Appendix 5B

Specimen Handling, the Processing of Blood Spots Using Specimen Gate for Newborn Screening Tracking No. CN 003 Version 7.1

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I. Title: Specimen Handling, the Processing of Blood Spots Using Specimen Gate

II Principle

After the specimens are barcoded, they are processed through the Specimen Gate workstation, an automated system for positive specimen identification, punching, eluting, and distributing. The workstation utilizes the DBS Puncher, the Symbol barcode scanner, the Sato barcode printer and the Specimen Gate software which requires that the barcode of a specimen is first scanned and then the specimen is punched into a 96 well plate. Each plate has a barcode that when read into the system will provide a link with the specimens for the plate. A worklist is generated and downloaded to the analytical workstations. The analytical workstations, fully integrated into the system, are the AutoDELFIA TSH/17-OHP/IRT and the API-300 workstation for TRA/BIO (Transferase/Biotinidase). The API-300 workstation is duplicated in the laboratory to enhance its productivity. Although the hemoglobin workstation is not fully integrated into the system, eluates for hemoglobin testing are still processed on the Specimen Gate workstation, along with the eluate for the API-300 system. The system requires, at this time, that the specimens be processed separately for the AutoDELFIA TSH/17-OHP/IRT workstation and the API-300 workstation.

A copy of this protocol is in the Supervisor's PC. To access, login, click **NBS Assays SOP** and select the Specimen Handling protocol.

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III. Specimen Collection and Type

Specimens are bloodspots collected from a heel stick puncture and dried on filter paper. The collection procedure is performed on newborns greater than 12 hours of age or at the time of discharge from the hospital but no later than after 6 days of life. The dried specimen is transported to one of the State's contract laboratories immediately after collection.

IV. Equipment and Supplies:

Equipment

A. Two Specimen Gate Workstation PCs

B. 2 DBS Punchers: one with a 6.0-mm punch head, the other with a 3.2mm (1/8") punch head

C. One Sato barcode printer

D. Two Symbol barcode readers

E. One Apricot GTPS-96 Pipetting System

F. Four Fisher Vortex Genies with platform inserts

G. One CHRONTROL digital timer

H. One vacuum pump with vacuum tubing

Supplies

A. Supplied by WALLAC

1. Zerostat anti-static device

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2. Endust Duster
3. Round deep well plates
4. Square deep well plates
5. Microtiter assay plates
6. TSH coated assay plates, part of AutoDELFIA Neonatal hTSH Kit
7. 17-OHP coated assay plates, part of AutoDELFIA Neonatal 17-OHP Kit
8. IRT coated assay plates, part of AutoDELFIA Neonatal IRT L Kit
9. Millipore filter plates (1.2um) with covers
10. Water reservoir
11. Colored barcoded sleeves for microtiter assay plates
12. Diskette
13. Anti-static pad

B Supplied by NAPS

1. Water trap for vacuum pump
2. Small test tube brush

V. Reagents

A. Supplied by GDL

1. Tray quality control
2. Special proficiency control

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B. Supplied by NAPS

1. Distilled water or NCCLS Type I water
2. Commercial bleach

VI. Calibration and Quality Control-NA

VII. Procedures

A. Start Up for Specimen Gate

1. Turn on the Specimen Gate workstation PC and log on to the Specimen Gate system by entering user name and password.
2. Turn on the DBS Puncher using the power switch on the back of the unit. If the puncher is turned on later in the Specimen Gate program, it may not respond. If this happens, you must exit Specimen Gate by clicking on **File** then **Exit** or the close **[X]** button and then repeat steps 1 and 2. After the puncher performs its initialization procedure, the display on the puncher will read:

Select mode	
S/A	Slave

Display 1

3. Press Slave. This mode of operation allows the Specimen Gate software to control the puncher. The display on the puncher now reads: "Under External Control" . Note the two configurations of the Specimen Gate workstation: one, the DBS Puncher with the 1/8"(3.2 mm) punch head is connected to the Specimen Gate workstation PC and the

barcode printer and two, the DBS Puncher with the 6.0 mm punch head is connected only to the Specimen Gate workstation PC.

4. Double-click the Puncher Workstation shortcut in order to start the Specimen Gate punching program.

B Preparation of Plates

1. API-300

    a. Gather the plates required for the number of specimens barcoded. A square deep well elution plate ("grandmother"), a round deep well distribution plate ("mother"), and two microtiter assay plates ("daughters") constitute a set of plates required to process a plate of blood spot samples. A maximum of four sets of plates may be used per run. Table 1 lists the number of sets of plates required for a maximum number of specimens.

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Table1. The Number of Sets of Plates required for the API-300

Number of specimens (≤N) N	Number of Sets of Plates	Run #
76	1	1
167	2	1
258	3	1
349	4	1
440	1	2
531	2	2
622	3	2
713	4	2

b. Place the "grandmother" plates on the left side of the plate holder and the "mother" plates on the right side.
c. Place a barcoded color sleeve around the edge of each "daughter" plate: a gold sleeve for the TRA/BIO "daughter" plate and a black sleeve for the Hb "daughter" plate.
d. Place a group of "daughter" plates, TRA/BIO and Hb, on top of each "mother" plate.
e. Double-click the NBS Barcoder shortcut to start the barcoder program.

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f. Click on the **MOTHER** button. The "mother" barcodes are used to identify the square deep well plates used in eluting the blood spots and the round deep well plates used in distributing the blood eluates for the API-300/Hemoglobin assays. The barcodes are also used to identify the storage bags containing the specimens corresponding to the deep well plates with the same barcodes.

g. Click on the **START** button. "Mother" barcode labels are printed in quadruplicate.

h. Place one barcode label on a square deep well elution plate ("grandmother") and another on the corresponding round deep well distribution plate ("mother"). Place the label along the numbered side of the plate, near the A1 well. Place the two remaining labels on two slips of paper and place the papers in a plastic bag with the barcodes readable from the sides of the bag. Place the deep well plates back on the plate holder and repeat for subsequent sets of deep well plates. Keep the sets in order throughout the entire process.

i. Click on the **STOP** button when the required number of bar-code labels is printed.

j. Click on the **EXIT** button to leave the barcoding program.

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2. AutoDELFIA TSH, 17-OHP and IRT

    a. Remove the required number of plates for a run from the refrigerator. The plate map of a four-plate run given below can be used to determine the number of plates needed in the run.

    b. Open the sealed packets of the TSH plates, 17-OHP plates and IRT plates and remove the plates

    NOTE: The plates are now dry coated.

    c. Use the plate map of a four plate run to determine the number of strips needed on the last plate of a run. Remember to account for the end tray control and the three end system controls.

    d. Remove any excess strips from the backside of the last plate. This is the side away from the barcode.

    e. Place the excess strips in the original packet. Write the date on the packet and store in the refrigerator for future use.

    f. Add a “dummy” strip to the last plate to give an even number of strips.

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STD	STD	STD	STD	STD	STD	STD	STD	STD	STD	STD	STD
SC	SC	SC	QC	1	2	3	4	5	6	7	8
9	10	11	12	13	14	15	16	17	18	19	20
21	22	23	24	25	26	27	28	29	30	31	32
33	34	35	36	37	38	39	40	41	42	43	44
45	46	47	48	49	50	51	52	53	54	55	56
57	58	59	60	61	62	63	64	65	66	67	68
69	70	71	72	73	74	75	76	77	78	79	QC
QC	80	81	82	83	84	85	86	87	88	89	90
91	92	93	94	95	96	97	98	99	100	101	102
103	104	105	106	107	108	109	110	111	112	113	114
115	116	117	118	119	120	121	122	123	124	125	126
127	128	129	130	131	132	133	134	135	136	137	138
139	140	141	142	143	144	145	146	147	148	149	150
151	152	153	154	155	156	157	158	159	160	161	162
163	164	165	166	167	168	169	170	171	172	173	QC
QC	174	175	176	177	178	179	180	181	182	183	184
185	186	187	188	189	190	191	192	193	194	195	196
197	198	199	200	201	202	203	204	205	206	207	208
209	210	211	212	213	214	215	216	217	218	219	220
221	222	223	224	225	226	227	228	229	230	231	232
233	234	235	236	237	238	239	240	241	242	243	244
245	246	247	248	249	250	251	252	253	254	255	256
257	258	259	260	261	262	263	264	265	266	267	QC
QC	268	269	270	271	272	273	274	275	276	277	278
279	280	281	282	283	284	285	286	287	288	289	290
291	292	293	294	295	296	297	298	299	300	301	302
303	304	305	306	307	308	309	310	311	312	313	314
315	316	317	318	319	320	321	322	323	324	325	326
327	328	329	330	331	332	333	334	335	336	337	338
339	340	341	342	343	344	345	346	347	348	349	350
351	352	353	354	355	356	357	358	QC	SC	SC	SC

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C. Selection of Assays

When the barcodes of the trays for blood spot punching are scanned, the Specimen Gate system will automatically create worklists for the correct analysis.

D. TRA/BIO/HB Assay

1. Punching of Blood Spot Specimens
    a. Click the **Load** button at the top of the screen.
    b. Scan the barcode of the square deep well tray then select plate Map 1, and click **OK** to accept.

        NOTE: Plate Map 1 is used for the first tray in a worklist. Use Plate Map 2 for Tray 2, Plate Map 3 for Tray 3, and Plate Map 4 for Tray 4.
    c. Install the correct punch head. In addition, make sure to use the computer and puncher system that is used for TRA/BIO/HB, since the TRA/BIO/HB system and the TSH/17-OHP systems are no longer interchangeable. The API-300 requires the 6.0 mm punch head. If the wrong punch head is installed, the display on the puncher reads:

Install punch head: 6 mm

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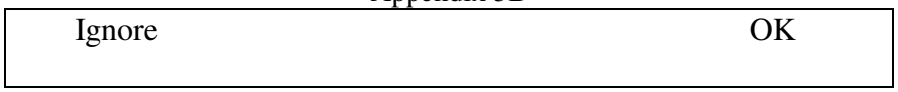

Display 2

1) Elevate the plexi-glass with the thumbs so the cover is parallel to the bench top and position the index and middle fingers on both sides of the trigger plate.
2) Pull the trigger plate straight out. Set the trigger plate assembly aside.
3) Pull down on the cover plate to remove it from the puncher.
4) Grasp the punch head installed on the puncher with your right hand.

   NOTE: Fingerprints on the punch piston can cause enough friction to prevent it from working correctly. Fingerprints on the outer brass piece can cause corrosion easily. Wear gloves at all times when handling the punch head. Remove fingerprints using an alcohol wipe.
5) Press in on the spring loaded button on the left side of the punch head with your thumb.
6) Pull the punch head straight out from the puncher. Keep the punch head upright when handling it in order to avoid dropping the puncher piston. Place the punch head on its side in its storage box.
7) Grasp the correct punch head with your right hand, being careful to keep the punch head in its upright position.

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8) Push in on the spring loaded button on the left side of the punch head with your thumb.

9) Align the rubber ring on the top of the puncher piston with the metal fork in the "notched" section on the puncher cover.

10) Push the punch head straight back towards the puncher so the rubber ring engages with the metal fork, and the positioning rods on the puncher insert into the holes in the back of the punch head. Release the spring-loaded button.

11) Replace the cover plate by aligning the rectangular protrusion on the inside surface of the cover plate with the "notched" section on the puncher cover and then pushing up on the cover plate until it clips in place.

12) Replace the trigger plate by aligning the "notched" region on the trigger plate with the punch head assembly, sliding the trigger plate over the screws heads on the side of the punch head, and pushing the plate towards the puncher until it clicks into place.

13) Press **OK**.

d. Load the tray into the DBS puncher in the correct orientation: well A1 located in the upper right corner. The display on the puncher now reads:

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	Load Plate	
Eject	Cancel	OK

Display 3

e. Use the Zerostat anti-static device by:

   1) Holding device about 12 inches away from plate.

   2) Slowly squeezing and releasing the trigger slowly.

   Lower the cover back onto the puncher.

f. Press **OK** on the puncher. The platform will move to position the plate correctly beneath the punch head. The display on the puncher now reads:

Pos:xy	Disk 1/1

Display 4

Where xy are the alphanumeric coordinates of the well position on an 8 x 12 plate just beneath the punch head and Disk 1/1 indicates that the system is waiting for the first and only punch.

g. Click the **Specimen** button at the top of the screen.

NOTE: Take from the freezer only the number of TQC strips needed for a run. If the spots are sufficiently large, punch two 6mm disks from each spot; then discard. Once thawed, TQC strips must not be returned to the freezer for future use, store in the refrigerator.

h. Click the **Patient** button to begin punching patient samples.

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i. Scan the barcode for each patient blood spot specimen then punch (6 mm diameter) into the corresponding well of the tray. Follow the plate map to complete the punching of the tray.

The display on the puncher now reads:

Pos:xy		Disk 1/1
Punch	ID	Check

Display 5

j. If the puncher detects no disk dropping from the punch head during the punching process, the display will read:

Disk Not Detected			
Retry	Check	Ignore	Head

Display 6

Press **Retry**. Activate the punch head again to dislodge the disk. If Display 6 reappears, then select **Check**. The platform will move to position the plate for removal from the puncher and the display on the puncher will read:

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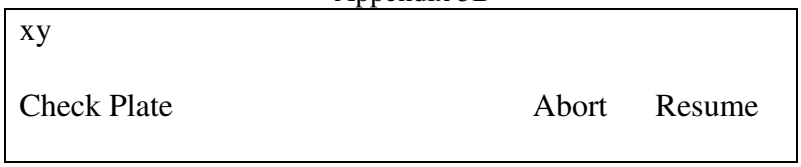


Display 7

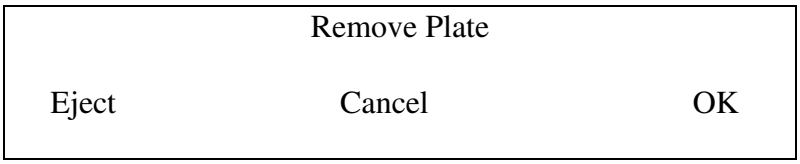
Remove the plate and check to see that the well in question contains a disk. Place the plate back onto the loading platform, use Zerostat as you did when loading the plate, and then press **Resume**. Display 6 reappears; press Ignore and continue with the next specimen.

k. Click **No** when**,** after punching is complete for a full tray, a message box appears on the PC screen asking if you want to load the next tray,

l. Click the **Control** button to begin punching the QC samples.

m. Scan and punch the controls according to the plate map.

n. Click **Yes** when, after controls have been punched, a message box appears asking if you want to load the next tray. The display on the puncher now reads:



Display 9

o. Unload tray from puncher then select **OK**.

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p. Use the Zerostat anti-static device as you did when loading the plate. Remove the plate and press **OK**. Place the plate on the anti-static pad. Check each well to verify that it has a blood spot. Immediately, without moving the plate, place a TOMTEC THIN LID on top of the plate by peeling away the center portion of wax paper from the TOMTEC THIN LID; leaving the paper intact at the narrow edges. Grasp the THIN LID by the narrow edges and cover the square deep well plate. Align the first row of circles nearest you on the lid with the first row of wells in the plate. Then lay the lid carefully onto the plate one row at a time. The circles must align with the wells. Place tray on metal tray-holder.

   Place the blood specimens in the appropriate storage bag.

q. Scan another plate to continue or select **Cancel** and exit the puncher application.

r. When all patient specimens have been punched for the last partial tray select **Cancel** from the Patient Barcode Entry screen.

s. Right click on the plate map and select **Mark as complete plate *PlateBarcode***.

t. Click **Yes** when message box appears stating that the controls for that tray need to be punched and asks if you want to punch them now.

u. Follow steps #m-q above.

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v. Complete the NBS checklist with the pertinent information.

2. Punching of Blood Spot Specimens for Hemoglobin Only Analysis

   a. Double-click **DBS Workstation.**

   b. Select **Hemoglobin** protocol with **Start New** option. Click **Start Punching**.

   c. Scan barcode of black colored sleeve for microtiter assay plate.

   d. Load Grandmother Tray onto DBS puncher and press **OK** on puncher screen.

   e. Scan Tray Control barcode and punch Tray Control into well B04. Continue scanning and punching patient specimens into following wells.

   f. If well E01 is reached, scan and punch Proficiency Control into that well.

   g. After last patient specimen is scanned and punched, scan and punch a water barcode (W1001).

   h. Right-click on first empty well after well with water barcode and select **End Plate Here**. Scan and punch Tray Control.

   i. Click **Plate Completed** at lower right corner. Remove Grandmother Tray from DBS puncher and press **OK** on puncher screen.

   j. *Do you want to punch more plates to this worklist?* query appears. Click **Yes** if you have more trays to punch and **No** if you do not.

   k. *Is this worklist to be added to the current run?* query appears. Click **No**.

   l. Double-click **Transfer HB**. Select appropriate worklist. Worklists are named according to the year, month, and day when they are created.

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m. Insert floppy disk and click **OK**. Give floppy disk to Hemoglobin analyst.

3. Processing Punched Blood Spots for Analysis Using the Apricot GTPS-96 and Specimen Gate.

a. Sending the worklist to the API system.

1) Start the NeoGO program.
2) Scan the barcode of the first square deep well plate. Verify that the barcode has been read correctly by comparing the barcode in the Mother Tray barcode box with the one on the plate. Scan again if the barcode is incorrect.
3) Click on the **NEXT** button. The section of the NeoGO program that handles the punching will be skipped.
4) Select the system (workstation) where you want the worklist to be sent. This option is only available when the first plate of a run has been punched. Click on the **NEXT** button.
5) Continue building the work list by selecting
Yes, I have more trays to punch, and then
Append to the current work list.
When the work list is completed, proceed to step 7).
6) Click on the **NEXT** button. Repeat the scanning process for subsequent plates.
7) End the work list by selecting
NO, I have punched all my trays.
8) Click on the **NEXT** button. A copy of the worklist is printed; click **YES** on the message box if the copy is satisfactory. Click **NO** to print multiple copies. Give a copy of the worklist to the hemoglobin analyst. Specimens out of sequence will be flagged (***). (This will aid the analyst in preparing the worklist at the hemoglobin workstation if manual accession entry is used.)
9) Scan the barcodes of the first set of plates in the following order:
   a) Square Deep Well Elution Plate (“grandmother”),
   b) Round Deep Well Distribution Plate (“mother”),
   c) TRA/BIO “Daughter” Plate,
   d) HEMOGLOBIN “Daughter” Plate.

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If a mistake is made in the scanning order of the daughter plates, click on the color-coded assay box to remove the bar-code and then scan the correct daughter plate.

10) Click on the **NEXT** button. The Export HB Work list window appears. Select the worklist using the left mouse button and then click the **OK** button.

11) Insert the correct diskette, by day, into drive A and click the **OK** button on the Wallac NeoGO message window. If a worklist for the current day already exists on the diskette, a second message appears indicating two options: click on the **YES** button to use the diskette or click on the **NO** button to change the diskette. Select the desired option to complete the export of the Hb worklist.

12) Remove the diskette from the drive and give it to the hemoglobin analyst.

13) Click on the **NEXT** button. The Congratulation screen appears displaying a jubilant Baby Eric. At this point, the worklist and the barcodes of the daughter plates for the run should have been transmitted to the workstations. The processing of blood spots is now completed. A worklist may be retransmitted to a workstation if the initial transmission went awry. A worklist may also be retransmitted if one wishes to send a worklist to a different workstation. For details on retransmitting worklists, see § VII. G., Restoration of Worklists.

14) Click on the **EXIT** button. A window appears asking whether you wish to restart NeoGO. Click on the appropriate button.

b. Place the plates on the plate holder with the “grandmother” plate in the left front position and the “mother” plate in the right front position with the “daughter” plates on top of the “mother”.

c. Repeat Step 2b for trays two through four as needed, placing the sequential trays behind tray one on the plate holder. It is critical that a set of grandmother, mother and daughter trays remain together.

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d. Turn computer that controls the Apricot GTPS-96 Pipetting System on. Select **Apricot** username and double-click **TPS** shortcut.

   Note: Supervisor may log into Apricot program as an administrator by clicking on **Tools**, then **User Information**. Enter Username and Password, then click **OK**.

e. *Initialize Pipettor* window appears. Enter *Operator* name. Be sure that **Initialize** checkbox is selected. Select **Yes** for *Do you want to connect machine?*

f. Positions on surface of Apricot are numbered 1-9 from left to right, starting from the back left position. Items to be placed in positions during normal operation are follows:

1. Water Reservoir	2. Empty Box for Discarded Tips	3. Falcon Tray for TRA/BIO	4. Multiscreen Filter Plate
5. Falcon Tray for Diluent	6. Grandmother Tray	7. Falcon Tray for Hb	8. Mother Tray
9. Pipet Tips to be Loaded			

Before loading Grandmother tray on Apricot, remove Thin Lid and set aside.

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g. Fill water bottle with DI water. Place in position upside down suspended from upper left side of Apricot. Water will drain down into reservoir as needed.

h. Lubricate the pipette tip head with the tip head lubricator.

i. Click on **File**, then **Open.** Select **Fill_Grandmother.tps** protocol.

j. Click on **EXE** tab, then click on **Run** button.

k. A *Warning!* window appears. Make sure that the tips are not already loaded on the head and that the plates are in the correct positions. Select checkboxes for **Check Head, No Tips On Head**, and **Check Plates and Plastic Tip Box(es)**. Click **OK**.

l. Apricot will load the tips onto the head and transfer 425 uL of DI water to Grandmother tray. After DI water transfer, replace Thin Lid on Grandmother tray.

m. Tap the plate gently on the bench top to dislodge air bubbles.

n. Place the plate in the "cut-out" region of the platform on the Vortex Genie and secure it with the safety strap. The first plate of the run should be placed on Vortex Genie 1, the second plate of the run on Vortex Genie 2, *etc*. The shaker setting on the Vortex Genie is calibrated by the vendor for a specific RPM. **DO NOT CHANGE THE SETTING.**

o. Use the CHRONTROL programmable timer to initiate the 30-minute elution program by entering the key sequence:

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Program /N (N=Vortex Genie number)/On.

The plate is shaken for 1-minute, follow by a 5-minute rest period. This cycle is repeated five times for a total elution time of thirty minutes. A circuit light on the timer for Vortex Genie N comes on only during the 1-minute shaking step. To ascertain the end of the elution program, synchronize a 30-minute alarm with the CHRONTROL timer. To prematurely stop a Vortex Genie enter the key sequence:

Circuit/N/Off.

p. Repeat the elution steps for subsequent square deep well plates in the same order they were punched allowing at least 20 minutes between subsequent plates. The time interval is to reduce the idle time that the plates sit on the sampler.
q. Remove the first square deep well plate from the VORTEX GENIE at the end of its 30-minute elution.
r. Load Grandmother tray back on Apricot. Remove tray seal from Grandmother tray. Make sure that multiscreen filter plate, Mother tray, and all Falcon trays are in correct positions.
s. Click on **File**, then **Open**. Select **Filter_Dist_TRABIO&Hb.tps** protocol.
t. Repeat steps j-k.

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u. Apricot will load tips onto head, transfer eluate from Grandmother tray to multiscreen filter plate, filter eluate into Mother tray, and distribute undiluted eluate to TRA/BIO Falcon tray and diluted eluate to Hb Falcon tray.

v. If only a TRA/BIO Falcon tray is required for analysis, select **Filter_Dist_TRABIO_Only.tps** during step s. If only a Hb Falcon tray is required for analysis, select **Filter_Dist_Hb_Only.tps** during step s.

4. Processing Punched Blood Spots for Analysis Using Apricot GTPS-96 Onboard Shaking Function

   a. Perform steps 3. a. – 3. h.

   b. Click on **File**, then **Open**. Select **Complete_With_On_Board_Shake_TRABIO_Hb.tps** protocol.

   c. Click on **EXE** tab, then click the **Run** button.

   d. After the Apricot transfers 425 uL of DI water to the Grandmother tray, the monitor will display a **Waiting** window. Cover the Grandmother tray with a tray seal and click **OK** to begin the on-board shaking.

   e. After 30 minutes, the on-board shaking will cease. The monitor will display another **Waiting** window. Remove the tray seal from the Grandmother tray and click **OK** to proceed with the filtration and distribution steps.

f. If only a TRA/BIO Falcon tray is required for analysis, select **Complete_With_On_Board_Shake_TRABIO_Only.tps** during step b. If only a Hb Falcon tray is required for analysis, select **Complete_With_On_Board_Shake_Hb_Only.tps** during step b.

5. Shut Down Procedure for the Apricot GTPS-96

   a. Dispose of discarded pipet tips in proper waste container.

   b. Empty bottle that supplies DI water reservoir.

   c. Open stopcock on DI water reservoir and drain into sink or other container.

   d. Clean the Apricot GTPS-96. Any blood spills should be cleaned immediately.

E. Punching for the AutoDELFIA TSH/17-OHP and IRT Assays

1. Follow steps under VII. D.1. with the following exceptions:

   a. The puncher uses a 1/8" (3.2 mm) punch head.

   b. Do not use a thin lid to cover the plates.

   c. Before step VII.D.1.l.(small case L), click **Calibrator** button, scan barcode on calibrator blood spot card, and punch calibrators into designated wells, with Std A going into A01 and A02, Std B into A03 and A04, and so on.

   d. Do not split the end of run system controls over two plates. This cannot be done with Specimen Gate software. For the last tray, if the end or tray TQC in a worklist falls on well H10 or H11, punch

water/reference samples in those two wells and put the end of tray TQC in well H12.

e. When tray is marked as completed, scan low, medium, and high SQC barcodes and punch System Control blood spots into designated wells.

2. Send worklists to AutoDELFIA.

a. Click **ADPlates** desktop shortcut on the AutoDELFIA computer that the worklists will be sent to.

b. The barcodes of the plates that have just been punched will be seen near the bottom of the window and highlighted with a light yellow background.

c. Highlight plate barcode and click **Include**. If there are multiple plates for an analysis, include the plates one at a time in the order in which they were punched. If there are multiple analyses, group the plates from the same analysis together.

d. Click **Create Worklist**.

e. A message will appear asking if you want to create 1 worklist from x number of plates. Click **OK** to create.

f. Select Run ID number. Click **OK**.

g. A confirmation will appear in the format:

01 = 042884031343 → N17OHP.W01

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Click **OK**.

3. Take the plates to the appropriate AutoDELFIA for TSH, 17OHP and/or IRT analyses. An error in the transmission of the work list may be corrected by restoring the worklist. For details on retransmitting a worklist, see Vll.G., Restoration of Worklists.
4. Do not do the following when handling plates after punching.
    a. Do not stack plates that could cause spots to stick to the upper plate.
    b. Do not put the plate down with excessive force that could cause spots to jump.
    c. Do not bump or disturb the plates when loading onto the Auto-DELFIA.
5. Complete the NBS checklist with the pertinent information.
6. Do not store the plates overnight after punching; please begin analysis as soon as possible after punching is completed.

F. Repeat Assay

1. Check the local and GDL repeat lists.
2. Add individual specimens that require retesting for TRA/BIO and/or Hb to the current day's API-300 run. Specimens requiring retesting for TSH/17-OHP/IRT are added to the current day's AutoDELFIA TSH/17-OHP/IRT run.

G. Restoration of Worklists

Appendix 5B

1. BIO/TRA

   a. Restore a worklist by using NeoGO software at either punching station.

   b. Select **Restore Worklist** at the Assay Selection screen.

   c. Select the worklist's filename by date and run, *i.e.* 971103.01(yr/mo/day/run).

   d. Select **Restore**.

   e. Confirm the restoration of the worklist by clicking on **YES** on the Wallac NeoGO message box.

   f. Select the system where you wish to send the worklist and the assay(s) to be run. The assays must be in agreement with the initial worklist.

   g. Click on the **NEXT** button. A copy of the restored worklist is printed. Click **YES** on the message box to continue.

   h. Scan the original barcodes of the "grandmother" and the "mother" plates.

   i. Scan the barcodes of the "daughter" plates for the selected assays. Click on the **NEXT** button.

   j. Click on the **NEXT** button again. The restoration of the BIO/TRA work list is now completed.

Appendix 5B

2. TSH/17-OHP/IRT

a. Double-click **Worklist Restore** shortcut on desktop of computer that controls the AutoDELFIA

b. Highlight the worklist to be restored.

c. Click **Restore**, and then **Exit**.

H. Maintenance

1. Apricot GTPS-96

a. Daily – see Shut Down Procedure for the Apricot GTPS-96.

b. Monthly

1) Cleaning the Apricot GTPS-96

a) Clean the unit with bleach (diluted 1:10) in areas where blood spills are common, *i.e*. within the vacuum box and on the stage.

b) Wipe the entire unit with a moist towel.

2) Perform Accuracy on 38 µL and 176 µL volumes.

a) Label 2 Falcon plates A (38 µL) and B (176 µL). Accuracy and precision should be done in triplicate, so 6 plates are required, 3 labeled A and 3 labeled B.

b) Weigh the plates to two decimal places. This is the tare weight. Good laboratory technique demands that gloves be worn during the accuracy and precision procedure.

c) Place plate A onto position 7 and plate B onto position 3.

Appendix 5B

d) Click on **File**, then **Open**. Select **Accuracy_Test_38uL_176uL.tps** protocol.

e) Click on **EXE** tab, then click on **Run** button.

f) A Warning! Window appears. Make sure that tips are not already loaded on head and that plates are in correct positions. Select checkboxes for **Check Head**, **No Tips On Head**, and **Check Plates and Plastic Tip Box(es)**. Click **OK**.

g) Apricot will load tips onto head, dispense 38 uL of DI water into Falcon tray A, and 176 uL into Falcon tray B.

h) Calculate the volume (uL) dispensed each time into each well of plate A and B by the formula:

$$\mu L \text{ dispensed} = \frac{\text{(final weight (g) – tare weight (g))}}{96 * \text{Density of water at room temp (g/mL)}} * 1000 \frac{uL}{mL}$$

Table with densities of water at various temperatures is located at end of protocol (p. 38).

i) Repeat the accuracy procedure 2 more times.

j) Determine the acceptability of the accuracy measurement. See Tomtec Quadra 96 Accuracy Limits below.

3) Perform Accuracy on 425-µL volume.

a) Label 3 “grandmother” plates C.

Appendix 5B

b) Weigh each plate to two decimal places. This is the tare weight. Good laboratory technique demands that gloves be worn during the accuracy and precision procedure.

c) Place plate C onto position 6.

d) Click on **File**, then **Open**. Select **Fill_Grandmother.tps** protocol.

e) Click on **EXE** tab, then click on **Run** button.

f) A Warning! Window appears. Make sure that tips are not already loaded on head and that plates are in correct positions. Select checkboxes for **Check Head**, **No Tips On Head**, and **Check Plates and Plastic Tip Box(es)**. Click **OK**.

g) Apricot will load tips onto head, dispense 425 uL of DI water into plate C.

h) Calculate the volume (µL) dispensed into each well of the plate by the formula:

$$\mu L \text{ dispensed} = \frac{(\text{final weight (g)} - \text{tare weight (g)})}{96 * \text{Density of water at room temp (g/mL)}} * 1000 \frac{uL}{mL}$$

Table with densities of water at various temperatures is located at end of protocol (p. 38).

i) Repeat the accuracy procedure two more times.

j) Determine the acceptability of the accuracy measurement. See Tomtec Quadra 96 Accuracy Limits below.

Appendix 5B

4) Tomtec Quadra 96 Accuracy Limits

Plate	Target (uL)	Range (uL)
A	38	36.8 to 39.2
B	176	173 to 179
C	425	418.6 to 431.4

Each of the three means must fall within the appropriate range. If one or more is out, repeat weighing(s). If they are still out of range, call Perkin Elmer Wallac for service.

5) Perform Quadra Well to Well Precision, 200 uL and 27 uL

To perform the procedures you will need the following items:

HCG tracer

Enhancement solution

HCG diluent cups

Special barcodes for uncoated plates *

Uncoated plates *

Falcon plates

A special reagent cassette label with special barcode for 200ul*

Appendix 5B

A special reagent cassette label with special barcode for 27ul*

Wash reservoir, not use for routine work *

Special Station 5 plate without seating pins *

* Supplied by Perkin Elmer Wallac

a) Perform the 200 uL procedure first.
   i. **Dilute** the hCG tracer using the diluent cup label for 200 uL by adding 100 uL hCG tracer to 100 mL of enhancement solution. Mix well. Solution **must** be homogeneous and free of air bubbles or foam. After mixing, wait until solution is clear, takes about 30 mins. This diluted tracer solution should have 800-900K counts when 200 uL is dispensed into each well.
   ii. Use the Apricot to **dispense** the diluted tracer solution into wells of an uncoated plate.
   iii. Add the diluted hCG tracer solution from step i. to the wash reservoir and place the wash reservoir on Position 6.
   iv. Label an uncoated plate with the special barcode for 38 and 200 uL on the A1 side in the middle with letters facing up and close to the lower edge.
   v. Place the label plate on Position 4.
   vi. Run the Precision_Test_200uL.tps protocol. Apricot will dispense 200 uL from the wash reservoir into each well. Check the plate to be sure there are no air bubbles in the wells. Any air bubbles will cause an artificially high CV. If air bubbles are present, discard plate and run Precision_Test_200uL.tps protocol again.
   vii. Remove the plate, **immediately** read the plate on the AutoDELFIA. It takes about 15 minutes to read.
   NOTE: Do not touch the bottom sides of the strips on this plate. This will affect the proper reading of the counts on the AutoDELFIA. Hold the plate by only touching its white plastic frame.
   viii. **Read** the plate using a specific AutoDELFIA. Wallac will have a special software program loaded.

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ix. Click Automate, enter user name and password.

x. **Loading Wizard** appears. Click **Next**, then **Next**. If no response on the second **Next**, click on **Unload old samples**. Remove sample racks as needed, then click **Next** again.

xi. Click on **Create New** tab. Click on the + sign next to the **Service** option. Double click on **909 Tomtec Prec 200**, then **Next**.

xii. Under **tube types**, click on the drop down arrow and select the **Man. Pipetted** option.

xiii. Under **Code**, type **1**, and under **number of samples** type **96**. Then click on **Add Samples** tab.

xiv. Click **Next** (seven times) until the **Reagent Consumption** screen appears. Click **OK**, see **Liquid Consumption** box. Check to make sure liquid levels are adequate. Replace or replenish as needed. Click **OK**. Now the instrument scans the reagent bar code and the loader comes out.

xv. Load the prepared plate on the plate loader with the bar code facing the mirror. Press the lighted **IN/OUT** button.

xvi. Click **Next** when prompted and see **Wizard Completed** message.

xvii. Click **Start** and see **Process Initialization**.

xviii. Stand by the instrument until **Schedule** box appears.

xix. Look at the final schedule to know when reading is completed.

xx. Click **Unload** and unload the plate upon completion. The uncoated plate can be reused unless dirty.

xxi. Click **Start/Programs/Pr(in)t Assay/Tomtec 200ul** to print the MultiCalc printout. The printout shows the calculated mean, %CV, and outliers by position. Review the printout. If any data point is outside 0.85 – 1.15 of the mean, it is an outlier and the position is flagged. Repeat the procedure. If confirmed, call Proxy for service. The acceptable well to well %CV specified by GDL is 3.00%. If the %CV is greater than 3.00%, call Perkin Elmer Wallac for service.

xxii. **Wash** the wash reservoir. Dump the solution in the wash reservoir. Place the wash reservoir

under running tap water for 10 minutes, then thoroughly rinse with distilled water (or Type 1 water).

xxiii. Unload the tips.

xxiv. Dump the diluent cup. Store the uncoated plate. Uncoated plate is clean when unload from the AutoDELFIA

b) Perform the 38 uL procedure

i. **Dilute** the hCG tracer using the diluent cup label for 38 uL by adding add 250 uL of hCG tracer to 100 mL of distilled water (or Type 1 water). Mix well. Be sure there are no air bubbles. This diluted tracer solution should have 200-300K counts when 38 uL is dispensed into each well.

ii. Follow the steps for the 200 uL and use the Apricot to **dispense** the diluted tracer solution into each well of an uncoated plate label with the special barcode for 38 and 200 uL. In addition, place a reservoir containing 100 mL of Enhancement Solution into Position 8.

iii. Run the Precision_Test_38uL_New.tps protocol, to prime and dispense. Check the plate to be sure there are no air bubbles in the wells. Any air bubbles will cause an artificially high CV. If air bubbles are present, discard plate and run Precision_Test_38uL_New.tps protocol again.

iv. Remove and **immediately read** on the AutoDELFIA. Use caution when handling the plate by not touching the bottom of the strips. Select **909 Tomtec Pre 200** and use the reagent cassette label with the special barcode for 200 uL.

v. Review the printout. If there are outliers, repeat. If confirm ,call Perkin Elmer Wallac for service. The acceptable %CV specified by GDL is 7.40%. If the %CV is greater than 7.40%, call Proxy for service.

vi. **Wash** the wash reservoir. Dump the solution in the wash reservoir. Rinse under running tap water for 10 minutes, then rinse thoroughly with distilled water.

vii. Unload the tips.

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viii. Dump the diluent cup. Store the uncoated plate. Uncoated plate is clean when unloaded from the AutoDELFIA

2. Wallac DBS Puncher

a. Weekly

1) Remove the punch head from the puncher and place it on a clean bench top.

2) Remove the piston from the punch head and clean with a lint free cloth using 70% alcohol (*i.e*. isopropanol).

3) Clean the punch head cylinder and the disk outlet shaft with a soft test tube brush using 70% alcohol.

4) Place the piston back in the punch head cylinder.

5) Install the punch head back onto the puncher.

6) Test the Zerostat anti-static device.

a) Insert the ion-indicator provided with the device into the nose of the Zerostat.

b) Slowly squeeze and release the trigger. If the device is functioning correctly, a gentle glow should be observed.

b. Monthly

1) Remove the punch head from the puncher and place in its storage box.
2) Remove the second punch head from its storage box and clean it as described above.
3) Clean inside and outside of the puncher unit with a moist cloth to remove lint and debris from the unit.

Appendix 5B

4) Install the new punch head back onto the puncher. It is imperative that gloves be worn while performing maintenance on the puncher.

3. Maintenance Charts

Refer to Automated Dissociated Enhanced Lanthanide Fluoro-Immunoassay System (AutoDELFIA) for the determination of Thyrotropin (TSH), Version 4.7 . From **Equipment**, select either DBS Puncher or Quadra.

VIII. Calculations-NA

IX. Reporting Results-NA

X. Procedure Notes

If there is only one adequate bloodspot, enter a comment into the comment window when punching the bloodspot.

XI. Limitation of Procedure-NA

XII. References

A. Apricot Designs, Total Pipetting Solution, TPS384 Series and TPS96 Series, GUI Software Operation Manual, Ver. 2.03, 2011.

B. DBS PUNCHER Instrument Manual, EG&G WALLAC, July 1997.

Conversion of mass of water to volume of water at selected temperatures

Appendix 5B

	Mass	Volume
°C	**g/cm^3 ***	**g/mL ****
18.0	0.9985986	0.9986266
18.5	0.9985034	0.9985314
19.0	0.9984082	0.9984362
19.5	0.9983077	0.9983356
20.0	0.9982071	0.9982350
20.5	0.9981013	0.9981292
21.0	0.9979955	0.9980234
21.5	0.9978845	0.9979124
22.0	0.9977735	0.9978014
22.5	0.9976575	0.9976854
23.0	0.9975415	0.9975694
23.5	0.9974205	0.9974484
24.0	0.9972995	0.9973274
24.5	0.9971737	0.9972016
25.0	0.9970479	0.9970758
25.5	0.9969173	0.9969452
26.0	0.9967867	0.9968146
26.5	0.9966515	0.9966794
27.0	0.9965162	0.9965441
27.5	0.9963764	0.9964042
28.0	0.9962365	0.9962644

* density of water (CRC Handbook of Chemistry and Physics)

** conversion of cm^3 to mL (CRC Standard Mathematical Tables, 1.000028 cm^3/mL)

Appendix 5B

Who You Gonna Call?

For repair help on the OCR workstation, optical scanner, SIS computers, monitors, keyboards, connecting to the internet and generally all demographic data entry hardware and software problems

Follow this procedure

GDB is responsible for the OCR scanner, optical character recognition software, all data entry and editing software, transfer of data to and from GDB. If problems with any of the above items occur you should call the GDB Help Desk.

PerkinElmer is responsible for computer terminals, including the CPUs, monitors, keyboards and mice. If you have an obvious hardware problem i.e. the problem is isolated to only one computer, you can call the PerkinElmer's Proxy directly on your own to request service by PerkinElmer.

If you are not sure call the **GDB Help Desk** and let them decide if PerkinElmer needs to be called. GDB may request that you to call **Fujitsu** with whom GDB has a contract for servicing the scanner. GDB is supposed to contact PerkinElmer themselves for software installations, requests to turn on log files and items they cannot perform remotely. If there are problems related to connecting to the network, (so called "connectivity" problems), PerkinElmer can check their equipment very quickly by remotely connecting into your lab. You may then be asked by PerkinElmer to call GDB back for final resolution. Unfortunately this could entail calling GDB, then PerkinElmer, and then GDB again to finally resolve a problem.

Important Numbers

GDB Help Desk	1-510-307-8928
Fujitsu	1-866-3573788
Proxy	1-408-4560171

Appendix 5B

PerkinElmer™
life sciences.

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.

IMPORTANT → ***** 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 AutoDELFIA Plate Processor & PC	501	MM1 Q	MM1 Quattro Micro Mass Spec
152	AD2 P	AD2 PNS AutoDELFIA Plate Processor & PC	502	MM2 Q	MM2 Quattro Micro Mass Spec
153	AD3 P	AD3 NBS AutoDELFIA Plate Processor & PC	503	MM3 Q	MM3 Quattro Micro Mass Spec
154	AD4 P	AD4 NBS AutoDELFIA Plate Processor & PC	511	MM1 S	MM1 2777 AutoSampler
155	AD5 P	AD5 PNS AutoDELFIA Plate Processor & PC	512	MM2 S	MM2 2777 AutoSampler
156	AD6 P	AD6 PNS AutoDELFIA Plate Processor & PC	513	MM3 S	MM3 2777 AutoSampler
157	AD7 P	AD7 NBS AutoDELFIA Plate Processor & PC	521	MM1 P	MM1 Waters HPLC Pump
171	AD1 S	AD1 PNS AutoDELFIA Sample Processor	522	MM2 P	MM2 Waters HPLC Pump
172	AD2 S	AD2 PNS AutoDELFIA Sample Processor	523	MM3 P	MM3 Waters HPLC Pump
173	AD3 S	AD3 NBS AutoDELFIA Sample Processor	531	MM1 PC	MM1 Waters PC
174	AD4 S	AD4 NBS AutoDELFIA Sample Processor	532	MM2 PC	MM2 Waters PC
175	AD5 S	AD5 PNS AutoDELFIA Sample Processor	533	MM3 PC	MM3 Waters PC
176	AD6 S	AD6 PNS AutoDELFIA Sample Processor	561	PK1	MM1 PEAK N2 Generator & Argon Gas
177	AD7 S	AD7 NBS AutoDELFIA Sample Processor	562	PK2	MM2 PEAK N2 Generator & Argon Gas
201	CH1	Chrontrol Timer or Deep Well Plate Shakers	563	PK3	MM3 PEAK N2 Generator - Argon Gas
202	CH2	Chrontrol 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 5B

Prepared By: _____ Date: _____
Stephen Singh

Reviewed By: _____ Date: _____
Faezeh Samimi

Approved By: _____ Date: _____
George Helmer, Ph.D.

Appendix 5B

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: Specimen Handling, the Processing of Blood Spots using Specimen Gate, Tracking #CN003, Version 7.1

Revised by: Stephen Singh Date: February 2013

Sections	Page # of edited protocol	Deletion	Revision	Add-on
VII.D.1.b.	13		X	

Appendix 5B

Technical Performance Verification

Procedure: **Specimen Handling, the Processing of Blood Spots Using Specimen Gate**
Tracking No. CN 003 Version: 7.1

Employee's Signature[1]	Supervisor's Signature[2]	Completion Date of Training

1. Employee's Signature signifies that the employee feels he/she is confident in performing the procedure.
2. Supervisor's Signature signifies that department supervisor is satisfied with employee's competence to perform the procedure.

Appendix 5B

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: Specimen Handling, the Processing of Blood Spots Using Specimen Gate
Tracking number: CN 003, Version 7.1

Date	Supervi-sor's signature	Proce-dure Version	Re-view	Up-date	New Meth-od	Laboratory Director's signature	Date

Appendix 5B