level at a time to eliminate error of placing the wrong label on the tubes. The lot number is the current lot number and under the control of GDLB. Make sure the lot number on the barcode label matches the lot number on the vial.
6. Take a Universal (TMS) TQC vial, containing hCG, APF, uE3, and Inh from the freezer and thaw.
 - a. Dispense 500 uL into plastic tubes and cap with a color-

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- coded cap (different from the color-coded caps for all other controls).
- b. Place a barcode on each plastic tube encoded for the lot number. To print the TMS TQC barcode labels, open the **TMS Barcode software** icon and click the **Tray Control** icon and specify 10 (for 20 labels) as the number of labels. The lot number is the current lot and under the control of GDLB. Make sure the lot number on the barcode label matches the lot number on the vial.
 - c. Use for 7 days after thawing. Write the expiration date on each tube of TQC.
7. Take a single marker (PAPPA) TQC, containing PAPPA, from the freezer and thaw.
- a. Dispense 400 uL into plastic tubes and cap with a color-coded cap (different from the color-coded caps for all other controls).
 - b. Place a barcode on each plastic tube encoded for the lot number. To print the PAPPA TQC barcode labels, open the **First Trimester Barcode** icon and click the **Tray Control** icon, go to **Control type** and select **CT – Control 4**, and specify 6 (for 12 labels) as the number of labels. The lot number is the current lot and under the control of GDLB. Make sure the lot number on the barcode label matches the lot number on the vial.
 - c. Store in the refrigerator and use for 7 days after thawing. Write the expiration date on each tube of TQC.
8. Dispense Diluent I into tubes. Use a tube to dilute sample when the result is >Std.
- a. Remove bottle from refrigerator and open.
 - b. Aliquot 2 mL into each of 25 plastic tubes (12 X 75 mm).
 - c. Cap and use Parafilm to seal the tubes tightly. Store in refrigerator. Once dispensed, use for 3 months.
 - d. Remove a tube from refrigerator when needed. Use and dump.
 - e. Repeat with a new bottle when the last tube is used. Call GDL to ship a bottle to maintain your inventory at one bottle.
- E. Preparation for Analysis
1. Turn **ON** the AutoDELFIA plate processor. Allow 2.5 hours for the instrument to reach the set temperature.
 2. Turn **ON** the AutoDELFIA sample processor. Allow 30 minutes for the standard box to reach the set temperature.
 3. Turn **ON** monitor and process controller, PC.
NOTE: Perkin Elmer Wallac recommends that the system is powered up at all times.
 4. Print the “Repeats requested by GDL” list from the supervisor's PC.
 - a. Click **Login**.
 - b. Key enter **Name**, use the tab key to move the cursor to password, and enter **Password**. Click **OK**.
 - c. Click **Result Viewer**.
 - d. Click **Reports**.
 - e. Select by clicking from the window **Repeats requested by GDL**.
 - f. Click **Print**.
 - g. View the Repeats from GDL list on the screen. If any specimens are listed, print the list by clicking onto the **Print** icon on the left of the screen. Click **OK** and the printer will start to print.

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name ie. _HGC. HG1, _HG1, PAPP, and _PAPP are listed under **1st Trimester**. If you are running both 1st and 2nd trimester specimens, e.g., TMS and HG1, select all the analytes to be run. Each selected assay appears in the top section on the right.

NOTE: A run cannot contain PAPP and HG1 since both assays require dilution of the sample. Each assay uses a different diluent and the AutoDELFIA only has one position for one diluent cup. PAPP can be run with AFP and/or uE3. HG1 can be run with hCG, AFP, and/or uE3.

11. Make sure that the test or tests that you selected are in the box next to the **new**.
12. Click **new**. Click **next**.
13. Enter the number of samples, number of samples includes all patient specimens and all TQC, for TMS screening into **number of samples to add**. Enter **1** for **Code** (indicating the system is waiting for the 1st specimen to be added). Screen defaults to **0** for **predilution**, and **0** and **0** for **rack and position number**. On the bottom of the **Loading wizard** screen, check the assay box for hCG, AFP, and uE3, for TMS for 2nd trimester specimens and un-check the assay box for HG1 and PAPP for 1st trimester screening.

NOTE: If specimens to be tested include a repeat specimen for >STD, you must follow instructions in Section VII.P.3.c.1).
14. Click **Add Samples**. The **Code** automatically updates to the next available number.

NOTE: Only click on the **Add Samples** once. If you click more than once, the computer will add another cycle to be tested.

NOTE: If at any point in setting up the AutoDELFIA, you realize you have made a mistake, click **Cancel**. Click Yes when **Close the wizard?** is on the screen. You will return to the **Main Window** with access to the **Automate** icon to re-start. You must clear all information related to the run from the AutoDELFIA workstation program before re-starting. To clear the information, click **Options/Reset .../OK**.
15. Click **Next** to load the samples.

NOTE: The maximum number for N is 354, 343 specimens and 11 TQCs, 30 sample racks, 4 full trays for 3 markers. If you are setting up to run 4 markers, e.g., hCG, AFP, uE3, and HG1, maximum for N is 253, 253 maternal serum and 9 TQC, 3 full trays for 4 markers.

NOTE: If the run includes 1st and 2nd trimester markers, you can select either 2nd or 1st trimester screening first. Recommendation is to select the markers for 2nd trimester first.
16. Check to see if all the sample number has been loaded. To view the plates to be loaded, click on the **Plates** Tab. Click **Next**.
17. Open the lid of the sample processor and load the first 18 racks. Every rack has a readable number and a barcode ID. Racks can only be loaded one way, load the rack such that the barcode is facing the reader (to the right). Racks must be loaded in numeric order, 1, 2, 3, 4, 5, from right to left. Slide all the racks on the conveyor to be sure they are properly in place. DO NOT load more than a maximum of 18 racks at one time. Close the lid.

NOTE: Should not be any empty space in the racks.
18. The LOAD button will light on the sample processor. Press the **LOAD** button to begin reading of the barcodes of the sample racks, TQCs, and specimens. If a

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barcode cannot be read, a message is displayed. Click **RETRY**. The rack moves to the front conveyor. A red frame in the rack map shows the rack and position number of the tube with the unreadable barcode. Open the lid and check that the sample tube is placed correctly with the barcode exposed to the reader. Close the lid and press the **LOAD** button. The reader reads the barcode again. If the same error message appears after retrying, click **Retry**. This time, override the system and manually key enter the entire accession number, _ -- _ -- __/P-year-lab, where P (or A) must be upper case. The cursor is already in place in the Code bar. If the accession number is entered incorrectly, no error message will appear.

NOTE: Your manually entered barcode must be identical to the original barcode and in the correct format. Otherwise the result will not be assigned to the correct patient.

19. Repeat Steps 17 and 18 until all the racks in the run have been scanned. Make sure that you only load 18 racks at a time.
20. Once all the racks have been loaded, click **OK**. Click **Next**.
21. Open lid and place the control rack 72, the gray colored rack, onto the front conveyor. Close lid and press the lighted **LOAD** button. The sample processor will read the bar codes and send the rack to the front on the sample processor. Remove rack 72 and place it in position located in the center of the sample processor behind the dilution rack. Control rack 72 fits into its position only one way, with the number 72 to the left.

NOTE: The lot number being read is not the current lot in use, an error message appears on the screen. Click **OK** to replace the control tube and retry. If the controls are in the wrong position, an error message appears on the screen. Click **OK** to correct the error and retry.

22. Click **Next**.
23. Accept the schedule by clicking **Next**.
24. Open the standard tray holder by sliding the gray panel out to the first notch. If you slide it all the way out, it will detach from the AutoDELFI A sample processor. It can then be simply slide back into place. Remove the white lid. Mix gently and slowly to avoid foaming. Remove the caps and place the wet side up on the cap holder, tap the vials on the bench to remove any bubbles, and place the standard vials into the standard tray as shown from the screen. Use the pull-out drawer on the plate processor to store the cap.

NOTE: The caps are labeled with the analyte and a letter A - F representing the lowest to highest concentrations. The standard tray is maintained at refrigerated temperatures.

NOTE: A set of standards for TMS can be used for a maximum of four trays. If the standards had been used previously, the number of times it was used should be recorded to be sure sufficient volume remains. Analyst must keep track of this information by writing on the box the date and number of trays on each run. For TMS, each reagent unit has 2 sets of standards.

NOTE: PAPP A is a 1 plate reagent kit and contains one set of standards per plate. However, a bottle of standard can be used for 2 standard curves, i.e., can be sampled twice.

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25. Replace the white lid and slide the standard tray back to the closed position when all standards are in place.
26. Click **Next**.
27. Click **Show reagents**.
28. Open the AutoDELFIA plate processor lid by pulling it out and down. Remove the large black reagent rack. Make sure that there is enough of 1.25 mL Eppendorf tip pipette in the holder and dilution vessels are loaded for the run.
29. Place reagents into a reagent cassette. Remove the caps from the bottles of buffer and tracer. If the reagent unit contains buffer and tracer only, place into reagent cassette as shown.

Buffer	Tracer	Barcode

If the reagent unit contains buffer, tracer, and antibody, place into reagent cassette as shown.

Buffer	Tracer	Barcode
	Antibody	

30. Place the reagent cassettes into the reagent rack in the order the assays were selected. If this is the first time this reagent lot is used, label the reagent cassette with the barcode ID. The barcode is encoded for analyte, reagent lot number, and assay tray lot number. If this is not the first time, the cassette is already labeled with the barcode. It is not necessary to change the barcode label daily.
NOTE: Label the reagent cassette with the barcode such that the right end extends beyond the reagent cassette. Doing this will minimize barcode reading errors and make removing the barcode easier.
31. Place a black cap on the bottles of tracer and antibody.
NOTE: Bottles of buffer, tracer, and antibody for TMS contain enough reagents for 4 trays. If this is not the first time a bottle is used, the number of trays previously tested must be known to be sure sufficient volume remains for the number of trays in the run for the analyte. Analyst must keep track of this information by writing on the bottle the date and number of trays for each run. There are plenty of reagents. Each unit contains 4 trays with 2 bottles of buffer and 4 bottles of tracer (only two bottles each of tracer and antibody per uE3 unit). Extra vials of antibody and tracer are provided based on workload and the number of small runs.

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NOTE: A 1 plate PAPP A reagent kit is supplied with a bottle of buffer, tracer, and antibody. However, each bottle can be used for two trays. .

NOTE: It is acceptable to combine partially filled bottles of buffer. However, never fill the bottle above the normal liquid fill level. Discard any remaining after 1 week.

32. Check that the white cylindrical plug is in the left front position of the reagent rack. AutoDEL FIA uses this plug to verify that enough dilution vessels and tips are in place and that the caps are off the buffer bottles.
33. Put the reagent rack back into the plate processor aligning the two white lines with the transporting block. Slide the tray to make sure it is in place.
34. Close the plate processor lid.
35. Click **OK**. The plate processor moves the barcode reader into position to read the barcodes on each of the reagent cassettes for reagent and tray lot numbers.
 - a. If a barcode cannot be read, an error message will appear on the screen. Replace the barcode and click **Retry**.
 - b. If a barcode is for the wrong reagent lot, an error message will appear on the screen. Determine the cause of the error, correct the error, and click **Retry**.
 - c. If a barcode is for the wrong analyte an error message will appear on the screen. Determine the cause of the error, correct the error, and click **Retry**.
36. View the "Reagent consumption" window on the screen. Blue circles indicate the pipette tips needed for the assay. Blue rectangles indicate the dilution vessels needed for the assay. Red circle on the sample plate indicates the number of dilution strips that will be used. The window also shows the loading of the reagent cassettes. Check the reagent rack to ensure that this is the minimum quantity available. Click **OK** to continue.
37. View the "Liquid consumption" window on the screen. The top half of the window shows the volume of rinse solution (used to rinse the wash probes), wash solution (used to wash the trays), and enhancement solution needed by the plate processor. The window also shows that the waste container **MUST HAVE LESS** than the amount of waste shown. The bottom half of the window shows the volume of rinse solution (to rinse the sample probes at the end of the day), wash solution (to prime the sample probes and wash between samples), and sample diluent (to dilute the sample for hCG testing) needed by the sample processor. Again the waste container **MUST HAVE LESS** than the amount of waste shown. Check the volumes to ensure that there are substantially more than the volumes shown.
 - a. You must place the diluent cup into position on the sample processor with sufficient diluent. Place no more than 20 mL in excess of the volume on the screen. The cup holds 280 mL when filled to the rim.

NOTE: If a maximum of 354 samples (maternal serums and TQCs) are run, you will need to add 188 mL of diluent for hCG, 371 mL for HG1, and 91 mL for PAPP A. Based on the assay and number of samples, you may need to add diluent during the run.
 - b. Use the following information as a guide for diluent volumes needed. The AutoDEL FIA dilutes 12uL of specimen with 475uL of diluent for hCG, 10 µL

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of specimen with 990 μL of diluent, and 50 μL of specimen with 200 μL of Diluent 3.

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<u># of Trays</u>	<u>Amount of Diluent Required in mL</u>		
	<u>hCG</u>	<u>HG1</u>	<u>PAPPA</u>
1 tray	57	97	35
2 trays	101	188	54
3 trays	144	279	72
4 trays	188	371	91

38. Click **OK**. Click **Next**.
39. Wait until the "Plate loading" window appears on the screen and the AutoDELFI plate processor brings the first loading frame out to the loading position.
40. Load the trays onto the plate processor in the order shown on the "Plate loading" window which is in the order of the selection of the assays. For the first analyte, open the sealed packet and remove the tray, press down on the strips to be sure they are in place in the (tray) frame, and place it into the loading frame with the barcode side towards the plate processor (barcode facing right). The barcode is encoded for analyte, lot number, and serial number. Load the full tray onto the loading frame at the appropriate time.
41. Press the lit **IN/OUT** button and the tray will be taken into the plate processor. The barcode is read and checked against the corresponding reagents and tray lot numbers.
 - a. If the barcode cannot be read, an error message appears on the screen.
 - 1) Press **OK** to return the tray to the loading position.
 - 2) Make sure that the barcode is facing to the right.
 - 3) Press **IN/OUT** button to reload the tray.
 - b. If the wrong barcode is presented, an error message appears.
 - 1) Press **OK** to return the tray to the loading position.
 - 2) Determine the error and load the correct tray or place a new bar code label on the tray.
 - 3) Press **IN/OUT** button to reload the tray.
42. Repeat step 41 with the next tray for the first analyte, if any, when the loading frame reappears.
43. Repeat step 41 with each subsequent analyte. When the last tray is loaded, the "Plate loading" window disappears and the sample processor probes go to the home position in the wash station.
44. Click **Next**.
45. Click **Start**.
46. Schedule window appears. The "Process initialization" Window appears on the screen.

The AutoDELFI performs the following initialization steps:

 - a. Moves the sample racks into the front of the conveyor.
 - b. Primes the sample probes.
 - c. Rinses the sample probes.
 - d. Pressurizes the rinse and wash bottles.
 - e. Pumps enhancement solution through the dispense tip.

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- f. Rinses the washer.
- g. Moves the reagent rack to be sure it is in position.
- h. Counts the black caps for vials of tracer and antibody, counts the dilution vessels, counts the first and last pipette tips, and checks that the caps are off the bottles of buffer.
- i. Checks the incubator and shaker.

The systems temperatures and pressures are displayed. If the temperatures are in the red range, call Perkin Elmer Wallac. If the pressure is low, check for a leak in the rinse and wash bottles. Wait for the final assay schedule to appear

G. Walk Away

1. Have in mind the completion time to return to the AutoDELFIA for shutdown. The schedule for each assay step on each tray is displayed for each analyte. Assay steps are shown as a light blue bar for sample treatment, a red bar for action steps, a blue bar for incubation steps, and a yellow bar for sampling steps. Click on any assay step and see the details for start time, finish time, and total time (duration).
2. Click onto the last sampling step (last yellow bar) and determine the completion time for sampling.
3. Unload the sample racks as soon as possible. Do not wait for the end of the assay at the end of the day. Cover the tubes with plastic wrap/parafilm or proceed to the next assay, e.g. inhibin. You can unload samples (or set up another run) only during the light blue shaded portions of the time line.
 - a. Click **Loading wizard**, it becomes accessible only during the light blue shaded portions of the time line, changes from grey to black.
 - b. Enter username and password. Click **OK**.
 - c. Click **Unload old samples**.
 - d. Click **Cancel** to close the wizard when completed. You would click **Next** and proceed if you want to add another run. Do not click **Options/Reset**.
4. Have in mind the available times, blue portion of the time line, for loading an additional run.
5. Walk away and return as needed.

H. Shut Down

Proceed to shut down **before** the run is reviewed by the supervisor. The supervisor needs the outcome of the washer probe test to score the run correctly. Record any unusual occurrence to help your laboratory troubleshoot if needed.

1. Click **Close** when **Schedule completed** in on the bottom left of the screen. The screen returns the **Main Window** with **My Task** and **AutoDELFIA Workstation** screens.
2. Click **Loading/Unload samples**.
3. View the **Sample unloading** screen and follow the prompts on the screen.
4. Open the lid of the sample processor, and take out the sample racks from the front lane. Close the lid and click **OK**.

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5. Wait for the sample processor to move the sample racks from the back lane to the front. After the conveyor has stopped, open the lid, remove the sample racks, and close the lid.
6. Follow the prompt on the screen and unload the standards from the standard tray. Slide the standard tray holder out to the first notch and remove the white lid. Remove the standards and recap. Store in the refrigerator. Once open, standards can be used up to 14 days. Dump if the standards cannot be used again. Put the white lid back and close the standard tray. Click **OK**.
7. Click **Loading/Unload plates**.
8. Remove the first tray from the frame and press the **IN/OUT** button on the plate processor when lit. The unloading procedure continues until all trays have been unloaded. Click **Cancel**.
9. Unload the reagent rack.
NOTE: Reagent rack is instrument specific.
Remove the reagent cassettes. Remove the black caps. Cap and store in refrigerator or dump vials of tracers and antibody. It is your laboratory's responsibility to ensure that there is enough tracer and antibody for the remaining plates in the kit. Use discretion before discarding the tracer and antibody. Once open, use for 7 days. Cap bottles of buffer and store in the reagent cassettes refrigerated. Discard the used dilution vessels. Refill the empty pipette tip and dilution vessel positions.
NOTE: You must load the reagent rack back onto the AutoDELFIA, otherwise you cannot perform the washer test, Step 17 below.
10. Clean waste tip tray. Remove tray, empty, rinse with water to remove all salts, and dry. The circular button on the tray bottom of the tray must be clean, otherwise the tip cannot be removed from the reagent dispenser. Reload the waste tip tray and reagent rack.
11. Remove the diluent cup from the sample processor. Discard any remaining diluent.
12. Dump used strips and fill dilution rack with new strips sufficient for your lab's daily workload. This minimizes the amount of dust or particles collecting in tubes.
NOTE: Dilution rack is instrument specific. Do not interchange between AutoDELFIA's.
13. Check and replace the bottle of enhancement solution if needed. The bottle on the left should always be full since the system pumps from the right bottle to the left bottle, then to the coated wells. The bottle cap is threaded and should be screwed on straight. Remaining amounts can be combined with another bottle.
14. Click **OK**.
15. Click **OK** when the following appears on the screen:
All information related to the current run or sample load will be cleared. Press **OK** to Proceed, **CANCEL** to abort.
16. Fill or empty your wash, rinse, or waste containers. The pressure for the rinse and wash bottles for the plate processor has been released.
 - a. For the plate processor
 - 1) Open the wash and rinse containers for the plate processor using the open-end wrench provided by Perkin Elmer Wallac. Slide the wrench over the top of the cap. Use the handle on the bottle and the handle on

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- the wrench for leverage by turning each in the opposite direction (the handle on the cap goes counterclockwise).
- 2) Fill the wash container by adding 1 bottle of 250 mL wash concentrate and 6 liters distilled water.
NOTE: These volumes are not volumetric, but more appropriately thought of as "bucket chemistry".
 - 3) Fill the rinse container with distilled water.
 - 4) Close each container tightly.
- b. For the sample processor
- 1) Open and fill the wash container for the sample processor with 50 mL wash concentrate and 4950 mL distilled water.
NOTE: These volumes are not volumetric, but more appropriately thought of as "bucket chemistry".
 - 2) Open and fill the rinse container with distilled water.
 - 3) Close each container tightly.
- c. To empty the waste container is an automatic procedure triggered by the level of waste. You can use the PC to empty waste when necessary: click the **Maintenance/Plate processor/Waste pumping ... /OK** for the waste pump to empty the waste bottles. You can also do it manually: open and empty the waste bottles when the system is not pressurized.
17. Check the performance of the washer. Click **Options/Reset/Operate/Washer test** and follow the instructions on the screen. Follow the prompts and the following sequence of events will occur:
NOTE: If the reagent cassette is not loaded onto the AutoDELFIA, and error message appears:
Reagent rack missing
Put the rack onto the transport lane.
Push **Retry** to go on. **Abort** stops this dispenser process
Load the reagent cassette onto the AutoDELFIA and click **Retry**.
- a. System pressurizes.
 - b. System waits for a tray (with uncoated strips to be loaded). Load a plate and press the **IN/OUT** button.
 - c. Fills the wells and washes the wells. If some wells are not filled/emptied properly, clean the washer manifold according to the instructions in Appendix 3, Removing the AutoDELFIA Washer Manifold. If most wells are over filled and/or a lot of liquid is left after aspiration, call Perkin Elmer Wallac for service.
 - d. Remove plate, click **Close** and the system depressurizes.
 - e. Click **Options/Reset**.
- I. Storage of Maternal Serum Specimens After Completion of Testing
1. Cap the tubes tightly and place the sample racks into the refrigerator when unloaded from the AutoDELFIA until the specimen on the Local and GDL Repeat lists have been taken for repeat testing.

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2. Transfer the capped serum tubes, in order, from the sample racks to a specimen storage container or rack. The unfilled spaces in the last partial storage container or rack should remain unfilled for each accession day.
 3. Label each container or rack with lab site, accession day, test date, and container or rack number.
 4. Return specimens to its original storage by accession date after retesting.
 5. Store containers or racks in the freezer upright for 30 days. Discard after 30 days after removing all patient names and identifying information on the specimen. This can be achieved by defacing the identifying information with an ink marker, then followed by wrapping a thick label (2"x2.5") on the specimen tube which will cover all the defaced identifying information. A log needs to be maintained with the following information: Date of disposal, Julian date of samples being discarded or shipped to GDL, employee initials, and supervisor initials.
- J. Repeat Testing of Maternal Serum Specimens
1. Rules Used for Repeat Testing
 - a. If a run or tray is prevented from release, repeat the testing for all the maternal serum specimens from the run or tray for the single analyte.
 - b. If a result for a single specimen for a single analyte is prevented from a tray that was released, include the specimen in your next routine run. Reasons to repeat the testing for a single specimen include:
 - 1) Result is higher than the highest STD.
 - 2) Result is lower than the lowest STD.
 - 3) Results with an instrument flag.
 - 4) Two specimens had the same barcode.
 - 5) There was a pipetting error.
 - 6) Result judged invalid due to laboratory error.
 - 7) Result judged invalid by GDL.
 2. "Repeats Requested Locally" and "Repeats Requested by GDL" Lists
 - a. Use the two repeat lists to retrieve maternal serum specimens for repeat testing. The Repeats Requested Locally list should be printed immediately after the supervisor's review and release is completed. All maternal serum results prevented from release are listed and must be tested no later than the next day. The Repeats Requested by GDL list is downloaded to the screening laboratory after data review at GDL and printed each morning by your laboratory. Those listed must be included in the day's testing. The list shows the assay name, test date, run ID, instrument ID, status for the assay run, comments for run/tray/result. Listed under the assay name, by tray, are the specimens that need repeat testing. The list refers to the specimens by their original rack and position numbers. Specimens on the local repeat list will be tracked on the supervisor's PC. If on repeat testing, the result is released by the supervisor, the specimen drops off the list. If prevented or not tested, the specimen stays on the list. Specimens on the GDL repeat list are also tracked at the supervisor's PC. If released, the specimen drops off the list. If

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prevented, the specimen appears on the next local repeat list. If the specimen was not tested, the specimen stays on the GDL repeat list.

3. Setting up the Run When There Are Repeats
 - a. Place the specimens for repeat testing into sample racks with the day's new specimens.
 - b. Set up the AutoDELFIA for routine screening as previously described.
 - c. Handle special cases as follows:
 - 1) If repeat testing is due to >STD for hCG, AFP, uE3, and HG1, use the following dilution and setup procedures:

NOTE: If a specimen is >STD and it was not reclassified by SIS, see Section VII.Q., retest by diluting 1:5.

 - a) Dilute the specimen 1:5. Pipette 50 uL of sample and deliver into a tube. Add 200 µL of Diluent I and deliver to the same tube. Mix with the tip by aspirating and dispensing several times.
 - b) Label the tube with the same accession number as the undiluted sample. The accession number must be handwritten.
 - c) Place all specimens >STD, diluted, as the first group of specimens for the run immediately after the first TQC, then place the companion specimens >STD, undiluted, as the next group of specimens follow by maternal serum specimen that is of the same type, type P or type A.
 - d) Refer to Sections VII.F and G. for entering the number of specimens for testing into the system and loading the sample racks. When your run includes TMS specimens, 2nd trimester specimens >STD, and HG1, enter the number of specimens for testing as follows:
 - i. Enter **1** for the **number of samples to add**. This is the beginning TQC for Tray 1. Auto entry of **1** for **Code** and **0** for **predilution**. Be sure the correct analyte boxes are checked. Click **Add Samples**.
 - ii. Enter the # of 2nd trimester specimens >STD, diluted. Change entry from **0** to **5** for **predilution** and click **Add Samples**.
 - iii. Enter the # of 2nd trimester specimens >STD, undiluted, plus TMS specimens and all remaining TQC. Change the **5** to **0** for **predilution**. Click **Add Samples**.
 - iv. Enter the number of 1st trimester specimens, enter the rack number and **1** for position number for the first sample (is beginning TQC) for the 1st trimester assay, be sure the correct analyte box is checked and **0** is entered for **predilution**. Click **Add Samples**.
 - v. Click **Next** to load the sample racks. When the AutoDELFIA tries to read the barcode of the diluted sample, error message appears on the screen. Click

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Retry, pick up the tube and manually key enter the accession number including uppercase P, year, and lab site (with the “-” and “/”). See note on accuracy and formatting (VII.G.2.).

- 2) If testing for a specimen is repeated due to >STD for PAPP, repeat testing undiluted only. You do not have to run the specimen diluted.
- 3) If testing for a specimen is repeated because two specimens had the same barcode, use the following protocol to re-label the two specimens correctly:
 - a) Retrieve the tube and test request form for both specimens.
 - b) Match the tube to the test request form using the I number.
 - c) Re-label the tube and test request form with a new barcode for both specimens.
 - d) Reenter the test request forms using the new accession numbers. Call GDSP to have the original entry of the two TRFs deleted from the system. Include the two specimens with the day's new specimens.
 - e) Inform the supervisor of the two new accession numbers for the two specimens. The supervisor, during review and release of the day's run, **MUST** inform GDLB, using the Free comment line for both specimens, of the new accession numbers for the two specimens with the same barcode. Determine cause and take corrective action.
4. Setting up the Run When There Are Repeats for A Single/Double Analyte
 - a. Set up a separate run on the AutoDELFIA to repeat testing for a single analyte. If the second run is an overnight run, you must set up the system so that the AutoDELFIA will not stop the assays if it runs out of a reagent, e.g., enhancement solution, but will proceed to save as many of the trays as possible. This must be done before clicking AutoMate.

NOTE: The option must be clicked off before starting your routine run.

 - 1) Click **Settings/System**
 - 2) Click **Advanced**
 - 3) Select **Automatic exception recovery** (overnight run)
 - 4) Click **Routine**
 - 5) Click **OK**
 - b. Transfer the specimens from the original sample racks into another set of sample racks starting with rack #1 and with all the TQCs in the correct marked positions.
 - c. Set up the AutoDELFIA for routine screening as previously described for the specific analyte.

NOTE: Results downloaded to the supervisor PC will be gray if the repeat was not necessary.

K. Retesting Specimens That Were Reclassified by SIS, From 1st to 2nd or 2nd to 1st Trimester

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1. Print daily, before you set up a run, the list of specimens that were reclassified, what was a 1st trimester specimen is now a 2nd trimester specimen or vice versa, by SIS based on correct/true gestation age of the fetus. To print
 - a. Go to <https://sis.dhs.ca.gov>.
 - b. Click SIS Reports and enter user name and password.
 - c. Click SIS Production Reports.
 - d. Open Report #320, List of 1T TRFs with GA Greater Than 13 Weeks 6 Days, and print.
 - e. Open Report #321, List of 2T with TRFs with GA Less Than 15 weeks, and print.
2. Add specimens that were reclassified as 2nd trimester specimen to your TMS run, hCG, AFP, and uE3. (Also test specimen for Inhibin.) Use the original barcode accession number and do not give the specimen a new accession number.
3. Add specimens that were reclassified as 1st trimester specimen to your PAPP A and HG1 runs. Use the original barcode accession number and do not give the specimen a new accession number.

L. Inventory

An inventory system is provided so that you can easily update all of your consumables supplied by Perkin Elmer Wallac, Bio-Rad, or GDLB. Each time your inventory is updated, the new information is picked up by the GDLB QA system and when your supplies are low (below a set minimum amount) GDLB will place the order for your laboratory and the vendor will ship the supplies. It is the laboratory's responsibility to key enter into the system the exact number of reagent kits received. The inventory system will count down automatically from the number of kits received for hCG/HG1, AFP, uE3 and PAPP A using the number of strips tested. It is also the laboratory's responsibility to key enter the amount of all other supplies and reagents, received, used and removed. For these items, the countdown is **not** automatic.

1. Inventory Access
 - a) Login onto the supervisor's PC by entering your **login name** and **password**.
 - b) Click MANAGEMENT.
 - c) Select **Inventory icon**. View the Perkin Elmer Wallac Inventory window on the screen. The Inventory program is divided into several applications:
 - 1) **Menu bar**: This is a standard Windows menu bar. The commands appropriate for the current mode are Inventory, Routine, and Help.
 - 2) **Toolbar**. The toolbar is quite large with a large icon and a caption for each button. The different commands available match those available from the menus in the current mode. The different modes are Use, Remaining, Receive, Remove, Set Current Lot, View Lots, Settings, Events/Tasks, Print, and Exit.

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- 3) **Listbar.** The listbar is situated on the left side of the main screen. It is divided into several *listbar groups*. These, in turn, contain icons representing *item groups*. When a listbar groups bar is clicked, it is opened to reveal its contents. When one of the item group icons is clicked, the item list displays only items from that item group. The Headingbar is also updated. The Listbar can be customized in the Listbar Editor.
- 4) **Item list.** The Item list shows all items (and their part numbers) for the currently selected item group. Selecting an item will update the Lot drop-down list, the Headingbar, the Item picture and information as well as the Graph area.
- 5) **Lot drop-down list.** The Lot drop-down list contains all the lots for the selected item. Selecting a lot will update the Headingbar, the Item information and the Graph area. Just the lots at your site are shown. An entry in the list contains the lot's number, and its expiration date. If the lot is the current one, this is also indicated.
- 6) **Headingbar:** The Headingbar shows the names of the item group and item. Also, the item group's icon is displayed at the right end of the bar. Directly to the left of this icon, the currently selected lot number is displayed.
- 7) **Item description.** The current item is described in two places, the item picture beneath the lot list, and the Item information area at the right of the screen. The Item information area also displays information about the current lot. The information shown in order is,
 - i. Item title and supplier
 - ii. Names and amounts of the units in which the item is shipped and used
 - iii. Lot number and if the item is lot dependent
 - iv. Expiration date and days left (same as previous)
 - v. Stock level for the lot
 - vi. Whether or not this is the current lot (same as previous)
- 8) **Graph area.** The Graph area displays different kinds of graphs with matching description texts. To change which graph is shown, select a new one from the graph drop-down list. The basic graphs available are:
 - i. **Number of items.** This graph shows the number of items in storage. For lot-dependent items the selected lot is used. Item information text above graph lists lot expiration and quantity in inventory for selected lot. **You can view the sum of all lots of the item by checking the box for "Plot graph based on combined sum..."**.

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- ii. **Percentage of capacity.** This graph shows the percentage of the site's storage capacity for the selected item in use. For lot-dependent items the selected lot is used, for others the sum of all lots is used.

2. **Menu Structure**a) **Inventory**

- 1) **View Events & Tasks** Opens the (local) Event & Task Viewer window
- 2) **Print Graphs/ Reports** Opens the dialog for printing reports & graphs
- 3) **Exit** Exits the Inventory program

b) **Routine**

- 1) **Use Items** Opens the Use Items dialog
- 2) **Receive Items** Opens the Receive Items dialog
- 3) **Remaining Items** Opens the Remaining Items Dialog
- 4) **Remove Items** Opens the Remove Items dialog
- 5) **Set Current Lot** Sets the selected lot to current
- 6) **View Lots** Opens the View Lots window

c) **Help**

About Inventory Opens the about box

3. **Inventory Update**

- a) Select a group (**All Items, Instrument or Storage, etc.**) on Listbar to update inventory of reagents and supplies. Click the item to be updated from the white box on the screen, e.g., TMS-System control. The screen shows lot number, item information, and graph (percent remaining numerically/graphically).

All items, except hCG/HG1, AFP, uE3, and PAPP A reagent kits, require entry of amount used, remaining, received, and removed. Enter changes in inventory into the computer program each time that your laboratory **receives** shipments, **uses** items for testing or **removes** items (for example, to ship to another laboratory).

For hCG/HG1, AFP, uE3, and PAPP A reagent kits, only entries for amount received, remaining and removed are needed. There is automatic countdown of amounts **used**. However, **if a run is aborted**, the number of strips used for that run must be entered manually into the inventory program as **Used**.

- 1) Click Use (for every reagent, supply item, & reagent kit), check lot number and answer the prompt, "How

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many xxxx have you used?" to enter the amount used.
Enter the amounts in the units displayed on the screen.
Click OK.

- 2) Click **Remaining** to see the numbers entered by the system for the amount still in storage. If the number is different from the actual physical count of your inventory, proceed with the instructions in section 5., "Reconcile the Inventory", to adjust.
- 3) Click **Receive**, and select lot number from the drop-down list.

NOTE: All lot numbers are now entered by Wallac. When you receive items with lot numbers, use the **Lot** drop down menu to select the new lot number.

- i. Answer the prompt, "How many xxxx have received?" by entering the amounts received into the box.
 - ii. Select either "This shipment was received from the supplier, Wallac." Or "This shipment was received from another site."
 - iii. Enter a valid **Order ID** number from the shipment information if this is a shipment from the vendor.
 - iv. Click **OK**.
- 4) Click Remove and answer the prompt,

"How many xxxx have you removed from storage?" to correct an error in entry of number Received or to enter the amount redistributed to another NAPS laboratory.

- b) Continue as in a) above for updating all inventory items.
4. Conversion of Next (New) Lot to Current Lot
 - a) Select the Item that needs to be converted. Use the Lot drop down menu to select the new lot number.
 - b) Click **Set Current Lot** on Toolbar. You will see on the screen,

"Do you want to change the current lot
From: (current lot #)
To: (new lot #)?
Yes No

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- c) Click Yes.
 - d) Click OK. This converts the next lot to the current lot. If you now click the Remaining button, the inventory of what was the "next" lot now shows in the inventory for the current lot.
5. Reconcile Inventory
- Reconcile your inventory for discrepancies by **Monday**. It is important to use the correct option to reconcile the inventory so the usage rate will not be adversely affected. The usage rate feature enables GDL to calculate when to send additional inventory to the NAPS.
- a) Select the item that has a discrepancy by clicking on it so that it is highlighted.
 - b) Click **Remaining** on Toolbar for item that has a listed amount in **excess** of actual physical count when the item was used for testing. Click **Remove** when the item was removed from your inventory for other reasons such as shipment to another laboratory or the reagent was spilled. Click **Receive** on Toolbar for item that has an amount listed which is **less** than the actual physical count.
 - c) Enter the **discrepancy amount** from actual **physical count** to adjust the inventory.
 - d) Click **OK**.
6. Settings
- a) GDL will set the Maximum or Minimum limits for inventory items from the central site. To view the limits, click the Settings button and select the item from the Item list.
 - b) GDL will set the Warnings for inventory items. To view, select item from the Item list.
7. Events/Tasks
- a) Click **Events** to review actions taken by and entries made by staff.

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- b) Click **Tasks** to review notices of needed orders recorded. This table will also show when a GDL person has **acknowledged** the notice for a needed order and placed the order.
8. Print
To print inventory reports:
 - a) Select item to be printed.
 - b) Click **Print** on Toolbar menu.
 - c) Select type of printout needed.
 - d) Click **Print**.
 - e) Click **Close**.
 9. Exit
 - a) Click **Exit** Icon on Toolbar.
 - b) Click **EXIT** on Main Menu.
 - c) Select **LOG-OFF** .

M. Maintenance

The AutoDELFIA keeps track of the required maintenance. The system will not allow you to complete setting up a run unless all maintenance has been performed. There is a two day grace period. If maintenance is not completed by midnight of the second day of the grace period, a warning message appears on the screen.

Warning: This system will not allow you to complete setting up the run. The AutoDELFIA system has not maintained. You must perform the required maintenance before you can set up the next run. Click **OK** to perform maintenance.

If you proceed to set up the run, the system will close after you load the standards with the following message on the screen.

The AutoDELFIA system has not been maintained. The system is closed, to continue you must perform the maintenance procedures.

If this cannot be done please contact Perkin Elmer. Click **OK** to close.

Proceed with maintenance. Only under extreme circumstances, you may call Perkin Elmer to override the due date.

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You must click **Options/Reset** before the start of a maintenance session and click **Options/Reset** at the completion of maintenance session. This gives you a clean database to perform maintenance and a clean database to start the next run.

1. Daily
As described when performing daily set up and shut down of the AutoDELFIA.
2. Weekly
 - a. Turn off the computer
 - 1) Click **START** button on Window Screen.
 - 2) Click **SHUTDOWN** and select **YES** to shutdown the computer.
 - 3) Click **YES** to exit AutoDelfia workstation.
 - 4) Click **OK** to quit the program windows before you close down the Window.
 - 5) Wait until the “Closing- Multicalc AutoDELFIA-WIACALC” dialogue appears on the screen.
 - 6) Click **END TASK** to turn the computer off.
 - b. Turn on the computer
 - 1) Push **BUTTON** to turn the computer on.
 - 2) Type in **USERNAME** and **PASSWORD** and press **ENTER**.
 - 3) 1235 AutoDelfia Initialization dialogue appears on the screen, then click **OK**.
 - 4) Click **YES** to process for the initialization.
 - c. Clean sample probe wash wells.
Clean the sample probes wash well using a small brush with 70% Alcohol or distilled water.
 - d. Full backup.
Once a week, e.g., every Friday, AFTER shutting down the system to reinitialize the sample probes (see Section VII.S.1.), perform a **full** backup where all of the data from your three PCs (supervisor's and the two for the AutoDELFIA systems) are backed up. Run data are backed up everyday to the internal DAT drive installed on the file server PC. Backup procedures store all run data on DAT tape for emergency retrieval. Four tapes labeled as Tape #1, #2, #3, and #4 are used. One tape is used for one week. Rotate the use of the tapes so that the order of use is #1, #2, #3, #4, # 1, #2, #3, #4, etc. Even though backup is done everyday, user intervention is required only once a week.
 - 1) Press the **Eject** button on the DAT tape drive to remove the previous week's tape, and place it as the last tape in the tape rotation queue.
 - 2) Insert the next tape from the rotation queue into the DAT tape drive. When the tape is loading, a green light blinks. When loading is

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- completed, the green light is lit. This tape will be used for one week until next Friday when this procedure is repeated.
- 3) Hit the Windows key, after the tape has initialized, and select **Backup**. The Backup program window comes up and the program starts to backup the data. Procedure takes about 30 minutes. A printed report is generated automatically for your files.
 - 4) Click **Logout**.
3. Biweekly
- a. Wash and disinfect the sample probes.
 - 1) Use 16 x 125 mm tubes, with an internal diameter of 9-14 mm.
 - 2) Fill 8 tubes with at least 5mL of wash solution, diluted 1:20, and place into a sample rack.
 - 3) Click **Maintenance/Instrument Cleaning/Wash Sample Processor probes**. Click **Off** any other options that may be selected.
 - 4) Click **OK** and follow instructions on the screen to wash the probes.
 - 5) Click **No** when , “Would you like to record this maintenance procedure in the maintenance record history?” shows on the screen. The Autodelphia workstation with the Automate icon shows up on the screen. If by mistake, **YES** is clicked, the weekly, biweekly and monthly maintenance screen will be displayed, but you have no access. Click **Close** to bring you back to the Autodelphia workstation with the Automate icon on the screen. Proceed with the next maintenance.
 - 6) Fill the first 8 tubes of a sample rack with at least 5 mL of 70% alcohol and the last 4 tubes with water.
 - 7) Click **Maintenance/Instrument Cleaning ... /Disinfect Sample processor probes**. Click **Off** any other options that may be selected.
 - 8) Click **OK** and follow instructions on the screen to disinfect the probes.
 - 9) Click **No** when the computer asks you if you like to record this maintenance procedure in the maintenance record history.
 - b. Disinfect the plate processor washer.
 - 1) Click **Maintenance/Instrument Cleaning ... /Disinfect Plate processor washer**. Click **Off** any other options that may be selected.
 - 2) Click **OK** and follow instructions on the screen.
 - a) Empty the waste bottle.
 - b) Click **No** when asked if you want to disinfect the disk remover.
 - c) Place a single reagent cassette with a buffer bottle containing 50 mL of a 0.5% bleach solution, and 2 Perkin Elmer Wallac pipette tips in the left most positions of the reagent rack.
 - d) Load a full tray with uncoated strips and press the **IN/OUT** button. The disinfecting process begins. The dispenser adds bleach to the wells, aspirates strips C to H, and soaks in strips A and B, after which the wash tips are rinsed.
 - e) Click **NO** when the computer asks you if you like to record this maintenance in the maintenance record history.

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NOTE: This biweekly maintenance item, when performed, triggers the automatic registration onto the AutoDELFLIA. When registered, the system adds 16 (due date plus 2 day grace) to the date registered and sets the next due date, e.g., every other Monday with a 2 day grace period. Do not wait until the end of the grace period, but perform on the Monday due date. If maintenance is performed at the end of the grace period, the next maintenance is due 16 days from date performed, and therefore, would lose the concept of every other Monday as biweekly maintenance. It is acceptable to perform additional maintenance as part of troubleshooting when needed. However, do not consider the extra maintenance when deciding the next due date. The next due date is the same due date as if the extra maintenance was not done. This also maintains the schedule in the maintenance module of the supervisor's PC.

- c. Check the enhancement solution outlet for precipitate.
 - 1) Obtain a flashlight, flash it on dispense outlet tip and check for precipitate. There is a small ring right above the end of the tip that may look like precipitate.
 - 2) Wipe the tip with a cotton swab that has been moistened with enhancement solution if there is build-up.
 - d. Empty and refill the wash and rinse bottles.
Refer to instructions about loading consumables, section VII.M. 1-3 and 13-14.
4. Monthly
- a. Wash the sample and plate processor wash solution bottles.
 - 1) Empty both the sample and plate processor wash solution bottles.
 - 2) Rinse well with distilled water.
 - b. Disinfect the sample and plate processor waste bottles.
 - 1) Empty waste bottles and add 1 liter of 0.5% bleach solution. Let it stand for 30 minutes with occasional swirling.
 - 2) Rinse well with water.
 - c. Clean the waste bottle liquid sensors and bottle caps by wiping them with a damp cloth if there is build-up of crust.
 - d. Wash and disinfect the sample processor tubing.
 - 1) Fill a 250 mL beaker with wash solution, diluted 1:20.
 - 2) Open the rinse bottle and put the tubing into the beaker.
 - 3) Click **Maintenance/Sample Processor/Rinse ... /OK.**
 - 4) Fill a 250 mL beaker with distilled water and put the tubing into the beaker.
 - 5) Click **Maintenance/Sample Processor/Rinse ... /OK.**
 - 6) Fill a 250 mL with 70% ethanol or isopropyl alcohol and put the tubing into the beaker.

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- 7) Click **Maintenance/Sample Processor/Rinse ... /OK.**
- 8) Repeat with distilled water.
- 9) Put the tubing back into the rinse bottle and tighten the cap.
- 10) Click **Maintenance/Sample Processor/Rinse ... /OK.**
- e. Clean covers, racks, and mirrors.
 - 1) Wipe the surface of the lid over the conveyor of the sample processor and of the plate processor with 70% alcohol.
 - 2) Wash the black and white standard tray covers with water, rinse with distilled water, and dry.
 - 3) Wash the reagent rack and sample racks.
 - 4) Clean mirrors on the plate holders. The plate holders can be brought out from the incubator by using the Unload plates command. Replace the plate holder with the mirror on the right, and check that it is properly seated.
- f. Compare color card
Compare the working copy of the color standard to the reference copy to ascertain that the color of the two copies are the same. If not, start using the reference copy as the working copy. Call GDL for a replacement.
5. Quarterly
Perform accuracy and precision determination for the pipettes.
 - a) Weigh 30 tubes.
 - b) Use the 1500 uL pipette to dispense 1500 uL of distilled water into the first 10 tubes.
 - c) Set the adjustable pipette to 50 uL and dispense 50 uL of distilled water into the next 10 tubes.
 - d) Set the adjustable pipette to 200 uL and dispense 200 uL of distilled water into the last 10 tubes.
 - e) Reweigh the 30 tubes.
 - f) Calculate the volume dispensed.
 - g) Determine the average volume and CV for each set. Results are acceptable if within the following ranges:

Volume	Average	cv
50 uL	49 - 51	1.5%
200 uL	195 - 205	1.0%
1500 uL	1470 - 1530	1.0%
6. Periodic
 - a. Refer to Appendix 4 for maintenance of the barcode printer.
 - b. Enhancement Solution Flush
 - 1) Flush the Enhancement Solution tubing if
 - a) System was not used for 4-5 days.
 - b) Enhancement Solution is contaminated, or mistakenly replaced with a bottle of Wash Concentrate.
 - 2) Replace the Enhancement Solution with distilled water and screw the bottle tightly in place.

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- 3) Click **Maintenance/Plate Processor/Enhancement Solution Flush ... /OK**.
 - 4) Replace the distilled water with Enhancement Solution, screw the bottle tightly in place, and repeat the above step “3”.
7. Maintenance Charts
- Maintenance software and charts are programmed into the Supervisor’s PC. Use the software and charts to document performance of routine maintenance. To access the maintenance software when performing weekly, biweekly, and monthly maintenance,
- a. Login with name and password.
 - b. Click **Management/Maintenance**. This opens the Wallace Maintenance Screen. To document routine maintenance performed,
 - 1) Select **Task/All Tasks**. (If **Scheduled, Performed, or Overdue** is selected, you will have a partial list of task by status.) Select **Equipment** from the three bars (you have access to) on the left of the screen (Equipment, Tasks, and SOP).

NOTE: Click the **SOP** icon to view the assay protocols which are now loaded into the supervisor’s PC. Click the assay icon to access the protocol.

Select the equipment type for which you are performing maintenance, using the equipment list on the left of the screen, e.g., AutoDELFIA. Information for the equipment type is automatically entered into the **Tasks-All Tasks** screen.

2) View **Tasks-All Tasks**. Entered on the screen are **Equipment type, Equipment**, identification by system #, and **Description**, assay for which it is used.

3) Select from **Equipment** the specific instrument for which you are performing maintenance. Information for the specific instrument is automatically entered into the **Tasks for Selected Equipment** screen.

4) View **Tasks for Selected Equipment**. Entered on the screen are **Type**, i.e., checklist **Status**, i.e., whether it is scheduled, performed, partial, or overdue, **Equipment**, name of specific instrument by system #, **Due**, date maintenance is to be performed, **Grace**, number of days after the due date before maintenance becomes overdue, **Description**, whether it is weekly, biweekly, or monthly, and **Responsible**, who has the responsibility to perform the task.

NOTE: Click on any one of the name items, e.g., **Due**, to arrange the list by the due date, each time you click the list, it goes to earliest date first or latest date first, or **Description**, to arrange the list with all weekly, biweekly, monthly maintenance listed together.

5) Double click on the checklist for either weekly, biweekly, or monthly. This opens the **Operator Maintenance Checklist** screen.

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- 6) View the **Operator Maintenance Checklist** screen. Under the **Subtasks** bar are **Description**, description of each required routine maintenance step, **Comment**, available space for comment specific to the step, **Performed by**, automatic entry with login name after the step is checked as performed, and **Date**, automatic entry with date the step is checked.
- 7) Click on the checkbox for each maintenance step performed. Use the **Move Up** and **Move Down** icon to scroll up and down the list. When all steps are checked off, the same checklist will appear for the next due date using the scheduled date, not the performed date. Additionally, if your laboratory wants to add steps to the provided maintenance list, use the **New** icon. Click **New**, enter the new description, and click **OK**. Your laboratory has no access to the **Delete** icon.
- 8) Enter any comment in the **Comment for Selected Subtask** area for the highlighted step and it will appear in the **Comment** for the list of subtasks.
- 9) Enter any general comment in the **General Comment** area regarding the maintenance.
- 10) Click **OK**. In the **Tasks for Selected Equipment**, software automatically enters **Performed by** and **Date**. When all steps are checked off, **Status** changes to “Performed”. If incomplete, the status changes to “Partial” within the grace period. If no steps are checked or is incomplete, the status changes to “Overdue” after the grace period.
NOTE: As long as the status is “Overdue”, the next scheduled maintenance for that periodicity will not appear on the **Tasks for Selected Equipment**. When status changes to “Performed”, the next maintenance that appears will be the one after the completion date so that there may be missing maintenance(s). **Do not** have a gap in your maintenance schedule. Checking off or not checking off the **Supervisor Review** box does not affect the appearance of the next scheduled maintenance.

After all tasks are performed by the analyst, supervisor checks the **Supervisor Review** box which changes the status to **Supervisor** with the supervisor’s logon name listed under **Performed by**.

- 11) Click **Print** to print the Operator Maintenance Checklist.
 - 12) Click **Exit**.
- N. Troubleshooting
1. Reboot the System (system freezes during setup)
 - a. Turn the plate processor power **OFF**.
 - b. Hold the four sample probes up and turn the sample processor power **OFF**.
 - c. Move the sample probes to the waste position and gently lower position of the probes.
 - d. Shut down AutoDELFIA PC:
 - 1) Click the **Close** button to close down the application.
 - 2) Select **Yes** to exit AutoDELFIA Workstation.

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- 3) Double click on **MultiCalc AutoDELFIA** application on tool task bar.
- 4) Press **Esc** key twice and select **X** to exit to MS-DOS.
- 5) Press **F1** for **Yes** to exit.
- 6) Click **Start/Shut Down**.
- 7) Select **Shut down the computer** and click **Yes**.
- 8) Turn PC power **OFF**.
- e. Turn the plate processor power **ON**.
- f. Turn the sample processor power **ON**.
- g. Start up AutoDELFIA PC:
 - 1) Turn PC power **ON**. A “Enter Network Password” dialog will appear. The screen defaults to:

User name - “system”
Password remains as blank (do not enter password)
Domain - “lab’s name”

 - 2) Click **OK**.
 - 3) Click **OK** on “1235 AutoDELFIA Initialization” dialog.
 - 4) Click **Yes** on AutoDELFIA Initialization. It takes about 10 min to complete the initialization process.
2. Interrupted flow of data files to the supervisor’s PC
Use the Dataflow Monitor to determine where a data file is stalled
 - a. Logon at Level 2 and enter password.
 - b. Select **Programs/Dataflow Monitor**. This opens the

NAPS Dataflow Monitor window. (The **Universal Eluent Worklists**, **TSH worklists**, and **FASpac result files** applies only to NBS.) The **MultiCalc input files** window contains the worklist and raw data for a PNS run. The **MultiCalc results files** contain the calculated data. Files flow from the “input” window to the “result” window to the Database window, at which point the files are in the supervisor’s PC. Whenever a completed run is not available in the supervisor’s PC, open **Dataflow Monitor** to determine where a file is stalled. Call Perkin Elmer Wallac for service and provide the information to Perkin Elmer Wallac.
3. See also Appendix 9, Troubleshooting Common Assay Problems for PNS. To call Proxy, refer to Appendix 5.

VIII. Calculations**A. Calibration**

A STD curve is constructed daily from the duplicate responses for each of the STDs on the first tray. The standard curve is a spline fit line with log concentration on the x-axis and log response on the y-axis.

B. Patient Results

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At the completion of each run, the data file is downloaded to the supervisor's PC where the MultiCalc software program automatically converts the instrument readout for each sample to a concentration for each analyte using the STD curve. Two levels of calibrators are run in duplicate on each subsequent trays. Therefore, samples on these trays are quantitated using the "corrected" STD curve.

IX. Reporting Results

Results are validated using a quality control program.

A. Quality Control Program

The quality control program is used to validate results before reporting. The program is designed to 1) monitor the day to day performances of the AutoDELFIA and 2) monitor the day to day performance of the methods including the dilution step, e.g., hCG assay.

1. Monitor Performance of the AutoDELFIA

- a. Three TMS system controls and 3 PAPP A system controls are provided by Perkin Elmer Wallac to monitor the performance of the AutoDELFIA. The judgement of whether or not the results for the system controls are acceptable is made using the limits established by Genetic Disease Laboratory Branch in conjunction with the application of the Westgard rules.
- b. The Westgard rules are applied to the pair of results for the low, medium, and high SQC. By definition, the run is out of control if "3SD 1" (one is outside 3 SD limits), "2SD 2" (two are outside 2 SD), or "R4s" (range of SD exceeds 4 SD). The system issues a warning if "2SD 1" (one is outside 2SD), or if the assay median is outside acceptable limits. These limits and Westgard rules are part of your quality control software.
- c. The analytical run is scored automatically as being in control (green light), out of control (red light), or as a warning (yellow light). Each result which is outside the ± 2 and 3 SD limits is appropriately flagged.
- d. System control results can be used to aid in troubleshooting the entire method in the event the results are outside acceptable limits. Specifically, the results of the system controls together with that of tray controls will assist in determining the source of error, such as the degradation of standards and/or reagents, inaccurate reconstitution of reagents, instrument malfunction, operator error, sample dilution, or inaccurate sampling.

2. Monitor Performance of the Method

- a. Two tray controls are provided by the department to monitor the performance of the PNS methods. One tray control is formulated to contain AFP, hCG/HG1, and uE3 at a clinically appropriate concentration. The second tray control is formulated to contain PAPP A at clinically appropriate concentration.
- b. The judgement of whether or not the results for the tray control are acceptable is made using the limits established by Genetic Disease Laboratory in conjunction with the application of the Westgard rules. The same Westgard

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rules in use for the SQC are in use for the two TQCs bracketing the patient specimens. These limits and Westgard rules are part of your quality control software.

- c. The tray is scored automatically as being in control, out of control, or as a warning. Each result is outside the +/- 2 or 3 SD limits is appropriately flagged. If tray control results fall outside the acceptable limits, the supervisor, in consultation with Genetic Disease Laboratory Branch, will determine the source of error, interpret its meaning, and take corrective action.

3. Quality Control Plots

The quality control software will plot for each AutoDELFIA the results of the system and tray controls showing the mean and acceptable limits. Access the plots using the supervisor's PC.

- B. Repeat Testing

Test must be repeated when results are prevented from release. Refer to the Repeat Testing of Maternal Serum Specimens (Section VII. P).

- C. Completed Worksheet

The completed worksheet is reviewed and released on the supervisor's PC and is automatically transferred overnight, or can be transmitted immediately, to the GDLB QA system. Worksheet can be printed with sequence of analysis, well/rack/position number, accession number, counts, concentration, and pertinent flags.

X. Procedure Notes

- A. The AutoDELFIA workstation software is necessary for successful use of the AutoDELFIA kit. The reagents supplied with the kits are intended for use as an integral unit. Do not use kit reagents after the expiration date printed on the kit label.
- B. Frozen patient specimens should be brought to room temperature and mixed by hand. Mix by gently inverting each sample 3-4 times. Do not vigorously vortex or mix patient specimens. Foam in the samples will cause liquid level detection errors. Re-centrifuge if needed.
- C. The avoidance of europium contamination and resulting high fluorescent background is important. Changing the Enhancement Solution bottle should be done with care without touching the tubings.

XI. Limitations of Procedure

Plasma containing EDTA or citrate cannot be used due to chelating effects of europium. Heparin plasma however can be used. However, icteric serum samples may cause an increase and lipemic serum samples may cause a decrease in estriol concentrations and therefore should not be used.

XII. References

1. Hemmila, I., Dakubu, S., Mikkala, V-M., Siitari, H. and Lovgren, T., Europium as a Label in Time-resolved Immunofluorometric Assays, *Anal. Biochem.*, 1984, 37, 335-343.

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2. AutoDELFIA hCG kit, Time-resolved Fluoroimmunoassay Kit B007-112 for "in vitro" diagnostic use.
3. AutoDELFIA hAFP kit, Time-resolved Fluoroimmunoassay Kit B079-112 for "in vitro" diagnostic use.
4. AutoDELFIA Unconjugated Estriol Kit, Time-resolved Fluoroimmunoassay Kit B083-212 for "in vitro" diagnostic use.
5. AutoDELFIA PAPP-A, Time-resolved Fluoroimmunoassay Kit B098-201 for in vitro diagnostic use.
6. AutoDELFIA **TM** User Manual, for AutoDELFIA Workstation software version 1.4, Perkin Elmer Wallac, November 1996, 235-920-08.
7. AutoDELFIA **TM** Maintenance Manual, for AutoDELFIA workstation software version 1.4, Perkin Elmer Wallac, November 1996, 1235-924-01.

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Appendix 1

EXPANDED AFP INADEQUATE AND UNKNOWN ADEQUACY SPECIMENS

DATE: PERSON FILLING OUT FORM:

LAB:

ACCESSION NUMBER	APF-TRF FORM NUMBER	PATIENT LAST NAME IF DIFFERENT NAME ON TUBE USE LINE 2	COORDINATOR CONTACT		ADEQUACY CODE			DATA ENTRY DATE	INITIALS
			AGC CALLED	AGC FAXED	AFP	hCG	uE3		
		1							
		2							
		1							
		2							
		1							
		2							
		1							
		2							
		1							
		2							
		1							
		2							

ADEQUACY CODES: H = Hemolyzed; N = No blood specimen; B = Broken tube; Q = Quantity insufficient;

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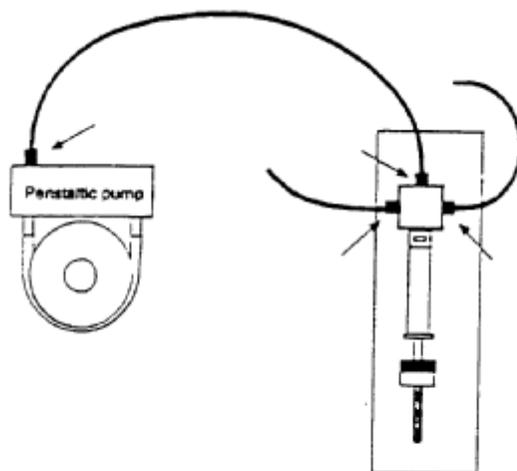
O = Other; C = Call coordinator to confirm match; M = mismatched / mislabeled

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Appendix 2

A. Drops from the tips or bubbles in the tubing

If there are bubbles in the tubing or drops on the end of the tip check and tighten the tubing connections in the peristaltic pump and four valves as shown in the figure.



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Appendix 3

A. Removing the AutoDELFIA washer manifold

From time to time a need may arise for the washer manifold to be removed and cleaned e.g. foreign material lodged in a needle. If you intend to perform this procedure the following points must be noted;

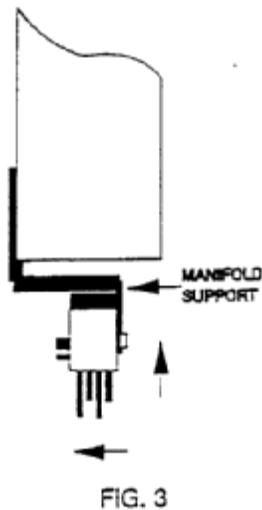
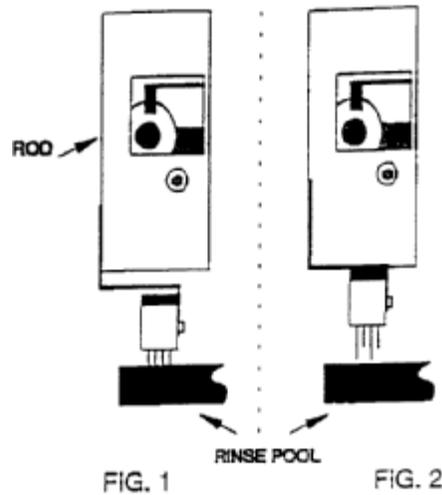
We strongly advise that you wear latex gloves when carrying out any service procedure on the AutoDELFIA system.

1. The washer manifold must be put through the disinfection procedure prior to carrying out any form of maintenance.
2. Removal of the washer manifold must be done with care as the dispensing and aspiration needles are easily damaged. Remove the Enhancement Solution bottles first.
3. Follow the washer manifold removal procedure as shown at the end of this appendix.
4. The rubber top seal should be removed and all walls of the aspiration cavity cleaned.
5. The aspiration needles should be cleaned using the pin with the corresponding diameter supplied in the maintenance kit.
6. The dispensing needles should be cleaned with the pin with the corresponding diameter supplied in the maintenance kit.
7. The four silicon caps sealing the dispensing cavity (two at each end) can be removed by using a small pin. (The fifth cap on the side should not be removed). The cavity should then be thoroughly cleaned with the brush supplied in the maintenance kit. Do not use this brush for anything, else otherwise you might contaminate it and hence the manifold.
8. The whole unit should be flushed with distilled water.
9. Both dispensing and aspiration cavities should have excess water shaken out and the whole manifold either allowed to dry or wiped dry with lint free paper.
10. Reinstall the rubber top seal ensuring it is properly positioned.
11. Install new silicon caps (found in the maintenance kit) ensuring that
 - a) **the cavity access holes are dry.**
 - b) **all caps are inserted and positioned properly so that the top of the cap is slightly below the outer wall of the manifold.**
12. Check that ail needles are straight and correctly positioned.
13. Reconnect the tubing and position the manifold back in its support, checking that it can move freely up and down.
14. Perform a washer test to ensure all functioning and coordinates of the washer unit are correct.

B. Removing the AutoDELFIA Washer Manifold.

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1. Open the lid of the instrument.
2. With your left hand turn the rod (fig.1) of the washer until the manifold is completely above (fig.2) the rinse pool.
3. Pull the manifold outwards until it is above the X-conveyor.
4. Remove the manifold from the support by lifting it up and moving left (fig.3).
5. Before disconnecting tubing from the manifold, put a paper towel on the X-conveyor so that liquid which may possibly be in the tubing, does not drop inside.



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Appendix 4

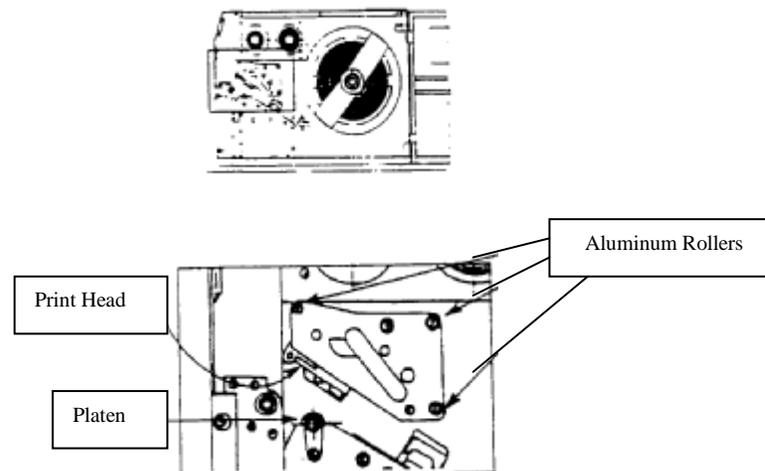
A. Cleaning the Printhead

Barcode Printer Model M-8400 (d:\docs\setup\barload.doc)

Printer Maintenance and Loading Instructions

Step Action

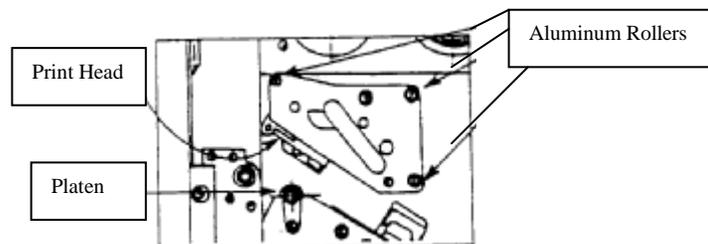
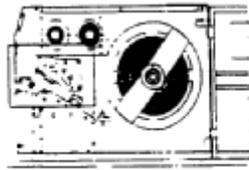
1. Power off the printer.
2. Open the printer side and top doors.
3. Open the print head assembly using the lever on the side of the assembly.
4. Apply rubbing alcohol to cotton swab.
5. The print head faces downward along the front edge of the assembly. Pass the end of the dampened swab along the entire width of the print head. (You may need to move the ribbon out of the way to accomplish this.)
6. Check for any black or adhesive on the swab after cleaning.
7. Repeat if necessary. The print head should be cleaned at least every time the ribbon is changed.



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B. Cleaning the Platen and Rollers

Step	Action
1.	Power off the printer.
2.	Open the printer side and top doors.
3.	Open the print head assembly using the lever on the side of the assembly.
4.	Apply rubbing alcohol to a clean wipe.
5.	The platen is the rubber roller directly below the print head. It should be cleaned of any ribbon or label residue.
6.	Note the aluminum rollers at the corners of the print head assembly and clean these as well. (It may be necessary to temporarily move the ribbon to clean those areas.)
7.	Repeat if necessary. The platen and rollers should be cleaned whenever foreign matter such as dust or adhesive is present.

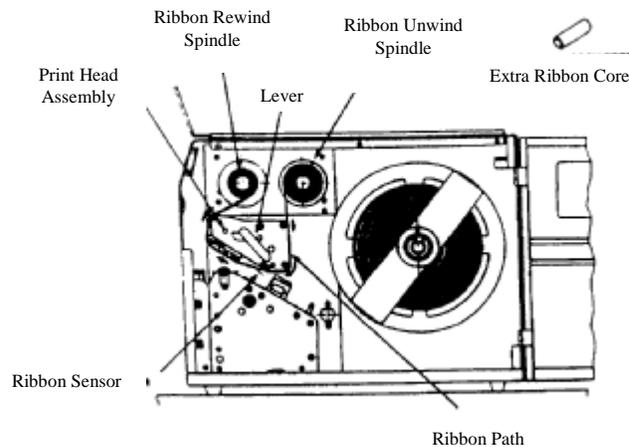


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C. Loading the Ribbon

Step Action

1. Open the side and top door.
2. Open the Print Head assembly by turning the Lever (on the side of the assembly) to the “head open” position.
3. Locate the extra ribbon core supplied with the printer (note that the new empty core of each subsequent roll becomes the next rewind core.) Place the core on the ribbon rewind the spindle, pushing it all the way to the inside of the spindle.
4. Load the ribbon onto the ribbon unwind spindle, also pushing it all the way to the inside of the spindle. The dull side of the ribbon should be facing down as it ravel through the print head assembly.
5. Feed the lead of the ribbon through the ribbon sensor (located at the inside wall of the print head assembly area) through the print head assembly, and up to the ribbon rewind spindle. Ensure the ribbon goes between the ribbon sensor and the metal bar directly beneath the sensor.
6. Load the ribbon behind and over the top of the ribbon rewind spindle and tape it to the extra ribbon core. Ensure that it matches the ribbon path in the diagram.
7. Manually turn the ribbon onto the rewind spindle 1 to 2 turns to secure it.
8. Close the print head assembly by turning the level to the label position. (note: Run a test, print patient sample to ensure the labels and ribbon were loaded correctly.)

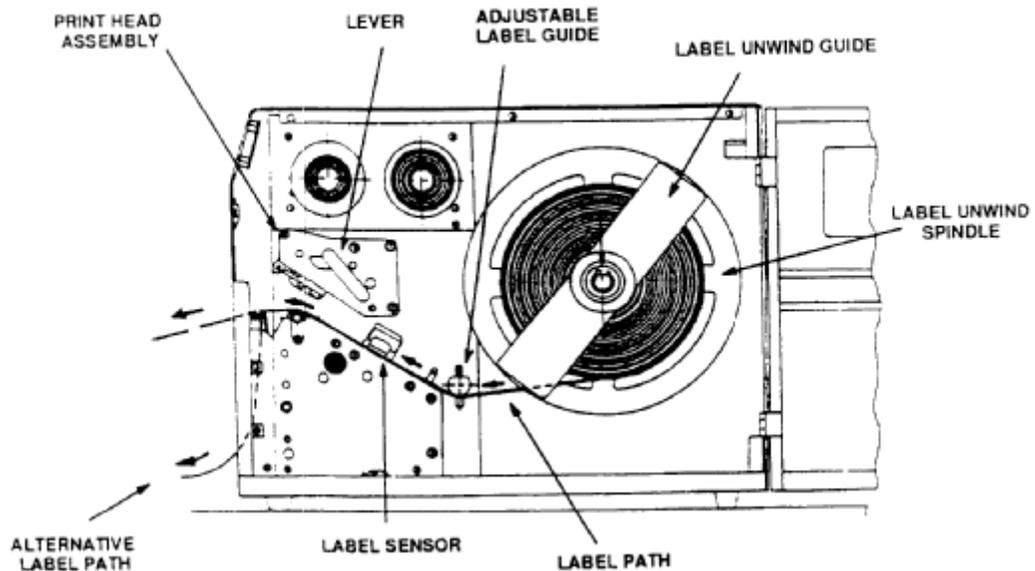


Appendix 5M

D. Loading Labels

Step Action

1. Open the side and top door.
2. Open the Print Head assembly by turning the Lever (on the side of the assembly) to the “head open” position.
3. Remove the label unwind guide from Label unwind spindle.
4. Load the roll onto the label unwind spindle so that the printed side of the label faces upward as it unwinds from the roll. Push the roll all the way to the inside of the printer, and replace the label unwind guide.
5. Feed the labels under the adjustable label guide, under the label sensor, through the print head assembly, and out the front of the printer. Remove 6 to 8 labels and feed the backing through the alternate label path as shown in the diagram.
6. Close the print head assembly by turning the lever to the label position.



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Call Proxy

(510) 215 - 1318

PerkinElmer LAS
 855 Marina Bay Pkwy
 Ste 31
 Richmond, CA 94804
 Phone: 510-237-9405
 Fax: 510-237-9492
<http://las.perkinelmer.com>

NAPS TELEPHONE INSTRUCTIONS

1. Follow the prompts, **Press 1** for California then enter your **Lab Number** from **Table 1**.

Lab Number	Site
11	Western Clinical Laboratory
12	Allied Laboratory
21	Fresno Community Hospital
31	Quest Diagnostics
32	Memorial Med Center of Long Beach
62	Genetic Disease Lab – Laboratory
65	Genetic Disease Branch
69	Genetic Disease Lab- QA Room
71	Kaiser Permanente North
72	Kaiser Permanente South

Table 1: Lab Number

2. Wait until prompted then **Enter the System ID** from Listing on page 2.

***** See Page 2 for Complete 3 Digit System ID Listings *****

3. At prompt **enter a call back phone #** , follow it by pressing the pound key (#).
4. After the Beep, **leave a voice message** give your name and a description of the problem. Press pound key (#) to end you voice message.



***** Before hanging up *****

5. **Wait to hear this confirmation**, “A PerkinElmer Engineer will return your call”.
6. **Done**

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Listing - Enter the 3 digit ID					
ID	SYS	Description	ID	SYS	Description
151	AD1 P	AD1 PNS AutoDELFLIA Plate Processor & PC	501	MM1 Q	MM1 Quattro Micro Mass Spec
152	AD2 P	AD2 PNS AutoDELFLIA Plate Processor & PC	502	MM2 Q	MM2 Quattro Micro Mass Spec
153	AD3 P	AD3 NBS AutoDELFLIA Plate Processor & PC	503	MM3 Q	MM3 Quattro Micro Mass Spec
154	AD4 P	AD4 NBS AutoDELFLIA Plate Processor & PC	511	MM1 S	MM1 2777 AutoSampler
155	AD5 P	AD5 PNS AutoDELFLIA Plate Processor & PC	512	MM2 S	MM2 2777 AutoSampler
156	AD6 P	AD6 PNS AutoDELFLIA Plate Processor & PC	513	MM3 S	MM3 2777 AutoSampler
157	AD7 P	AD7 NBS AutoDELFLIA Plate Processor & PC	521	MM1 P	MM1 Waters HPLC Pump
171	AD1 S	AD1 PNS AutoDELFLIA Sample Processor	522	MM2 P	MM2 Waters HPLC Pump
172	AD2 S	AD2 PNS AutoDELFLIA Sample Processor	523	MM3 P	MM3 Waters HPLC Pump
173	AD3 S	AD3 NBS AutoDELFLIA Sample Processor	531	MM1 PC	MM1 Waters PC
174	AD4 S	AD4 NBS AutoDELFLIA Sample Processor	532	MM2 PC	MM2 Waters PC
175	AD5 S	AD5 PNS AutoDELFLIA Sample Processor	533	MM3 PC	MM3 Waters PC
176	AD6 S	AD6 PNS AutoDELFLIA Sample Processor	561	PK1	MM1 PEAK N2 Generator & Argon Gas
177	AD7 S	AD7 NBS AutoDELFLIA Sample Processor	562	PK2	MM2 PEAK N2 Generator & Argon Gas
201	CH1	Chronrol Timer or Deep Well Plate Shakers	563	PK3	MM3 PEAK N2 Generator - Argon Gas
202	CH2	Chronrol Timer or Deep Well Plate Shakers	611	HB40	HEATBLOCK 40 Degree VWR
211	TOM1	Tomtec 96 Well Pipettor	612	HB60	HEATBLOCK 60 Degree VWR
212	TOM2	Tomtec 96 Well Pipettor	621	PP1	Apricot Personal Pipettor
221	NG1	DBS Puncher for TSH, MSMS	622	PP2	Apricot Personal Pipettor
222	NG2	DBS Puncher for Universal Eluent - Hb	631	PP1 PC	Apricot PC
223	NG3	DBS Puncher for TSH, MSMS	632	PP2 PC	Apricot PC
224	NG4	DBS Puncher for Universal Eluent - Hb	641	SVR	Server PC
225	NG5	DBS Puncher for TSH, MSMS	651	TX1	3 Plate Thermomix INC/SHAKE
226	NG6	DBS Puncher for Universal Eluent - Hb	652	TX2	3 Plate Thermomix INC/SHAKE
241	BC1	PNS Barcode Printer	653	TX3	3 Plate Thermomix INC/SHAKE
242	BC2	NBS Barcode Printer	661	INC1	9 Plate Incubator Shaker
243	BC3	PNS Barcode Printer	662	INC2	9 Plate Incubator Shaker
244	BC4	NBS Barcode Printer	663	INC3	9 Plate Incubator Shaker
251	WAP1	PerkinElmer Wallac AutoPuncher	671	HS1	Heat Sealer MSMS
252	WAP2	PerkinElmer Wallac AutoPuncher	691	BR1	Branson Model 2510 Sonicator
301	SG1	Specimen Gate, Server, Router, File Transfer	692	BR2	Branson Model 2510 Sonicator
302	SG2	Specimen Gate, Server, Router, File Transfer	701	UT1	UT1 Transferase/Biotinidase System
303	SG3	Specimen Gate, Server, Router, File Transfer	702	UT2	UT2 Transferase/Biotinidase System
304	SG4	Specimen Gate, Server, Router, File Transfer	703	UT3	UT3 Transferase/Biotinidase System
311	RPC1	Specimen Gate Review Station PC	704	UT4	UT4 Transferase/Biotinidase System
312	RPC2	Specimen Gate Review Station PC	901	SS1	SIS- Data Terminal,Server or Scanner-PC
313	RPC3	Specimen Gate Review Station PC	902	SS2	SIS- Data Terminal,Server or Scanner-PC
314	RPC4	Specimen Gate Review Station PC	903	SS3	SIS- Data Terminal,Server or Scanner-PC
			904	SS4	SIS- Data Terminal,Server or Scanner-PC
			905	SS5	SIS- Data Terminal,Server or Scanner-PC
			906	SS6	SIS- Data Terminal,Server or Scanner-PC
			907	SS7	SIS- Data Terminal,Server or Scanner-PC
			908	SS8	SIS- Data Terminal,Server or Scanner-PC
			952	SC2	SIS - Optical Scanner
			951	SC1	SIS - Optical Scanner

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Appendix 6

Flow Chart for AutoDELFIA hCG Assay

Access and barcode specimens

Take reagents from refrigerator for warmup/reconstitution

Centrifuge specimens for 10 minutes at 1000-1300g

Transfer specimens from centrifuge racks to sample racks and determine adequacy due to hemolysis

Remove caps, check for air bubbles

Print repeats from GDL list

Use the Local Repeats and Repeats from GDL to pull specimens for repeat testing

Add specimens for repeat testing to sample racks as appropriate

Place SQC in rack 72 and TQC in designated positions in the sample racks

Login

Set up AutoDELFIA

Load sample racks

Load standards

Load reagents

Load plates

Perform last system checks

Walk away as the AutoDELFIA automatically performs the assay steps

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Dilutes 12 uL of specimen with 475 uL of diluent

Aspirates 25 uL of diluted specimen and dispense into reaction well and adds 200 uL buffer

Incubates , 1 hour

Washes and aspirates, 2x

Dilutes the tracer, 1:50

Adds 200 uL tracer

Incubates, 30 minutes

Washes and aspirates, 6 x

Adds 200 uL enhancement solution

Incubates, 5 minutes

Reads fluorescence

HCG Assay

Shut down

Appendix 5M

Appendix 7

Flow Chart for AutoDELFIA AFP Assay

Access and barcode specimens

Take reagents from refrigerator for warmup/reconstitution

Centrifuge specimens for 10 minutes at 1000-1300g

Transfer specimens from centrifuge racks to sample racks and determine adequacy due to hemolysis

Remove caps, check for air bubbles

Print repeats from GDL list

Use the Local Repeats and Repeats from GDL to pull specimens for repeat testing

Add specimens for repeat testing to sample racks as appropriate

Place SQC in rack 72 and TQC in designated positions in the sample racks

Login

Set up AutoDELFIA

Load sample racks

Load standards

Load reagents

Load plates

Perform last system checks

Appendix 5M

Walk away as the AutoDELFIA automatically performs the assay steps

Aspirates 25 uL of specimen and dispenses into reaction well

Dilutes the tracer, 1:75 with buffer

Adds 200 uL tracer to reaction well

Incubates, 1 hour

Washes and aspirates, 6x

Adds 200 uL enhancement solution to reaction well

Incubates, 5 minutes

Reads fluorescence

AFP Assay

Shut Down

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Appendix 8

Flow Chart for AutoDELFIA uE3 Assay

Access and barcode specimens

Take reagents from refrigerator for warmup/reconstitution

Centrifuge specimens for 10 minutes at 1000-1300g

Transfer specimens from centrifuge racks to sample racks and determine adequacy due to hemolysis

Remove caps, check for air bubbles

Print repeats from GDL list

Use the Local Repeats and Repeats from GDL to pull specimens for repeat testing

Add specimens for repeat testing to sample racks as appropriate

Place SQC in rack 72 and TQC in designated positions in the sample racks

Login

Set up AutoDELFIA

Load sample racks

Load standards

Load reagents

Load plates

Appendix 5M

Perform last system checks

Walk away as the AutoDELFIA automatically performs the assay steps

Dilutes the antibody, 1:50, with buffer

Aspirates and dispenses 150 uL diluted antibody into reaction well

Incubates, 1 hour

Aspirates 50 uL of specimen and dispenses into reaction well

Dilutes the tracer, 1:50, with buffer

Aspirates and dispenses 50 uL of diluted tracer to reaction well

Incubates, 1 hour

Washes and aspirates, 6X

Adds 200 uL of enhancement solution to reaction well

Incubates, 5 minutes

Reads fluorescence

UE3 Assay

Shut down

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Appendix 9

Flow Chart for AutoDELFIA PAPP A Assay

Access and barcode specimens

Take reagents from refrigerator for warmup/reconstitution

Centrifuge specimens for 10 minutes at 1000-1300g

Transfer specimens from centrifuge racks to sample racks and determine adequacy due to hemolysis

Remove caps, check for air bubbles

Print repeats from GDL list

Use the Local Repeats and Repeats from GDL to pull specimens for repeat testing

Add specimens for repeat testing to sample racks as appropriate

Place SQC in rack 72 and TQC in designated positions in the sample racks

Login

Set up AutoDELFIA

Load sample racks

Load standards

Load reagents

Load plates

Perform last system checks

Walk away as the AutoDELFIA automatically performs the assay steps

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Dilutes 50 μ L of specimen with 200 μ L of Diluent 3

Dilutes the Biotin labeled antibody, 1:13.5, with buffer

Aspirates and dispenses 100 μ L diluted antibody into reaction well

Incubates, 30 minutes

Dilutes the tracer, 1:13.5, with buffer

Aspirates and dispenses 100 μ L of diluted tracer to reaction well

Aspirates 50 μ L of diluted serum and dispenses into reaction well

Incubates, 2 hours

Washes and aspirates, 6X

Adds 200 μ L of enhancement solution to reaction well

Incubates, 5 minutes

Reads fluorescence

PAPPA Assay

Shut down

Appendix 5M

Appendix 10 Troubleshooting Common Assay Problems for TMS

Event	Cause	Action
I. Outlier for STD curve		
A. 1 random outlier	Insufficient volume in vial	Label vial with # of trays used, do not create bubbles to avoid removing liquid from vial
	Dirty sample probe, wash well	Disinfect sample probe and wash well with 70% alcohol
	Air bubble in sample line, in vial	Check lines for air bubbles and flush, call Proxy if leak in tubing/syringe, mix gently to avoid creating bubbles
	Particulate matter in vial	Store tubes in clean space
B. 2x for 1 Std	Std not mix	Mix gently per protocol
	Bad Std, contaminated Std	Reconstitute new set of uE3 Stds, use Type 1 water, use clean pipets, do not touch with dirty gloves, recap immediately after removing from AD
	Insufficient volume	Label vial with # of trays used
	Wrong Std	Read label on vial before loading onto block
	Poor reconstitution of uE3 Std	Use Type 1 water, use accurate and precise pipets
	Air bubble in vial, film in vial	Mix gently per protocol, check for air bubbles before loading onto block, remove bubbles if present, break the film with disposable tip
C. 2 random outliers	Same as IA	
	Poor instrument performance with wash or sample probe	Call Proxy
D. Curve does not match to prior, flat or max/min incorrect	Stds placed in wrong order	Read label for Std name before putting into Std block, check order of Stds once loaded in block
	Wrong lot # used	Read lot # before use, store only the current lot
	Compromised stds, e.g., left on AD all day, contaminated	Store in refrigerator, unload from AD as soon as sampling is completed, recap
	Poor reconstitution of uE3 Stds	Use Type 1 water, use accurate and precise pipets
	Std block loaded onto AD incorrectly	Slide block all the way into the closed position
	Compromised reagents, e.g., poor storage	Store all reagents in refrigerator, recap reagents immediately after use

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	Problem with detector	Call Proxy
II. Blank is high	Dirty sample probe, dirty wash station, dirty wash probe	Disinfect sample probes and clean wash station with 70% alcohol, disinfect washer probes with 0.5% bleach, call Proxy if it persists
	Outside sample probe dirty	Clean the outside of probe with 70% alcohol, follow with a flush cycle
	Wash bottle for sample/plate processor dirty	Clean bottle and rinse with distilled water
	Contaminated Std A	Store in refrigerator, recap immediately after use, use only clean disposable pipets to remove air bubbles, do not touch with dirty gloves
	Dirty/Nosiy measurement unit area	Call Proxy
III. Assay aborted	Insufficient tips	Check number of tips on the screen with the reagent cassette during last system checks
	Cannot dump tip into waste tip tray	Dump used tips after each run, clean the tray, call Proxy
	Insufficient reagents	Label vial with # of trays used, do not create bubbles to avoid removing liquid from vial
	Computer/Instrument problems	Call Proxy
	Dirty sensor on waste container cap	Remove any build up by washing or wiping with clean damp cloth
	Curve fitting failed	PE and GDL are making an effort to better understand this error message, call Proxy for PE to determine cause to aid this effort
IV. "Possible foam detected"		
A. Entire tray/run	Used dilution strips that had been used	Dump all dirty strips during shut down
	Liquor sensor not working properly	Call Proxy
	Dilution strips placed higher in the rack	Check height of all strips, push strips as far down into the rack as possible, use system specific rack, load rack on AD correctly
B. Single sample	Computer/Software fluke	Call Proxy
	Non-uniform thickness in dilution strips resulting in varying liquid level	Occurs rarely, check inventory for affected strips and call GDL if found
C. Multiple samples	Same as IV.B.	
	Liquor sensor not working for a sample probe	Call Proxy
V. "Liquid loss detected"	Dilution rack loaded onto AD incorrectly, tilted up	Load rack on AD correctly
	Air bubbles in tubing of sample probes	Check for air bubbles before starting a run, flush the system
	Leaks in dilution strips	Clean area, visually inspect strips before using, check inventory for affected strips, switch to new lot of strips if needed
	No diluent	Verify volume of diluent displayed on the screen during last system check is at least the

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		volume on the AD
VI. “Liquid surface unstable”	Air bubbles in tube	Check for air bubbles before loading onto sample rack, remove if present
	Bad liquid sensor	Call Proxy
VII. “Barcode rack”	Marginal barcode – dirty, faded	Inspect barcodes and replace when needed
	Barcode reader problem	Call Proxy
VIII. “No liquid found”	Empty tube	Verify serum volume before loading onto sample rack
IX. “Too little liquid”	Volume < 250 uL	Judge volume for adequacy carefully, match volume of serum against a tube with line markers for volume
X. “Clot detected”	Fibrin in serum	Remove and re-centrifuge, retest
XI. “Liquid Moving Failed”	Sample probe was unable to transfer liquid from source to target	Verify liquid level in Std vials, verify liquid level in specimens and dilution strips, check sample probes for any damage, call Proxy if it persists
A. With Stds	Std block loaded onto AD incorrectly	Slide block all the way into the AD
B. With samples	Bad tip	Check reagent dispense tip, call Proxy if it persists
	Bad sample probe	Check for leaks, check connection from tubing to syringe to probe, check for any liquid on system, call Proxy if it persists
XII. Controls outside limits	Incorrectly reconstituted SQC	Use Type 1 water, use accurate and precise pipets
	Dispense TQC into tubes when partially Thawed	Allow sufficient time to thaw completely, check vial before dispensing
	Controls poorly mix when results are low	Mix after removing from refrigerator, mix before loading onto sample rack
	Contaminated tubes	Store tube in clean space, do not touch with dirty gloves
	Air bubbles in tubes	Check for air bubbles before loading onto sample rack, remove if present
	Wash/sample probe not working	Call Proxy
	System has hi/low bias	Isolate and eliminate each parameter to determine effects on the bias
XIII. Probe controls > limits		
A. One	One wash/sample probe not working	Call Proxy
	One probe hit gel and is plugged	Wash and disinfect sample probes per protocol, call Proxy if unable to clear gel

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	Tube was poorly mixed	Mix after removing from refrigerator, mix before loading onto sample rack
	Tube was contaminated	Store tube in clean space, do not touch with dirty gloves
	Air bubbles in tube	Check for air bubbles before loading onto sample rack, remove if present
B. Two	Same as XII.A	
XIV. Clot/no liquid found, QC	Empty tube	Verify liquid volume before loading onto sample rack
	Volume <250 uL	Dispense 18 tubes per vial to assure sufficient volume
	Particulate matter in tube	Store tubes in clean space
	Liquid sensor not working	Call Proxy
XV. System has hi/low bias	Poor maintenance	Follow protocol in all steps when performing maintenance
	Use expired reagents	Read lot #/expiration date before use, store only the current lot
	Detector problem	Call Proxy
	Analyst systematic errors	Identify steps and provide additional training, follow protocol
	Wash probes not working	Call Proxy
	Leak in sample probe tubing	Call Proxy
XVI. Repeats are inconsistent with initial	System has poor precision	Call Proxy
	Specimens poorly mixed	Mix after removing from refrigerator, mix before loading onto sample rack
XVII. Assay/Tray median out	System bias	Isolate and eliminate each parameter to determine effects on the bias
	Specimen compromised during mailing	Call PDC to correct collection errors
	Demographics	No action needed
	Specimens poorly mixed	Mix after removing from refrigerator, mix before loading onto sample rack

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Prepared by _____ Date _____

Renee Cullen, Supervising Chemist

Reviewed by _____ Date _____

T. Ho, Ph D, Research Scientist IV

Approved by _____ Date _____

George Helmer, Ph D, Acting Chief, GDLB

Appendix 5M

Procedure Revision Log

Enter section(s) and the page number(s) where deletion, revision or add-ons are found.
 Indicate whether this is a deletion, revision or an add-on by entering "X" in the appropriate column.

Procedure: Automated Dissociated Enhanced Lanthanide Fluoro Immunoassay System (AutoDELFIA) for Prenatal Screening Using Maternal Serum: 1st Trimester markers are PAPPA and HG1, 2nd Trimester markers are hCG, AFP, and uE3, Tracking 3CP002, Version 2.4

Revised by: R. Cullen

Date: Jan. 2013

Sections	Page #	Deletion	Revision	Add-on
VII.I.5	26		X	

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Technical Performance Verification

Procedure: Automated Dissociated Enhanced Lanthanide Fluoro Immunoassay System (AutoDELFLIA) for Prenatal Screening Using Maternal Serum: 1st Trimester markers are PAPPA and HG1, 2nd Trimester markers are hCG, AFP, and uE3, Tracking #CP002, Version 2.4

Employee's Signature	Supervisor's signature	Completion of Date of Training

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Procedure Review and Update Log

All procedures should be reviewed every 12 months. The Laboratory Director must also approve all new methods and procedural changes.

Procedure: Automated Dissociated Enhanced Lanthanide Fluoro Immunoassay System (AutoDELFIA) for Prenatal Screening Using Maternal Serum: 1st Trimester markers are PAPPA and HG1, 2nd Trimester markers are hCG, AFP, and uE3, Tracking #CP002, Version 2.3

Date	Supervisor's signature	Procedure Version	Review	Update	New Method	Laboratory Director's signature	Date

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