

Appendix 16
Required Validation Activities
NBS/PNS/MSMS

PROTOCOL FOR NEWBORN- AUTODELFIA SYSTEM VALIDATION

AutoDELFIA for TSH and 17-OHP (new instrument validation)

I. Barcoding

Use cards numbered 1001–1036 for the pools and the current TQC for TC.

II. Punching and eluting

(Make sure that PerkinElmer has certified the DBS puncher.)

- A. Punch off-center so that additional punches may be made for repeats, if necessary.
- B. Punch as you would for a screening run with Standards, SQC, TQC, PQC, etc., in their designated wells.
- C. Punch the samples in the following order:
B, G, A, TC, H, P, N; repeating until 12 cards of each pool has been punched. (This will give you 2 trays to test.) Use current TQC for TC.

Day 1: punch cards 1001 – 1012

Day 2: punch cards 1013 – 1024

Day 3: punch cards 1025 – 1036

- D. Return strips to the refrigerator immediately after punching.

III. Testing

- A. Set up the New AutoDELFIA in the **TRAINING MODE** and test according to protocol.
- B. Review and Release according to protocol.
Mark the checklist as a **VALIDATION RUN**.
- C. Calculate mean, SD and %CV for each blood spot pool including the TC pool with the TQC results.
- D. Enter the results into the comment section of the checklist.

IV. Repeat II and III for Day 2 and Day 3

- A. Punch samples from the cards for each day as designated above.
- B. At the end of Day 3, calculate the combined (all 36 results) mean, SD and %CV for each pool and all TQC results
- C. Enter the results into the comment section of the checklist or you can e-mail your completed excel tables (3 days summary tables for TSH and 17-OHP) to the lead RS IV of the NBS section.

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AutoDELFIA for IRT (new instrument validation)

I. Barcoding

Use cards numbered 1001–1036 provided for the IRT T1 – T5 pools and the current IRT TQC for TC.

II. Punching and eluting

(Make sure that PerkinElmer has certified the DBS puncher.)

- A. Punch off-center so that additional punches may be made for repeats, if necessary.
- B. Punch as you would for a screening run with Standards, SQC, TQC, PQC, etc., in their designated wells.
- C. Punch the samples in the following order:
T1, T2, T3, TC, T4, T5; repeating until 12 spots of each pool has been punched. (This will give you 2 trays to test). Use current IRT TQC for TC.

Day 1: punch cards 1001 – 1012
Day 2: punch cards 1013 – 1024
Day 3: punch cards 1025 – 1036
- D. Return strips to the refrigerator immediately after punching.

III. Testing

- A. Set up the New AutoDelfia in the **TRAINING MODE** and test according to protocol.
- B. Review and Release according to protocol.
Mark the checklist as a **VALIDATION RUN**.
- C. **Calculate** the mean, SD and %CV for each blood spot pool including the TC pool with the TQC results.
- D. Enter the results into the comment section of the checklist.
(You may use a separate sheet.)

IV. Repeat II and III for Day 2 and Day 3

- A. Punch samples from the cards for each day as designated above.
- B. At the end of Day 3, calculate the combined (all 36 results) mean, SD and %CV for each pool and all TQC results

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C. Enter the results into the comment section of the checklist or you can e-mail your completed excel table (3 days summary for IRT excel table) to the lead RS IV of the NBS section (Dr. Chow).

V. GDLB approval to start screening using new system.

Do not start to use this system to do production screening until GDLB completes data analysis and verifies that the new equipment system meets performance criteria for screening. GDLB will call you or send you a written notification of approval.

VI. GDLB contact

Dr. Chow Dr. Ho

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PROTOCOL FOR PNS - AUTODELFIA SYSTEM VALIDATION

Perform system validation for the new AutoDELFIA system for Prenatal Screen Biomarkers screening (AFP, hCG, uE3, HG1, PAPP) assays in your laboratory. System validation takes 6 runs. Each run consists of surrogate reference samples and maternal serum samples. Use the 3 levels of the lyophilized PT samples provided by GDLB for determination of accuracy and precision. They are labeled as Lot PN06 Levels 2, 3 and 6. You will be provided 10 vials for each level. Use the Lot PN 06 Levels 7 and 8 for checking system flags for low and high samples. You will be sent 2 vials each.

Store PT samples in freezer until ready to use.

A. Reagents

Use current lot of reagent kits for AFP, hCG, uE3, HG1 and PAPP.

If needed, GDLB will order extra reagent to be shipped to you.

B. Equipment

Use the newly installed AutoDELFIA.

Do not start validation until Perkin Elmer provides you with documentation to show that the instrument meets all specifications according to manufacturer's specification.

C. Sample preparation for surrogate reference samples

Barcode plastic tubes as follows:

72 tubes, P2001 through P2072 for PT Level 2

72 tubes, P3001 through P3072 for PT Level 3

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72 tubes, P6001 through P6072 for PT Level 6

6 tubes, P7001 through P7006 for PT Level 7

6 tubes, P8001 through P8006 for PT Level 8

Remove the Reference serum controls sent by GDLB from freezer storage. Let it thaw thoroughly and sit for additional 30 minutes and mix gently by inverting the vial. Aliquot 300 uL into each barcoded tube. Once reconstituted, store in refrigerator and use for up to 5 days. Use each tube once.

D. Maternal serum samples

Test maternal serum specimens after you have tested on current approved systems and have passed the QC. Keep the maternal serum sample in the sequence order when you do the validation run. Placing remaining maternal serum samples from prior day not tested at the beginning of the run followed by the 1st sequence of next day samples. This will help GDLB in data analysis. If there is a missing samples due to QNS or any other reason, fill the spaces with water tubes using barcode R9001, R9002, R9003 etc.

E. Analysis

Use Training Mode for system validation runs. Perform PNS testing per protocol. Set up 6 runs with the number of replicates as shown in Table 1 Place one each of P7 and P8 at the beginning of the run before the maternal serum specimens. Place 2 replicates of each level of PT samples, P2, P3, and P6 before and after the maternal serum specimens. See Table 2 and 3 for Example tray map. The last tray does not need to be a complete tray, but should contain at least 10 maternal serum samples.

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F. Supervisor's review

Lab Supervisor must review and release each run following protocol. Use separate checklist for each validation run and sign on checklist. Fax the checklist to GDLB everyday, attention to Dr. Ho or Dr. Sartippour. Indicate that it is validation run on the checklist. If the run passes all the QC criteria for release, proceed to next run. If the run fails QC due to operator error, correct the error and repeat the run. If the run fails QC due to instrument breakdown, analytical bias, or having too many flags, call Perkin Elmer service to correct the problem before proceed to next run. For validation run, you do not need to repeat any single sample to get a valid result.

G. GDLB approval to start screening using new system

Do not start to use this system to do production screening until GDLB completes data analysis and verifies that the new equipment system meets performance criteria for screening. GDLB will call you or send you a written notification of approval.

H. GDLB contact

Dr. Ho Dr. Sartippour

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Table 1 No of replicates of controls and reference samples on each run day

	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
No. of tray	1	2	3	4	4	4
No. of SCL	2	2	2	2	2	2
No. of SCM	2	2	2	2	2	2
No. of SCH	2	2	2	2	2	2
No. TQC	5	7	9	11	11	11
Reference-P2	4	8	12	16	16	16
Reference-P3	4	8	12	16	16	16
Reference-P6	4	8	12	16	16	16
Reference -P7	1	1	1	1	1	1
Reference -P8	1	1	1	1	2	1
Max./ Min No. of Maternal Serum Samples	59 59	137 69	215 147	293 225	293 225	293 225

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Table 2 Example Tray Map for the one tray run , Run No.1

Std A	Std A	Std B	Std B	Std C	Std C	Std D	Std D	Std E	Std E	Std F	Std F
SCL	SCM	SCH	TQC	P7	P8	P2	P2	P3	P3	P6	P6
MS											
MS											
MS											
MS											
MS	P2										
P2	P3	P3	P6	P6	TQC	TQC	TQC	TQC	SCL	SCM	SCL

Table 3 Example Tray Map for the last tray for 2-tray, 3 -tray and 4 -tray runs

Std A	Std A	Std B	Std B	TQC	P2	P2	P3	P3	P6	P6	MS
MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	MS	P2
P2	P3	P3	P6	P6	TQC	TQC	TQC	TQC	SCL	SCM	SCH

The last tray does not need to be a complete tray, but should contain at least 10 maternal serum samples. P7 and P8 are on Tray 1 only, not on subsequent trays.

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Protocol for Validation of Tandem Mass Spectrometer in Newborn Screening

I. Background

PerkinElmer will perform a comprehensive installation qualification (IQ), operational qualification (OQ) and performance qualifications (PQ) on new instrument prior to the testing of patient specimens. Upon completion of IQOQ of California method and loading of Master Project folders, instrument will be validated to check the performance (PQ).

II. Pre-Analysis

- a. Staff should read the MSMS instrument operation protocol "Tandem Mass Spectrometry Method for the Analysis of Amino Acids, Succinylacetone, and Acylcarnitines"
- b. Staff should be able to follow the protocol to run the assay
- c. Staff should be able to review and release the data following GDL protocol

III. Analysis

- a. Protocol:

Following protocol will be followed for the validation of the instrument

Run the MSMS instrument following the plate map below for five days for validation.

10 specimens of Low control
10 specimens of High control
10 specimens of Tray control
10 patient specimens
PT J1 through J6 in duplicate
6 Replicates of PT J3 and J6

Upon satisfactory completion of the study and evaluation of the data instrument will be commissioned for running patient specimens.

- b. Data analysis:

Inter- and Intra- day mean, standard deviation (SD) and coefficient of variation (CV) will be calculated for low, high and tray controls. Patient medians generated from all the runs will be compared. Linearity of the range will be generated using J1-J6 PT samples. Data collected will be analyzed using Microsoft Excel®.

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IV. Discussion

GDL will evaluate the results of the validation run.

V. Recommendations

VI. Corrective Action

VII. GDLB Approval to start screening using New System

Do not start to use the system to do production screening until GDLB completes data analysis and verifies that the new instrument meets performance criteria for screening. GDLB will call you or send a written notification for approval.

VIII. GDLB Contact

Dr. Neogi Dr. Ho

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MS/MS Instrument Validation Plate Map

Instrument Serial No. _____ Kit Lot No. _____

	1	2	3	4	5	6	7	8	9	10	11	12
A	IS1	IS2	IS3	CL	CH	CT	L0001	L0001	L0001	L0001	L0001	L0001
B	L0001	L0001	L0001	L0001	H0001							
C	H0001	H0001	T0001									
D	IS	P0001	P0002	P0003	P0004	P0005	P0006	P0007	P0008	P0009	P0010	J0001
E	CP	J0001	J0002	J0002	J0003	J0003	J0004	J0004	J0005	J0005	J0006	J0006
F	J0003	J0003	J0003	J0003	J0003	J0003	J0006	J0006	J0006	J0006	J0006	J0006
G	CT	CL	CH									
H												

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IS = Internal Standard

CL = Low System Control

CH = High System Control

CT = Tray Control

J0001-J0006 = GDL Proficiency Samples

L0001 = Low System Control

H0001 = High System Control

T0001 = Tray Control

P0001- P0010 = Patient

CP = Proficiency Control (Same as Tray Control)

Protocol for Validation of Prenatal Screen (PNS) DSX system

(DSX Inhibin-A Automated Immunoassay System)

I. INTRODUCTION

System verification fulfills requirements of the Clinical Laboratory Improvement Act of 1988 (CLIA88), and provides confirmation that results from contract laboratories are matched to those of other NAPS laboratories in the California Expanded AFP Screening Program. Matched performance is necessary to apply statewide quality control, cutoff and proficiency test standards. An analytical system for Inhibin-Assay, DSX Inhibin-A Automated Immunoassay system, has been installed in each of the NAPS laboratory. For verification purpose, test results from these systems will be compared to the reference analytical unit at GDLB. The new system is matched if test results obtained on selected specimen pools are within the acceptable limits established at GDLB.

II. METHOD

The system verification for each analytical system is performed on site by DSL staff and NAPS laboratory analysts. DSL staff performed first part of system verification after the installation to confirm DSX instrument is performing within the specifications of DSX and verify correct version of iConsole software have been installed. NAPS laboratory staffs then perform the analysis on the new system after returning from training at GDLB to confirm the analytical results from their new system is equivalent to the analytical results from GDLB's instrument. The procedures outlined here are for the NAPS laboratory analysts. Samples for the test of analytical performance are surrogate maternal serum samples pools spiked with Inhibin-A at various concentration levels covering the entire measurement range. Four replicates of reference sample at each level are tested on each plate. System verification will consists of 4 analytical runs with variable number of plates per run. Perform one to two runs on a day to complete 4 analytical runs. The number of reference samples and maternal serum samples per plate and per run are shown in Table 1

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A. Chemicals and Reagents

DSL 4--plate reagent kit (cat # 10-28100-4) for Inhibin-A is used. Report to GDLB if you have not received the reagent kit after DSL completed installation of DSX system for Inhibin-A in your laboratory.

B. Equipment

The DSX for Inhibin-A in your laboratory must have been checked out by DSL for meeting the specifications for instrument performance. Make sure you have the DSL's performance documentation. For record, fax a copy of the installation document signed by DSL service representative to Dr. Ho at GDLB.

If you have two DSX instruments installed at your lab, it is more efficient to perform system validation on both systems at the same time, using common specimens.

C. Software

There are two software programs for this analytical system. Confirm correct version is installed in your system.

Dynex Technologies Revelation software for control of analytical processes of DSX.

iConsole software for user interfacing to DSX and Supervisor PC software.

D. Samples

Four levels of reference samples. They are labeled as R1, R4, R5, and R6. You will be provided sufficient vials for each level. They must be labeled using reference barcode

System controls, which come with reagent kit are run with calibrator and does not have barcode.

Tray controls (TQC) which are provided by GDLB, are treated as patient samples and must be bar-coded. GDLB will send to you reference samples and TQC frozen via overnight courier. Store them frozen until analysis.

Maternal sera. Use only the maternal serum samples, which you have completed TMS testing to fill the space between the reference samples on each run.

E. Bar-coding

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Preprint barcode for references samples as shown below:

- Reference Sample R1: R1001 through R1040
- Reference Sample R4: R4001 through R4040
- Reference Sample R5: R5001 through R5040
- Reference Sample R6: R6001 through R6040

Preprint barcode for TQC as shown below:

TQC lot 13: T1001 though T1020

Use maternal serum which have been tested for TMS, thus no bar-coding needed

F. Preparing reference sample tubes and TQC for testing

For Day One testing, Thaw 1 vial of each level in the refrigerator the day before. Each vial contains 5 mL. Mix thoroughly by gently inverting the vial. Dispense 400 uL into 12 plastic tubes, 001-012, for each level. Reference samples are stable for one week in refrigerator. Use 4 tubes of reference samples on each plate for each level. Thaw new vial of reference sample as needed. Each vial of TQC contains 5 ml of serum samples. Thaw and dispense 400 uL into 12 plastic tubes with TQC labels T1001-T1012. Thaw new vial as needed.

G. Analysis

Perform the Inhibin testing per protocol.

H. Supervisor's review

Supervisor reviews and releases each run following the protocol. Repeat the run if the run fail QC.

Print worksheet for Run 1 from Tecan and from supervisor station. Verify results are the same. If there is any discrepancy between two printouts, report to GDLB.

Fax checklist to GDLB, attention Dr. Ho, marked: Inhibin Validation Run #X.

I. Data analysis

GDLB will perform data analysis to determine whether or not your system pass system verification criteria for screening maternal serum samples. Once notified by GDLB, you can start using the DSX instrument to report Inhibin results.

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J. GDLB approval to start screening using the new instrument system

Do not start to use this system to do production screening until GDLB completes data analysis and verifies that the new equipment system meets performance criteria for screening. GDLB will call you or send you a written notification of approval.

K. GDLB contact

Dr. Ho Dr. Sartippour

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TABLE 1 Number of Samples per Plate and per Run

Run#	Trays per Run	SCL	SCH	TQC	R-1	R-4	R-5	R-6	TQC & Ref.	MS	Total # of Sample
1	1	1	1	2	4	4	4	4	18	64	82
2	2	2	2	4	8	8	8	8	36	128	164
3	3	3	3	6	12	12	12	12	54	192	246
4	4	4	4	8	16	16	16	16	72	256	328
	10	10	10	20	40	40	40	40		640	

No. of MS samples and TQC samples on 1-plate through 4-plate runs

Plate /run	Maximum number of samples per run	TQCs per run	Maximum of MS samples per run
1	82	2	80
2	164	4	160
3	246	6	240
4	328	8	320

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ATTACHMENT 1

Example: Sample Rack Map for - One plate Run Inhibin-A

	R1	R2	R3	R4	R5	R6
1	TQC	MS	MS	MS	MS	MS
2	R-1	MS	MS	MS	MS	MS
3	R-4	MS	MS	MS	MS	MS#64
4	R-5	MS	MS	MS	MS	R-1
5	R-6	MS	MS	MS	MS	R-4
6	R-1	MS	MS	MS	MS	R-5
7	R-4	MS	MS	MS	MS	R-6
8	R-5	MS	MS	MS	MS	R-1
9	R-6	MS	MS	MS	MS	R-4
10	MS#1	MS	MS	MS	MS	R-5
11	MS	MS	MS	MS	MS	R-6
12	MS	MS	MS	MS	MS	TQC
13	MS	MS	MS	MS	MS	
14	MS	MS	MS	MS	MS	

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Attachment 2: Example plate map for 1 Plate run

Plate 1 Plate Map for Inhibin -A

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std A	Std F	R4	MS	R4							
B	Std A	Std F	R5	MS	R5							
C	Std C	Std G	R6	MS	R6							
D	Std C	Std G	R1	MS	R1							
E	Std D	Low Control	R4	MS	R4							
F	Std D	High Control	R5	MS	R5							
G	Std E	Tray Control	R6	MS #64	R6							
H	Std E	R1	MS #1	MS	R1	Tray Control						

Note: Low & High Control comes with the Inhibin Kit, Tray control are provided by GDLB