

Appendix 5E

**MS/MS Newborn Screening using NeoBase[®]
Supervisor's Daily Review and Release
Tracking Number: CN 008
Version: 2.4**

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I. Title**MS/MS Newborn Screening using NeoBase[®] Supervisor's Daily Review and Release****II. Principle**

At the completion of each testing day, the supervisor at the screening laboratory must review and release the data from that day. Data files from the Quattro Micro are downloaded to the PC. The data files are put through Specimen Gate, the QC software, to calculate results and score controls for determination of the run/tray/result status. Software provides QC plots, trend plots, specimen testing history, repeat list, and presumptive positive list. Security is maintained with different access levels to the data base. Staff with security level for read only can view the run data using the procedures below but cannot make any changes to the data. NAPS supervisor and any qualified CLS who is trained and authorized by the NAPS supervisor are allow to review and release results.

III. Specimen and Collection Type

Not applicable

IV. Equipment and Supplies

QA Workstations/Network Server

Laser Printer

Photocopier

Fax

Pens

Stapler

MS/MS Reference Materials

V. Reagents

Not applicable

VI. Calibration and Quality Control

Not applicable

VII. Procedures**A. REVIEW AND RELEASE****1. INSTRUMENT ACCESS**

- a. Use Ctrl. Alt. Delete to login.
- b. Enter user name and password.
- c. Supervisors performing review and release must log on at Level 2.

2. REVIEW OF GENERAL INFORMATION ON MS/MS CHECKLIST

- a. Obtain MS/MS checklist.

- b. Verify that the entries, i.e. tray ID, test date, instrument ID and kit lot # are correct on the checklist. If there is an error or omission, correct the entries by using red ink. Fill out the date and time on the checklist when the data is transferred to GDL prior to faxing it.
- c. If there are samples other than patients and/or CDC Proficiency samples on the tray, write comment on the checklist and also note in the system, under Assay Status, the sample types, e.g., “PT, Linearity, Peer Testing, etc...samples on this tray”.
- d. If there are special samples on the tray, enter comment: “Special sample on this tray” under Assay Status.

3. SELECTING A RUN/TRAY

- a. Click on Routine Programs (NBS-MSMS)
- b. Click on Result Viewer
- c. On the left of the Result Viewer main menu screen are icons for the different screens available under Work Flow.
- d. **Punched** button lists the trays that have been punched at the DBS puncher workstation but have not yet been processed.
- e. **AtWorklist** button lists trays that are in the process of being run.
- f. **Measured** button lists trays that have been run and need to be processed by the QC software.
- g. **NAPS Review** button has trays to be reviewed by the NAPS labs.
- h. To select MSMS tray to review, click on the dropdown arrow by Assay and select AAAC.

4. REVIEW OF A RUN/TRAY

- a. Click on NAPS Review under the Work Flow on the left side of the screen.
- b. Match the tray IDs of the trays to be reviewed on the checklist with the tray ID's listed on the PC screen.
- c. Select a tray to review by highlighting that tray on the list and click Open at the top left of the screen or just double click on the tray.

Note: If there is a tray to be reviewed on the Checklist but cannot find it on the PC screen, follow the “Phone the Proxy” instructions and fill out a Troubleshooting log. File checklist in the Not Transmitted folder.

- d. Once the tray screen has been opened, Result screen appears with following information under Plate Map:
 - 1) Assay number, Dialog box for comments, Test, Date, Instrument, Kit Lot, and Status.
 - 2) Plate information, tray barcode ID, and Dialog box.
 - 3) Well position, sample barcode ID, Dialog box, Sample, Concentration, Phase, and Determination.
- e. Match the barcode ID under the Plate section with the tray barcode on the checklist.

- f. The wells on the tray can be shown in green, yellow, red and blue. Below is a tabulation of what each color stands for:

Wells Color	Quality Controls	Internal Standard (IS) for well #3	Patient Samples
GREEN	QC results are within acceptable limits.	IS Intensity is within acceptable limits.	Results are normal.
YELLOW	N/A	IS Intensity is below acceptable limits for > 5 analytes.	More than 5 patient medians are outside of acceptable limits.
RED	QC results outside of $\pm 3SD$ and/or 2 of the same QC levels outside of $\pm 2SD$.	N/A	Results are below or above the QC ranges.
BLUE	N/A	N/A	Results are presumptive Positives, Out of Range, or Review.

- g. Verify that each control and internal standard is in the correct designated well positions by clicking on the Well Color field above the plate map and selecting Sample Role. To view the type of sample each color represents, click on the "...” button.
- h. In the Well Color field, select Severity to review the tray.
- i. Any well that has a flag and/or comment will have an "X" displayed on the well.
- j. In the Well Color field, select Determination to view presumptive Positives, Out-of-Range, and/or Review. These wells are blue in color.

5. REVIEW OF ASSAY STATUS

- Review the comments on the laboratory checklist.
- Review the general Assay information by clicking on View on the toolbar.
- Select Assay Parameters.
- Should see Blood Spot Volume = 3, Internal Standard Volume = 100, Instrument Software Version = 4.0 (Masslynx), Processing Software Version = 4.0 (Neolynx).

6. REVIEW OF TRAY STATUS

- Review the shape** and intensity of the total ion concentration (TIC) spectrum for each well. Compare the shape with the reference TIC's. Write comments for unacceptable TIC shape and/or intensity. To view the TICs, click on the All TIC's button at the top of the screen. Use the close button (X) in the upper right corner of the screen to close the TIC screen. Write appropriate comments if needed.
- Review** intensity of TIC spectrum of well # 3. TIC intensity of the well #3 internal standard must be $\geq 1.0 \text{ e}^6$. Write comment if it does not meet the acceptable criteria.

- c. **Review** intensity of TIC spectrum for controls and patient samples. TIC intensity must be $\geq 5.0 \times 10^5$. Write comment if any TIC does not meet the acceptable range.
- d. **Review the QC summary** to determine if QC violations, patient median violations and Internal Standard intensity violations are the same as listed under the tray status.
 - 1) Click the QC icon on the left side of the screen.
 - 2) At the top of the screen, select the type of Groups and Analytes to view from the drop down boxes.
 - 3) Click the QC Summary button at the top of the screen.
- e. **Review of QC Plots** Identify shifts and drifts for the analytes. Write the appropriate comments as needed.
 - 1) Click the QC icon on the left side of the screen.
 - 2) At the top of the screen, select the type of Groups and Analytes to view from the drop down boxes.
 - 3) Click the LJ (Levey-Jennings) Plot or Histogram button at the top of the screen.
- f. **Review of QC Violations**

Review the flagged analytes listed under the Plate comment section for QC violations (± 2 S.D. and ± 3 SD). For ≤ 8 QC violations, tray is scored green, for 9 -12 QC violations, tray is scored yellow and >12 QC violations, tray is scored is red.
- g. **Review of Patient Medians**

Review Patient Medians. Patient median violations are displayed under the Plate comment section. The tray status is yellow when > 5 analytes with patient medians are outside the acceptable limits.
- h. **Review of Internal Standards intensity**

Review Internal Standard intensities for well #3. Internal standard violations are displayed under the Plate comment section. The tray status is yellow when >5 internal standards are outside the acceptable limits.

7. SPECIMEN GATE SCORING OF RUN (ASSAY) STATUS

Run status will always be scored GREEN as it contains only the general information.

8. SUPERVISOR'S SCORING OF RUN (ASSAY) STATUS

Turn the run (assay) status yellow if there is any change in the general Information (except test date and analyst).

9. SPECIMEN GATE SCORING OF TRAY (PLATE) STATUS

- a. Release (Green)
 - 1) Total analyte QC violations are ≤ 8 .
 - 2) Total AA/AC IS intensities violations for well #3 are ≤ 5 .
 - 3) Total patient medians violations are ≤ 5 .
 - 4) The Patients results are normal.

- b. Hold (Yellow)
 - 1) Total analyte QC violations are >8 and ≤ 12 .
 - 2) Total AA/AC IS intensity violations for well #3 are > 5 .
 - 3) Total patient median violations are > 5 .
- c. Prevent (Red)
 - a) Total analyte QC violations are > 12 .

10. SUPERVISOR'S SCORING OF TRAY (PLATE) STATUS

a. Release (Green)

Same as Specimen Gate scoring. The supervisor may release a tray when:

- QC violations are ≤ 8 ,
- Well #3 Internal Standard has ≤ 5 intensities violation, and
- Patient median violations are ≤ 5 .

Add comment(s) indicating the reason(s) for release.

b. Hold (Yellow)

Same as Specimen Gate scoring:

- If there are > 8 but ≤ 12 QC violations.
- Well #3 has more than 5 analyte IS intensities outside of acceptable limits.
- The MRM TIC intensity for well # 3 is $< 1.0 \times 10^6$

Supervisor must also hold the tray if:

- (1) Intensity or shape of the TIC's for >10 patient wells is unacceptable.
- (2) Unable to judge the validity of the tray data.

Note: The NAPS supervisor may prevent the samples with unacceptable TIC's and release the tray if the tray data are otherwise acceptable.

c. Prevent (Red)

- 1) Same as Specimen Gate scoring: When there are >12 QC violations.
- 2) If total of 15 or more (≥ 15) patient wells are blue due to Presumptive Positive, Review, and/or Out-of-Range for one or more analytes anywhere on the tray. Do not include Cit/Arg ratio flags.

Note: When supervisor overrides the Specimen Gate scoring, he/she must enter a comment indicating the reason(s) for the change.

If tray status is changed by the supervisor to Hold or Prevent, the assay status also needs to be changed to the same status as the tray.

11. REVIEW OF INDIVIDUAL PATIENT RESULTS

- a. Review the flagged Patient wells by click on the Well Color field above the plate map. Select Determination. The Patient wells with flagged analytes are blue.
- b. Click on each blue color patient well. The flagged analyte(s) is/are listed under Details of Result for that well along with the status of the analyte(s) (e.g. Elevated or Low - outside of the analyte cutoff.)

NOTE: If consecutive blue colored patient wells have same analytes and/or disorder(s) flagged, enter comment at these wells level whether or not they are related, e.g., siblings, twin A, B, or C, etc. This will assure reviewer/releaser at GDL that these wells were not accidentally punched repeatedly from the same patient card.
- c. Flagged analyte(s) can also be viewed under Grid view.
 - a) At the top of the screen, click on the Grid button. The Grid screen is a table of all the analytes and their respective concentrations and ratio values.
 - b) Flagged values are displayed in blue.
- d. Review the shape of the MRM TIC and the intensity of each flagged analyte. The shape of the TIC must be acceptable and the TIC intensity must meet GDL criteria ($\geq 5.0 \text{ e}5$). Score the specimen yellow and write comments if any MRM TIC does not meet the acceptable criteria.

12. SPECIMEN GATE SCORING OF INDIVIDUAL PATIENT SPECIMENS

- a. Release:
 - Analyte concentrations are within cutoff levels. Refer to page 14 and 15.
- b. Hold:
 - Patient median of any analyte is outside acceptable limits.
- c. Prevent:
 - Analyte concentration is outside of the linearity ranges (below or above the ranges).

Note: If a specimen has an analyte result outside of the linearity range, prevent and repeat the specimen on the next run of the same day or the next day whichever available first. When supervisor reviews the tray, enter comment whether initial result is consistent or inconsistent with repeat result for that specimen. If the results are inconsistent on repeat, hold the specimen for GDL review.

13. SUPERVISOR'S SCORING OF INDIVIDUAL PATIENT SPECIMENS

- a. Release (Green)

Same as Specimen Gate scoring, except that the supervisor must make sure that the shape of the MRM TIC of the patient well is acceptable and

the intensity of the TIC must meet the minimum criteria established by GDL.

b. Hold (Yellow)

- 1) Same as Specimen Gate scoring.
- 2) IS intensity of an analyte is below acceptable limits.
- 3) Unable to judge the validity of the data.

c. Prevent (Red)

Same as Specimen Gate scoring in addition to the following:

- 1) Shape of the MRM TIC spectra is unacceptable.
- 2) MRM TIC intensity is $< 5.0 \times 10^5$.
- 3) **Four or more analytes** (ratios not included) in the same patient well are above the cutoff limits.

Note: *According to August 10, 2010 memo:* "A specimen flagged as overall positive and has multiple analytes elevation should be released if at least one of the secondary analyte is elevated for the same disease".

- 4) Four or more random initial patient sample wells on a tray are blue (in Determination status) as a result of having disorder(s) flagged as **Positive** (presumptive positive) and with **Overall = Positive, Urgent or Review** that identified by Specimen Gate.
- 5) QC failed for the initial result of an analyte that is above or below the cutoff. But if QC fails again for the repeat analyte of that specimen whether the result is above or below the cutoff, change the color to yellow and send to GDL for review.
- 6) No peaks for MRM-1, MRM-2 and/or MRM-3 spectra.
- 7) Flagged analyte has QC violations at $\pm 3SD$ or two of the same QC level at $\pm 2SD$.

Note:

- i. **If a Positive patient specimen is Held and sent to GDL for review, do not report to the coordinator until instructed by GDL.**
- ii. "Out-of-Range" does not represent any disorder.
- iii. Do not report/call "Review" cases.

14. PRINT TIC SPECTRA

- a. Double-click on the well.
- b. Select the TIC spectra from the spectra drop-down menu.
- c. Click on the Print button.
- d. Close window.

15. PRINT OF A TRAY

- a) Print the Plate map or any desired Well data:
 - 1) Click File
 - 2) Print Current View
- b. Print Grid (Only upon Request); not recommended

- 1) Click File
- 2) Print Current View
- c. Print Assay (Only upon Request); not recommended
 - 1) Click File
 - 2) Print assay
- d. Print Spectra i.e. TIC, MRM-1, MRM-2, MRM-3
 - 1) Click on the desired well
 - 2) Click on Spectra button on the top of the Screen
 - 3) Press (**Ctrl+Shift+P**)
- e. Print History
 - 1) Click on a desired well
 - 2) Click on History button on the top of the screen
 - 3) Click print

16. REPEATS

- a. Repeat of a Tray
 - 1) The failed tray is repeated on the same day or at the beginning of the next day, whichever is suitable.
 - 2) Print repeat list each morning from Supervisor's PC.
 - 3) Identify a repeat tray on the MS/MS checklist. When in review, add comment indicating that this is a repeat tray of failed tray ID #.
 - 4) Repeat tray is analyzed, reviewed and released in the same manner as the initial tray.
- b. Repeat of Patient Samples
 - 1) A prevented sample is repeated on the next tray of the same day or the first tray of the next day, and is placed right after CT1, before the new samples. When review, click on the History icon to compare initial and repeat result. Compare current flagged analyte(s) and/or disorder(s) to initial result, if any. If repeated result is inconsistent with initial result (except where analytical problems make the initial result completely invalid), enter comment "Repeat result inconsistent" in the well comment field. If repeat result concurs with previous result, enter comment "Repeat result consistent". Unless repeat result is suspected to be unreliable, it should not be prevented when it does not have the exact same analyte(s) and/or disease(s) flagged. Change valid repeat result status to "Held" for GDL to evaluate when in doubt.
 - 2) If a sample is prevented due to concentration of one or more analytes is beyond analytical range, repeat the sample as you would like any other prevented sample. Do not do dilution.

17. PRINT REPEAT LIST

- a. Bottom left of the Result Viewer main menu screen is the Special menu.
- b. Select the Special menu box

- c. Click Request List icon at the top of the screen.
- d. Select AAAC under the Test drop down menu.
- e. Select the Request Type drop down menu and choose Retest-All.
- f. Select File from the toolbar at the top of the screen.
- g. Click Print to print the request list.
- h. Click Exit.

18. RESTORE RUN DATA

If a tray is reviewed and released, the supervisor can restore the tray prior to sending it to GDL.

- a. Click on the QA Review icon on the left side of the main MS/MS review screen.
- b. Identify and open the tray to be restored.
- c. Click File on the toolbar.
- d. Select Restore Assay to NAPS Review. The tray will then disappear from the QA Review level and appear under NAPS Review.

19. VIEW ARCHIVE RUNS

- a. On the left of the Result Viewer main menu screen under the Workflow menu, click the QA Released Icon.
- b. All completed trays will be listed.

20. BACK UP PROCEDURES

Not applicable

21. TRANSFER DATA to GDL

- a. On the Specimen Gate main menu screen, select the Transfer to GDL option.
- b. Select Transfer NBS & MS/MS data to transfer the data to GDL the same day.

VIII. Calculations

Not applicable

IX. Reporting Results to ASC

- A. Identify the ASC to call to report the positives or “Panic Value” identified by the Specimen Gate as:
 1. “Overall Positive” either for (AA or AC) or both
 2. Analyte is marked as Urgent either (AA or AC)
 3. Analytes is marked as Positive

4. Analyte value out of linearity range is termed as “Panic Value”. Inform ASC that the value of the analyte is out of range and upon repeat result will be reported.
- B. Do not report “Review” and “Out of Range.”
 - C. Report results in $\mu\text{mol/L}$ for all analytes.
 - D. Follow the newborn screening Accession and Reporting at the NAPS Lab protocol for reporting presumptive positives.
 1. Review MS/MS trays at the Supervisor review and release PC.
 2. Right Click on the positive well.
 3. Click on “**Presumptive Positive Report**” tab.
 4. Click on the Printer Icon for printing the result.
 5. Call the area coordinator to report positive along with the disorder and concentration of elevated analytes.
 6. Complete the Confirmation of Contact (C of C) in SIS.
 7. Write the reporting information on the MS/MS Presumptive Positive log.
 8. Fax the positive specimen print out (containing the disorder and analyte concentrations) along with a copy of TRF to the coordinator.

Note: If a sample is positive for only one group (AA or AC) of analytes and not for the other, report out disease related to that group of analyte/s only.

X. Procedure Notes:**A. Analytical Linearity Range of Amino Acids, Succinylacetone
and Acylcarnitines in Dried Blood Spot**

Analyte	Linearity Range (μM)
Alanine	4.5- 4090
Arginine	0.6 - 3721
Citrulline	4.8 - 1683
Glycine	50.4 - 4487
Leucine	1.3 - 2545
Methionine	2.5 - 1185
Ornithine	0.6 - 3771
Phenylalanine	0.3 - 2341
Proline	4.7 - 3659
Succinylacetone	0.24 - 158.1
Tyrosine	1.2 - 2816
Valine	0.6 - 2358
C0	0.2 - 2274
C2	0.2 - 735
C3	0.03 - 88
C4	0.07 - 59.81
C5	0.04 - 59.1
C5DC	0.08 - 28.85
C6	0.08 - 61.54
C8	0.02 - 35.2
C10	0.04 - 28.86
C12	0.04 - 42.74
C14	0.02 - 41.81
C16	0.1 - 107.3
C18	0.04 - 32

B. Disorders Detected by MS/MS and their Biochemical Markers

Disorder	Abbreviation	Primary Analyte Elevation	Secondary Analyte Elevation	Urgent Disorder
CPT-II, CAT Deficiency	CPT-II	C16	C18, C18:1	
LCHADD, TFP Deficiency	LCHADD	C16OH, C16OH/C16, C18OH and C18:1OH	C14OH, C16	YES
VLCADD	VLCADD	C14:1	C14	YES
MCADD	MCADD	C8	C6,C10,C10:1	YES
IVA, 2MBCDD	IVA	C5[not C4] and C5/C3		YES
3MCC, MGA, HMG	3MCC	C5OH (not C5:1)		
BKT, 2MO3OHBCDD	BKT	C5OH	C5:1	
GA-I	GA-I	C5DC		YES
GA-II, EE	GA-II	C4, C5	C6, C8, C8:1, C10, C10:1, C12, C12:1, C14, C16, C16:1, C18, C18:1, C18:2	YES
SCADD, IBCDD	SCADD	C4[not C5]		
MCD	MCD	C3 and C3/C2 or C5OH		YES
PA/MMA	PA/MMA	C3 and C3/C2		YES
Malonic Aciduria	MAL	C3DC, C3DC/C10 and C5DC/C3DC (Low cutoff)		YES
Carnitine Transporter Deficiency	CTD	C0 ≤ Low cut off C2 ≤ Low cut off		
CPT1	CPT1	C0/(C16 + C18:1)		

(Continued...)

Disorder	Abbreviation	Primary Analyte Elevation	Secondary Analyte Elevation	Urgent Disorder
Maple Syrup Urine Disease	MSUD	Leu , Leu/Ala and Val/Phe		YES
Citrullinemia (I/II) ASL Deficiency	CIT	Cit and Cit/Arg		YES
Argininemia	ARG	Arg and Arg/Orn		
HHH Syndrome Gyrtae atrophy, Hyperornithemia w/ Gyrate atrophy	HHH	Orn	Orn/Cit	
5- Oxoprolinemia	Oxopro	5- Oxopro		
Hyperprolinemia	Hypro	Pro		
Homocystinuria	HCY	Met		
Non-Ketotic Hyperglycinemia	NKH	Gly		
Phenylketonuria	PKU	Phe, Phe/Tyr		YES
Tyrosinemia	TYR	Tyrosine		
Tyrosinemia type I	TYRO I	Succinylacetone (SA)		YES

C. MS/MS Cutoff for Free and Acylcarnitines

Analyte	Type of Analyte	Cutoff	Cutoff Type
C0	AC	7.0	Low
C0	AC	125	High
C2	AC	11	Low
C2	AC	80	High
C3	AC	6.3	High
C3DC	AC	0.38	High
C4	AC	1.7	High
C5	AC	1.0	High
C5:1	AC	0.6	High
C5OH	AC	0.9	High
C5DC	AC	0.6	High
C6	AC	0.95	High
C8	AC	0.6	High
C8:1	AC	0.7	High
C10	AC	0.65	High
C10:1	AC	0.45	High
C12	AC	2.0	High
C14	AC	1.2	High
C14:1	AC	0.8	High
C14OH	AC	0.2	High
C16	AC	10	High
C16:1	AC	1.4	High
C16OH	AC	0.10	High
C18	AC	4	High
C18:1	AC	7	High
C18OH	AC	0.10	High
C18:1OH	AC	0.10	High
C3/C2	AC Ratio	0.3	High
C0/(C16+C18:1)	AC Ratio	75	High
C16OH/C16	AC Ratio	0.07	High
C3DC/C10	AC Ratio	4.0	High
C5/C3	AC Ratio	0.45	High
C5DC/C3DC	AC Ratio	0.70	Low

D. MS/MS Cutoff for Amino acids and Succinylacetone

Analyte	Type of Analyte	Cutoff	Cutoff Type
Alanine	AA	1000	High
Arginine	AA	50	High
Citrulline	AA	60	High
Citrulline	AA	5	Low
Glycine	AA
Leucine/Isoleucine	AA	250	High
Methionine	AA	100	High
Methionine	AA	8	Low
Ornithine	AA	800	High
Oxoproline	AA	...	
Phenylalanine	AA	155	High
Proline	AA	1500	High
Tyrosine	AA	850	High
Valine	AA
Arg/Orn	AA Ratio	1.4	High
Cit/Arg	AA Ratio	6.0	High
Phe/Tyr	AA Ratio	1.5	High
Leu/Ala	AA Ratio	1.3	High
Val/Phe	AA Ratio	3.5	High
Succinylacetone	Ketone	4.5	High

E. Masses of Biochemical Markers and their Internal Standards

Function	Primary Markers in Blood		Internal Standards	
	Analyte Name	Analyte Mass (AMU)	Internal Standard Name	IS Mass (AMU)
MRM -1				
	Alanine	90.0	D ₄ -Alanine	94
	Arginine	175.1	D ₄ , ¹³ C-Arginine	180.1
	Citrulline	176.1	D ₂ -Citrulline	178.1
	Glycine	76.0	¹⁵ N, 2- ¹³ C-Glycine	78.0
	Isoleucine	132.1	D ₃ -Leucine	135.1
	Leucine	132.1	D ₃ -Leucine	135.1
	Methionine	150.1	D ₃ -Methionine	153.1
	Ornithine	133.1	D ₆ -Ornithine	139.1
	Phenylalanine	166.1	¹³ C ₆ -Phenylalanine	172.1
	Proline	116.1	¹³ C ₅ -Proline	121.1
	Tyrosine	182.1	¹³ C ₆ -Tyrosine	188.1
	Valine	118.1	D ₈ -Valine	126.1
	Succinylacetone (SA)	155.1	¹³ C ₅ -Methylpyrazolyl-propanoic acid (MMP)	160.1
MRM -2				
	C0	162.1	D ₉ -C0	171.1
	C2	204.1	D ₃ -C2	207.1
	C3	218.1	D ₃ -C3	221.1
	C3DC	248.1	D ₃ -C4	235.2
	C4	232.2	D ₃ -C4	235.2
	C4OH	248.1	D ₃ -C4	235.2
	C4DC	262.2	D ₉ -C5	255.2
	C5	246.2	D ₉ -C5	255.2
	C5:1	244.2	D ₉ -C5	255.2
	C5OH	262.2	D ₉ -C5	255.2
	C5DC	276.1	D ₆ -C5DC	282.1
	C6	260.2	D ₃ -C6	263.2
	C6DC	290.2	D ₆ -C5DC	282.1
	C8	288.2	D ₃ -C8	291.2
	C8:1	286.2	D ₃ -C8	291.2

MRM -3				
	C10	316.2	D ₃ -C10	319.2
	C10:1	314.2	D ₃ -C10	319.2
	C10:2	312.2	D ₃ -C10	319.2
	C12	344.3	D ₃ -C12	347.3
	C12:1	342.3	D ₃ -C12	347.3
	C14	372.3	D ₃ -C14	375.3
	C14:1	370.3	D ₃ -C14	375.3
	C14:2	368.3	D ₃ -C14	375.3
	C14OH	388.3	D ₃ -C14	375.3
	C16	400.3	D ₃ -C16	403.3
	C16:1	398.3	D ₃ -C16	403.3
	C16OH	416.3	D ₃ -C16	403.3
	C16:1OH	414.3	D ₃ -C16	403.3
	C18	428.4	D ₃ -C18	431.4
	C18:1	426.4	D ₃ -C18	431.4
	C18:2	424.3	D ₃ -C18	431.4
	C18OH	444.4	D ₃ -C18	431.4
	C18:1OH	442.4	D ₃ -C18	431.4

XII. Limitations of Procedure Notes

Not applicable

XIII. References

- A. NBS Supervisor's Daily Review and Release Protocol, Tracking number: CN007
- B. Tandem Mass Spectrometry Method for the Analysis of Amino Acids, Succinylacetone & Acylcarnitines Protocol, Tracking number: CN09.

Prepared by: _____ Date: _____
Trang Bui, CLS

Reviewed by: _____ Date: _____
Partha Neogi, Ph.D.
Research Scientist IV

Reviewed by: _____ Date: _____
CLIA Laboratory Director, M.D.

Approved by: _____ Date: _____
George Helmer, Ph.D.
Acting Chief
Genetic Disease Laboratory Branch

