Connecticut Newborn Screening: An Overview

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CT Newborn Screening

► CGS 19a-55 mandates screening of all CT newborns for select genetic and metabolic disorders

► The CT State Lab screens for 64 disorders including AA, OA, Urea Cycle, FAO, hemoglobin production, endocrine disorders, autoimmune & peroxisomal disorders

► 37,242 births in 2016

► 99.89% newborns screened

► CF Screening conducted at UCONN and Yale Laboratories

► DPH Family Health Section oversees hearing screening, CCHD screening and birth defect registry

Connecticut NBS Timeline

1964	1979	1983	1995	05/2004	09/2004	11/2004	01/2005	09/2010	10/2011	07/2016
• PKU	• CH • GALT	• MSUD • HCY • BIO • HGB S • HGB SC • Hgb C • Hgb SD • Hgb D • Hgb SE • Hgb E • Hgb Bart's • Hgb Sβ° Thal • Variant Hg	• CAH	• MSUD • HCY • MET • TYR • MCAD • LCHAD • VLCAD • TFP	• CPT1 • GAII • CPTII • CACT • CUD	• PPA • MMA • IVA • HMG • 3MCC • MCD • GA I • ßKT	• ARG • CIT • ASA • OTC • SCAD • DE RED • MMA • HHH* • NKH*	• M/SCAD • IBG • EME • FIGLU • 2MBG • 2M3HBA • 3MGA • CPS • PC • RMD • PHE • BIOPT (REG) • BIOPT (BS)	• SCID • T-Cell Lymph openia	• X-ALD

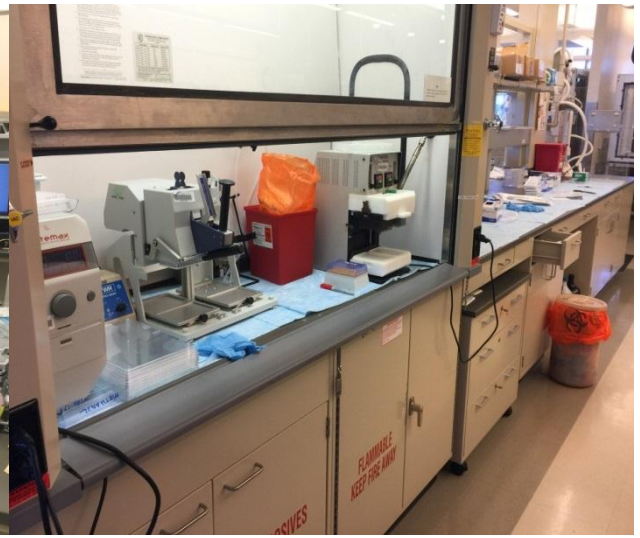
*removed 2016

CT Newborn Screening

LABORATORY

Responsibilities:

- Receipt, login, sample quality evaluation
- Creating worklists, punching of samples into 96-well plates
- Sample preparation
- Instrument maintenance and analysis set-up
- Sample interpretation
- Reporting of sample results



CT Newborn Screening

SHORT TERM FOLLOW-UP AND TRACKING

Responsibilities:

- Using the NBS database, assuring that all infants are screened
- Reporting abnormal results and
  - Requesting a repeat NBS specimen or
  - Referring to a regional diagnostic/treatment center
- Following up through diagnosis or exclusion of a disorder
- Maintaining and reporting of statistics
- Educating stakeholders
- Maintaining and trouble shooting the NBS database
- Collaborating with and supporting hospital and birthing center staff, diagnostic/ treatment center staff, primary care providers and parents

Disease	National incidence*	Results of Untreated Disease
Galactosemia	1 in 7,500 for some form of galactosemia	severe brain damage, developmental disabilities, death
PKU	1 in 17,000	developmental disabilities, seizures
Congenital Hypothyroidism	1 in 4,500	developmental disabilities, poor growth, low metabolic rate
MSUD	1 in 120,000	neonatal acidosis, developmental disabilities, coma, death
Sickle Cell Disease	1 of every 500 African Americans have disease; 12,000 in CT have sickle trait	anemia, septicemia, pneumonia, death
Biotinidase Deficiency	1 in 60,000	seizures, developmental disabilities, vision and hearing loss, skin infections
Homocystinuria	1 in 200,000	thromboembolism, seizures, developmental disabilities, osteoporosis
Ornithine Transcarbamylase Deficiency (OTC)	1 in 30,000	lethargy, coma, seizures, vomiting, poor feeding, hyperventilation
Congenital Adrenal Hyperplasia	1 in 21,500	salt wasting, ambiguous genitalia, dehydration, shock, death
MCADD	1 in 10,000	hypoketotic hypoglycemia, death
IVA	1 in 50,000	hyperammonemia, acidosis, seizures, coma, developmental disabilities, brain damage, lethargy
PPA	1 in 50,000	acidosis, seizures, coma, developmental disabilities, brain damage, lethargy

*All incidences as reported from Save Babies Through Screening Foundation website and www.ureacycle.com

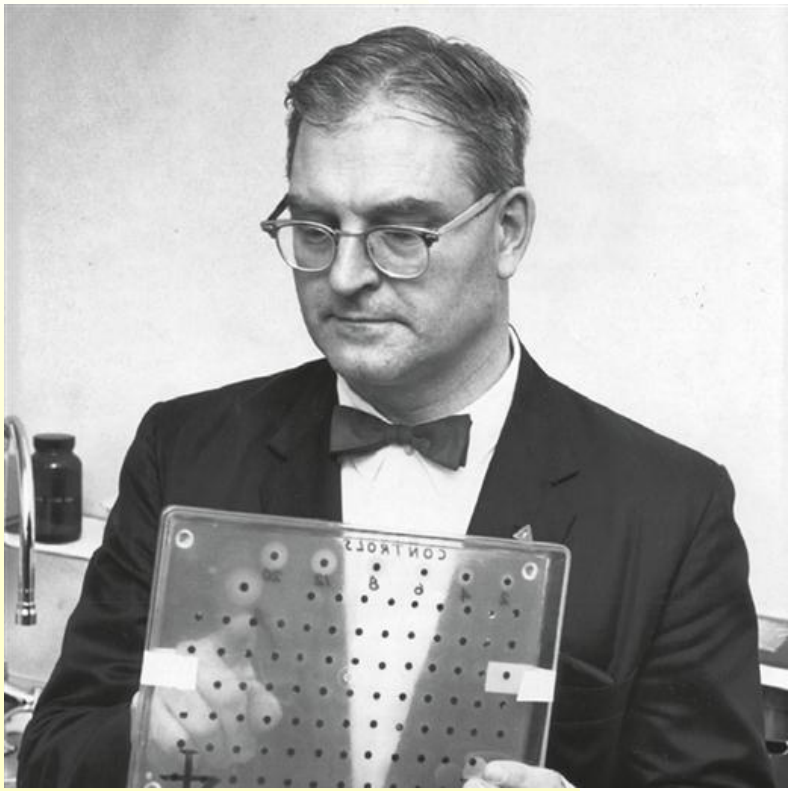
CT Newborn Screening

1964	1979	1983	1995
Phenylketonuria (PKU)	Congenital Hypothyroidism	Maple Syrup Urine Disease (MSUD)	Congenital Adrenal Hyperplasia
	Classical Galactosemia	Homocystinuria	
		Biotinidase Deficiency	
		Hemoglobin S	
		Hemoglobin SC	
		Hemoglobin C	
		Hemoglobin SD	
		Hemoglobin D	
		Hemoglobin SE	
		Hemoglobin E	
		Hemoglobin Bart's	
		Hemoglobin Sβ° Thal	
		Variant Hemoglobins	
		Hemoglobin Traits	
		Hemoglobin AS	
		Hemoglobin AC	
		Hemoglobin AD	
		Hemoglobin AE	
		Hemoglobin AOther	

Seventeen (17) Disorders and Five (5) Traits by 1995

Phenylketonuria (PKU) (1964)

This was the very first universal newborn screening assay used to screen for PKU, a disorder with an incidence rate of about 1:17,000 infants. This disease, if untreated, causes severe developmental disabilities. After decades of research demonstrating that early detection and modification of an infant's diet could reduce the affects of the PKU, Dr. Robert Guthrie developed this test and in the early 1960s this became the first screen to be used for newborn infants for the detection of PKU. The theory was simple: a blood spot was placed on an agar plate that is treated with a bacteria that cannot grow without the presence of Phenylalanine, the amino acid that cannot be digested by individuals with PKU. If no growth of bacteria is observed, the infant does not have an elevation of Phenylalanine and is therefore normal. However, if the area around the blood spot grows this bacteria, the patient has elevations of Phenylalanine and thus has PKU. This first universal newborn screening for a metabolic disorder has had such an impact that to this day the Newborn Screening testing is still often referred to as the "PKU test". In later years this test was modified to include two other amino acid disorders: Homocystinuria and Maple Syrup Urine Disease (MSUD).

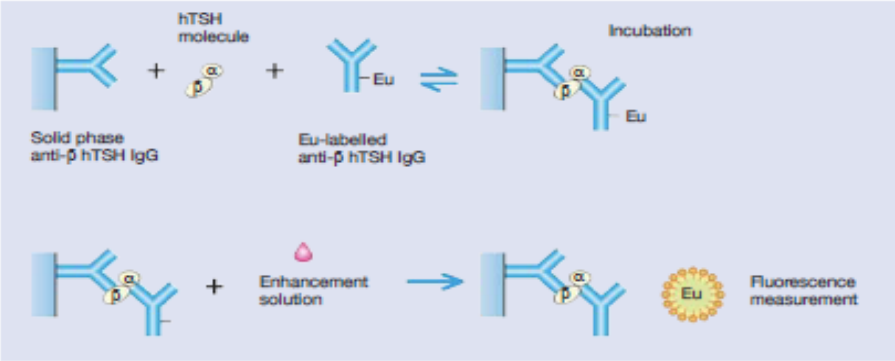


Classical Galactosemia (1979)

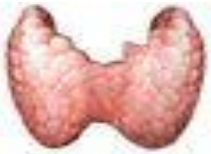
Galactosemia (GAL)

- GAL is a condition in which the body is unable to process galactose, the sugar present in milk. Accumulation of excessive galactose in the body can cause many problems, including liver damage, brain damage and cataracts.

Congenital Hypothyroidism (1979)



The Neonatal hTSH assay is based on a direct sandwich technique where two monoclonal antibodies recognize separate antigenic determinants on the hTSH molecule. The fluorescence signal is proportional to the analyte concentration in the sample.

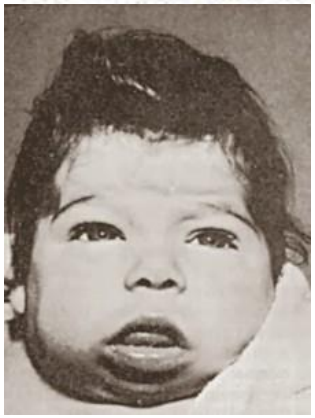


Congenital Hypothyroidism (Cretinism)

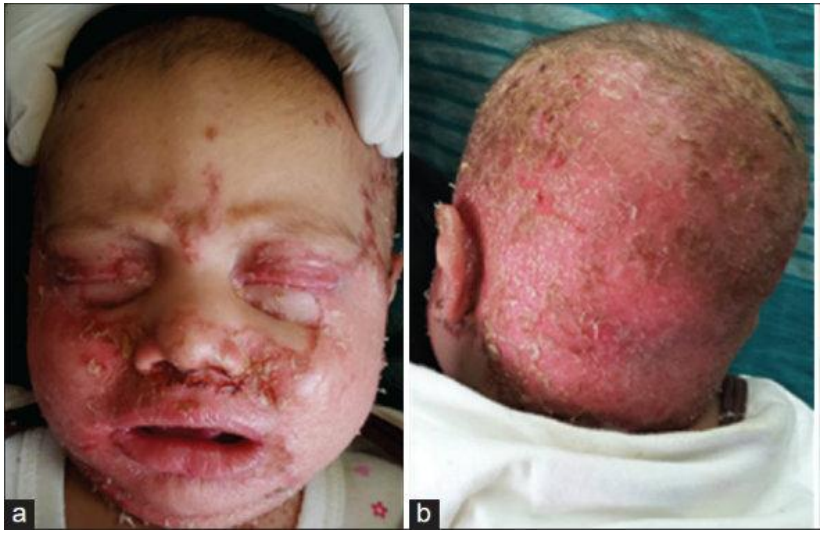
Almost all cases identified through **neonatal screening**

Clinical
- Constipation
- Hypotonia
- Hoarse cry
- Macroglossia

Delayed treatment can lead to
- Learning disabilities
- Cognitive deficits
- Clumsiness
- Diminished fine motor skills



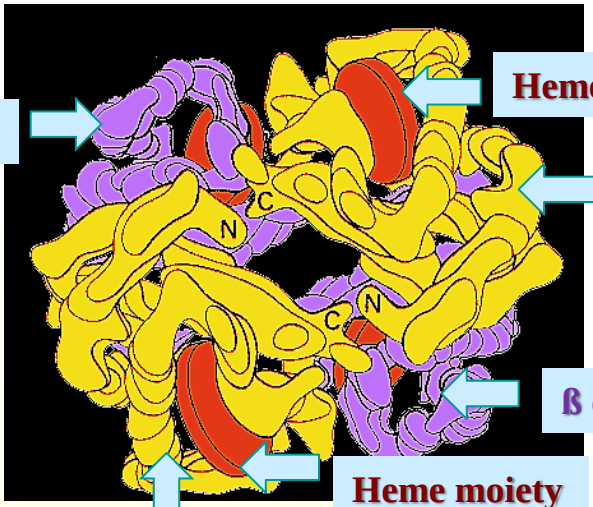
Biotinidase Deficiency (1983)



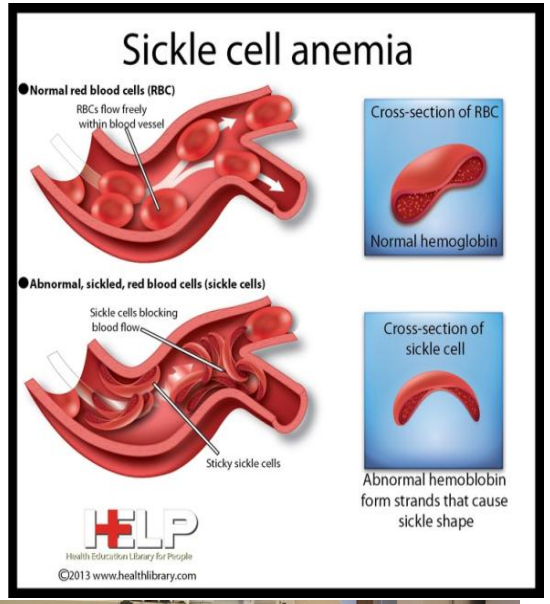
Biotinidase deficiency is a disorder in which the body is unable to reuse/recycle the vitamin biotin. Biotin is important for the body to be able make certain fats and carbohydrates and break down protein. If this condition is not recognized and treated, it can cause seizures, weak muscle tone (hypotonia), breathing problems, hearing and vision loss, problems with movement and balance (ataxia), skin rashes, hair loss (alopecia), and a fungal infection called candidiasis. Affected children also have delayed development. Easily treated with addition of large amounts of biotin to the infant's diet.

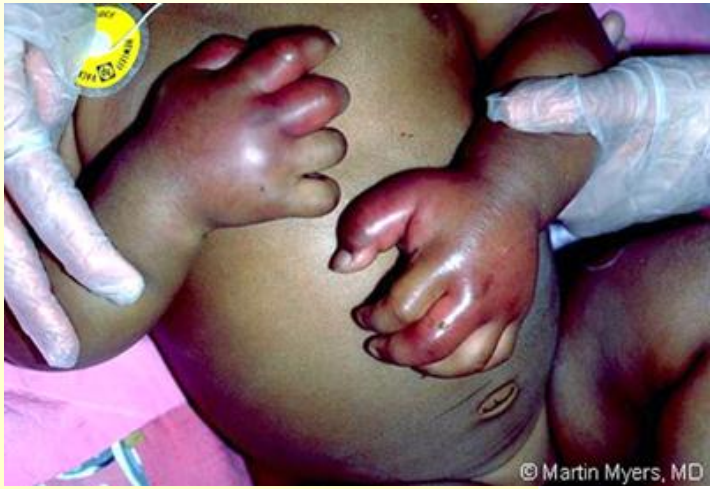
Hemoglobinopathies (1983)

Normal Hemoglobin (HbA) is a heterotetramer with 2 α and 2 ß chains bound to a heme moiety. Main function of hemoglobin is to deliver oxygen to cells within the body.



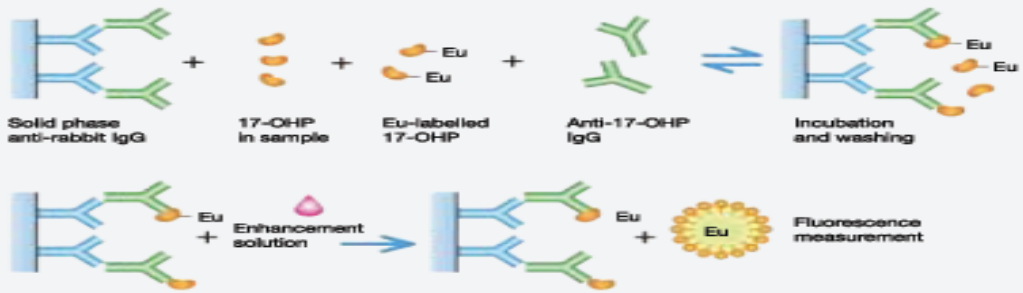
Most well known hemoglobinopathy is Sickle Cell Disease. Early testing can allow for penicillin prophylaxis to decrease the morbidity and mortality associated with pneumococcal septicemia.





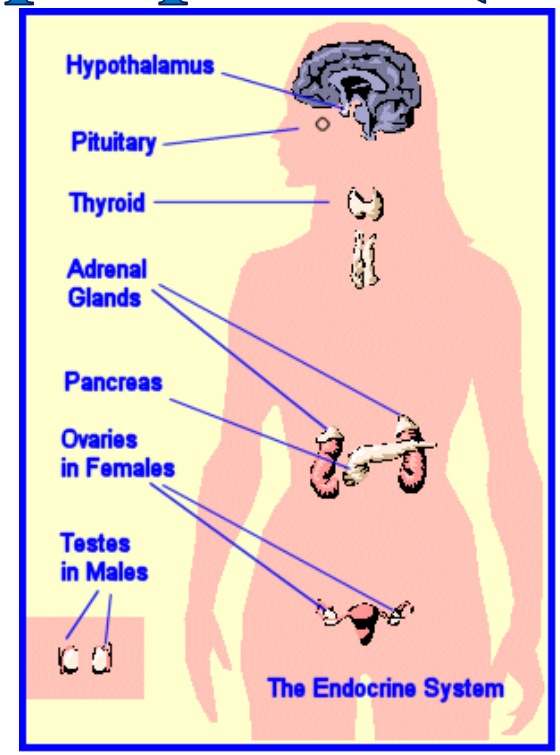
Hemoglobin schematic taken from NEPSSC

Congenital Adrenal Hyperplasia (1995)



Robust competitive type DELFIA® assay

The AutoDELFIA/DELFIA Neonatal 17OHP assay is based on the competitive binding of europium-labeled 17OHP, and 17OHP in the sample to 17OHP-specific antibodies.



CAH affects the adrenal glands resulting in reduced amounts of cortisol and aldosterone production and an overproduction of androgens. Babies with CAH are born with a number of physical changes. Their adrenal glands are often larger than normal, even at birth. Girls with CAH may be born with external sex organs that appear more masculine than they should. If not treated, both boys and girls will develop early sexual characteristics, well before normal puberty should begin. The most severe form of CAH is know as ‘salt-wasting’ CAH which will affect about 75% of babies with classic CAH. When not enough aldosterone is produced, the infant will start losing excess water and salt in their urine which can quickly lead to dehydration and very low blood pressure and often an adrenal crisis that can lead to coma or death.

Connecticut NBS Timeline Cont.

5/1/2004 TMS Initial Phase	9/1/2004 TMS Phase X	11/1/2004 TMS Phase Y	1/1/2005 TMS Phase Z
Phenylketonuria (PKU)	Carnitine Palmitoyl Transferase Deficiency (CPT I)	Propionic Acidemia (PPA)	Argininemia, Arginase Deficiency Arginase Deficiency (ARG)
Maple Syrup Urine Disease (MSUD)	Glutaric Acidemia Type 2 (GA II)	Methylmalonic Acidemia (MMA; includes Cbl A,B and Cbl C,D)	
Homocystinuria (HCY)	Carnitine Palmitoyl Transferase Deficiency (CPT II)	Isovaleric Acidemia (IVA)	Citrullinemia Type I/Citrullinemia Type II (CIT I/CIT II)
Hypermethionemia (MET)	Carnitine/Acylcarnitine Translocase Deficiency (CACT)	3-Hydroxy-3-Methylglutaryl CoA Lyase Deficiency (HMG)	Argininosuccinic Aciduria (ASA)
Tyrosinemia (TYR)		3-Methylcrotonyl CoA Carboxylase Deficiency (3MCC)	Ornithine Transcarbamylase Deficiency (OTC)
Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCAD)		Multiple CoA Carboxylase Deficiency (MCD)	Hyperornithinemia (HHH)
Long Chain Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD)		Glutaric Acidemia Type 1 (GA I)	Nonketotic Hyperglycinemia (NKH)
Very Long Chain Acyl-CoA Dehydrogenase Deficiency (VLCAD)		Beta-Ketothiolase Deficiency (BKT)	Short Chain Acyl-CoA Dehydrogenase Deficiency (SCAD)
Trifunctional Protein Deficiency (TFP)			2,4 Dienoyl CoA Reductase Deficiency (DE RED)
			Malonic Aciduria (MAL)

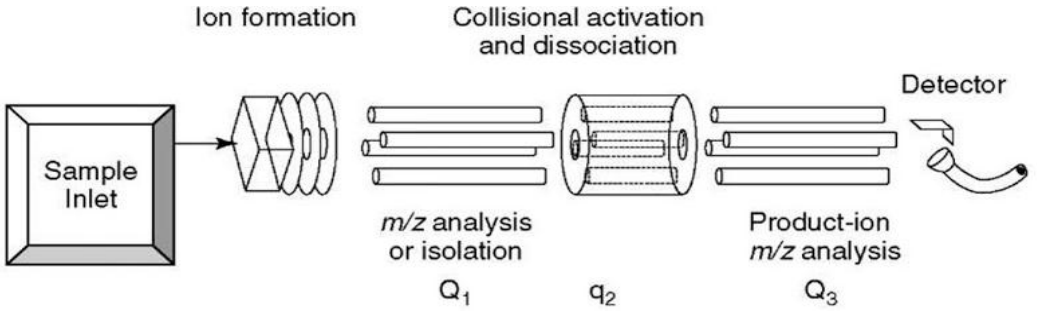
Fifty (50) Disorders screened for by 2005

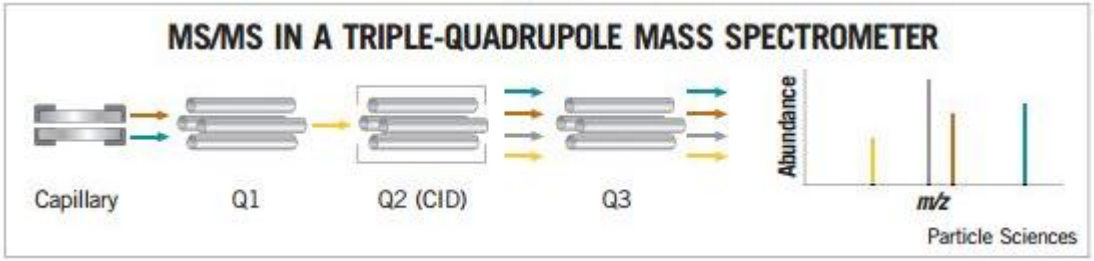
Connecticut NBS Timeline: Changes

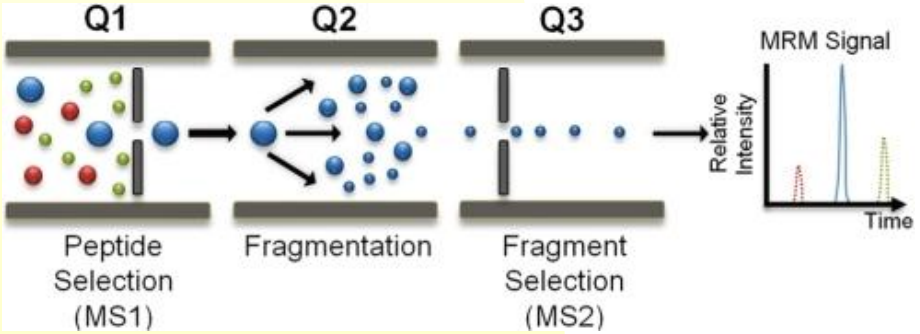
9/1/2010 Additional MS/MS Disorders Detected	10/1/2011	1/1/2015	7/1/2016	8/15/2016 Removal of Disorders Method Change	8/15/2016 Additional Disorder Markers Method Change	8/7/2017
Medium/Short Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency (M/SCHAD)	Severe Combined Immunodeficiency Disease (SCID)	Cutoff and Reporting Algorithm Changes Congenital Hypothyroidism	X-linked Adrenoleukodystrophy (ALD)	Removal of Hyperornithinemia (HHH)	Tyrosinemia Type I (TYR)	Testing Platform Addition (GSP) Congenital Hypothyroidism
Isobutyryl-CoA dehydrogenase deficiency (IBG)	T-Cell Lymphopenia	Cutoff and Reporting Algorithm Changes Congenital Adrenal Hyperplasia		Removal of Nonketotic Hyperglycinemia (NKH)	Arginosuccinic Aciduria (ASA)	Testing Platform Addition (GSP) Congenital Adrenal Hyperplasia
Ethylmalonic Encephalopathy (EME)					Ornithine Transcarbamylase Deficiency (OTC)	
Formiminoglutamic Acidemia, Glutamate Formiminotransferase Deficiency (FIGLU)						
2-Methyl Butyryl-CoA Dehydrogenase Deficiency (2MBG)						
2-Methyl 3 Hydroxy Butyric Aciduria (2M3HBA)						
3-Methylglutaconic Aciduria (3MGA)						
Carbamoyltransferase Deficiency, Carbamoyl Phosphate Synthetase I Deficiency (CPS)						
Pyruvate Carboxylase Deficiency (PC)						
Homocystinuria due to MTHFR (5,10-methylenetetrahydrofolate reductase (NADPH)) deficiency, Remethylation Defect (RMD)						
Hyperphenylalaninemia (PHE)						
Defects of Biopterin Cofactor Regeneration (BIOPT (REG))						
Defects of Biopterin Cofactor Biosynthesis (BIOPT (BS))						

Amino Acids and Acylcarnitine Analysis by LC-MS/MS (2004)

Allowed for the multiplexing of analysis for Aminoacidopathies, Urea Cycle Disorders, Fatty Oxidation Disorders and Organic Acidurias into a process that only requires 1.5min per infant for the instrument analysis of over 40 different disorders.







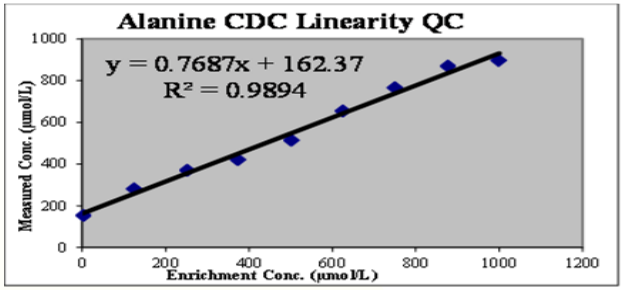
Amino Acids and Acylcarnitine Analysis by LC-MS/MS (LDT 2016)

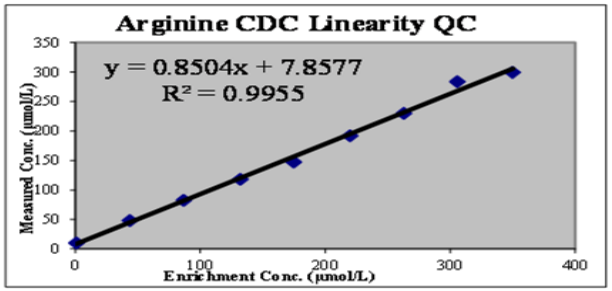
- From 2004-2016 CT LC-MS/MS used Perkin Elmer NeoGram Kit
- **Pros:**
  - FDA cleared kit
  - All components supplied by a single entity
  - Instrument Maintenance and Method Optimization covered under Service Contract
  - All tuning of instrumentation, method development carried out by vendor of kit
- **Cons:**
  - No Succinylacetone for Tyrosinemia Type 1 identification
  - Expensive
  - No ability to troubleshoot method since all components tied to an FDA kit
  - Lack of freedom to add analytes or internal standards
  - Changing to LDT Method: Validation **more** involved requiring: accuracy, precision, reproducibility, carry-over, drift, ruggedness, linearity and proper assessment and establishment of population-based analyte cutoffs for the disorders screened

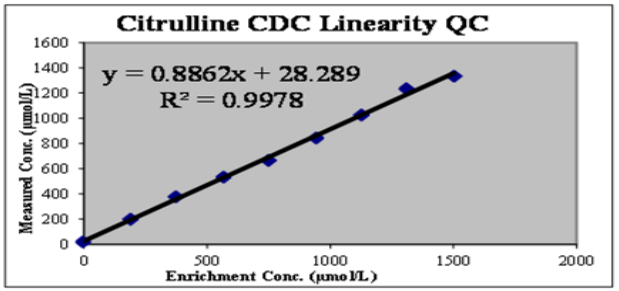
Amino Acids and Acylcarnitine Analysis by LC-MS/MS (LDT 2016)

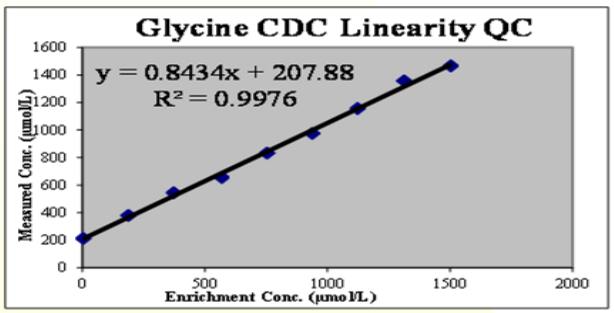
- 4 levels of QCs used for all analyses with exception of the Linearity study where 9 levels were used
- 11 Amino Acids in each level of QC
- 18 Acylcarnitines in each level of QC
- 14 Isotopically Labeled Internal Standard Solutions for Amino Acids analysis and 13 Isotopically Labeled Internal Standard Solutions for Acylcarnitine analysis

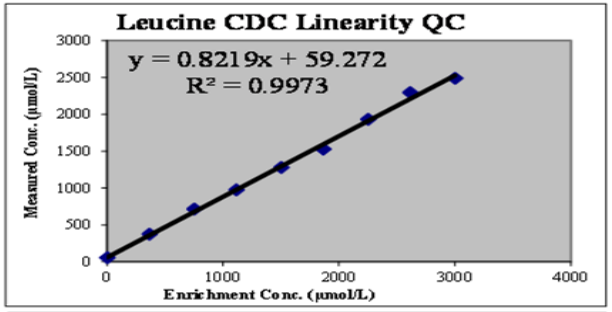
Amino Acids and Acylcarnitine Analysis by LC-MS/MS (LDT 2016)

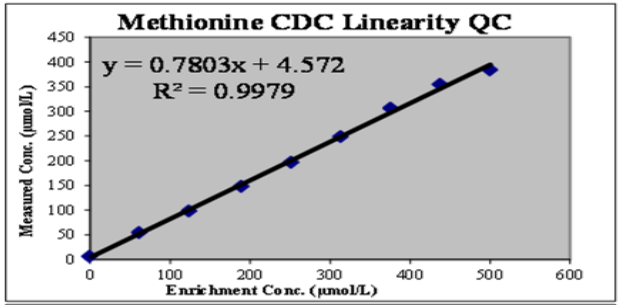


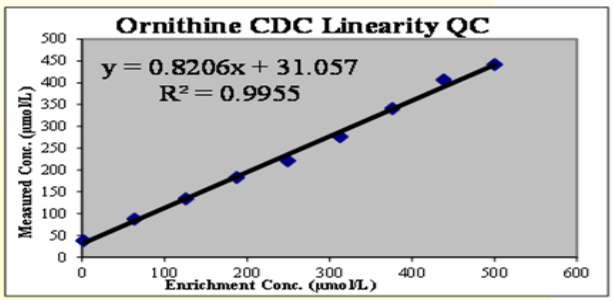


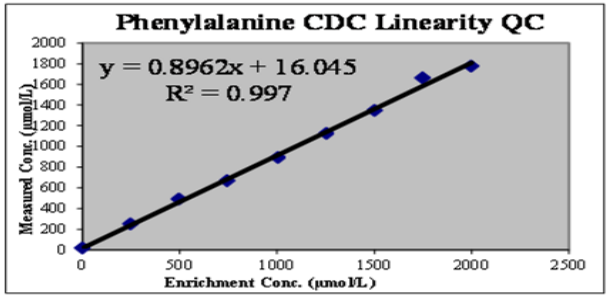


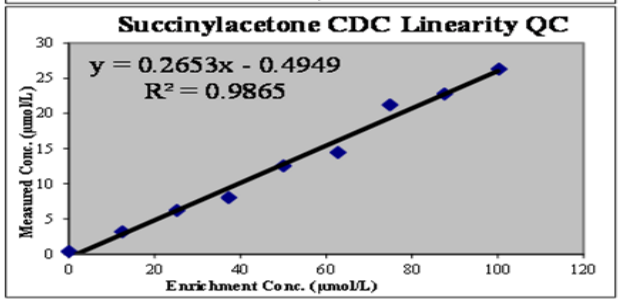


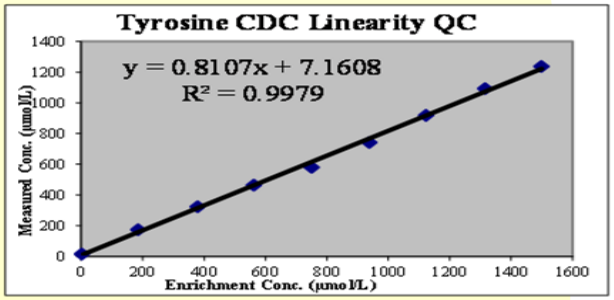


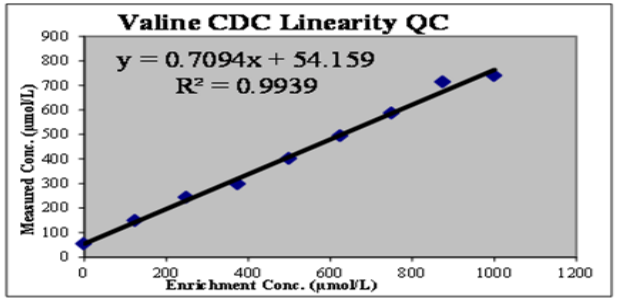




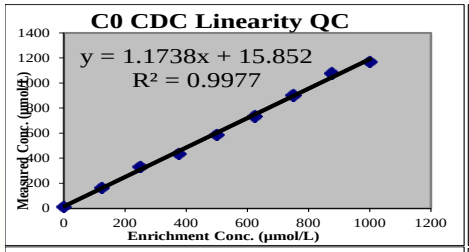


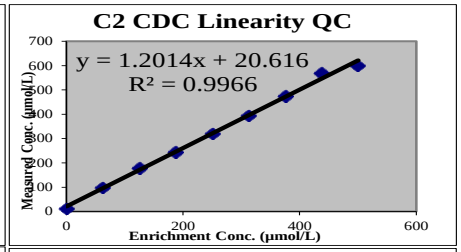


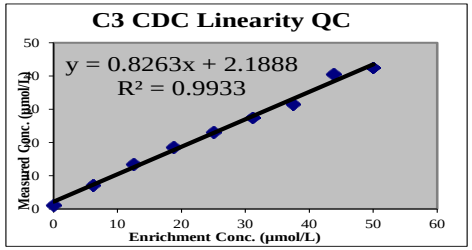


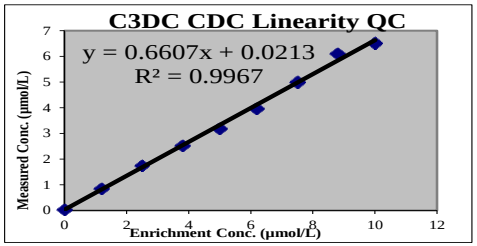


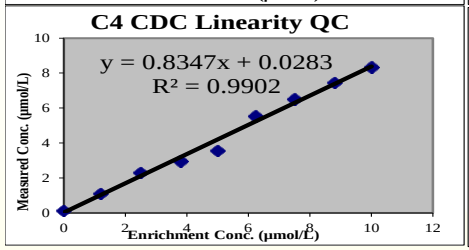
Amino Acids and Acylcarnitine Analysis by LC-MS/MS (LDT 2016)

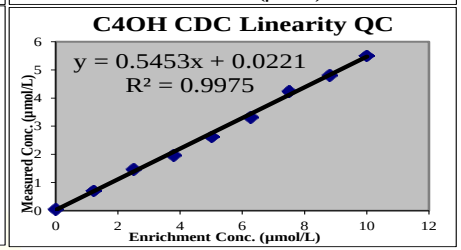


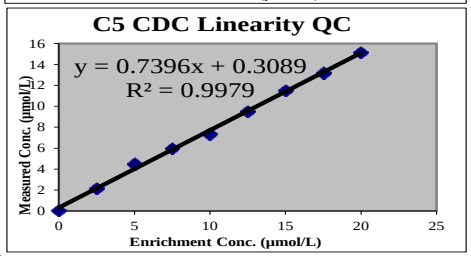


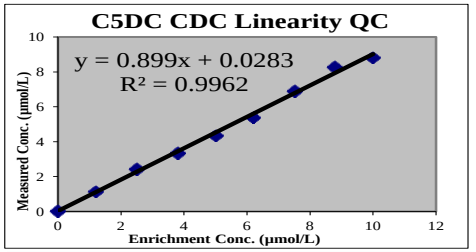


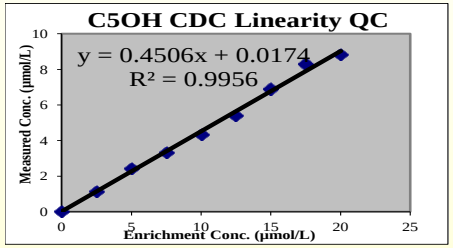


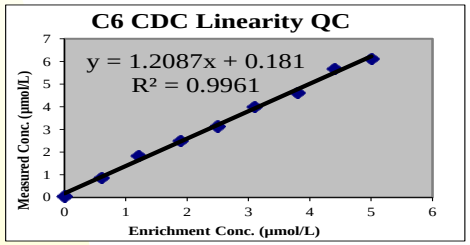


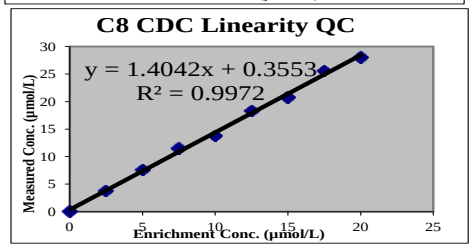


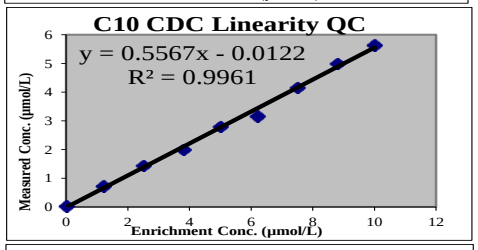


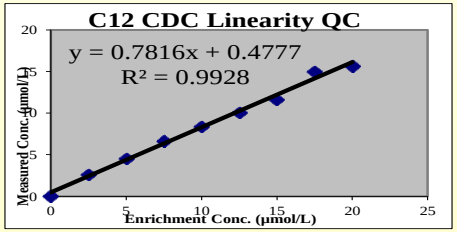


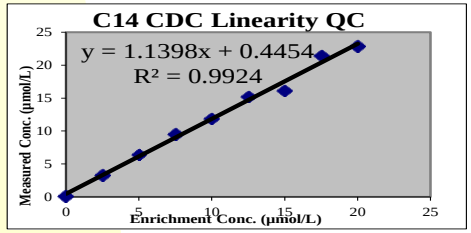


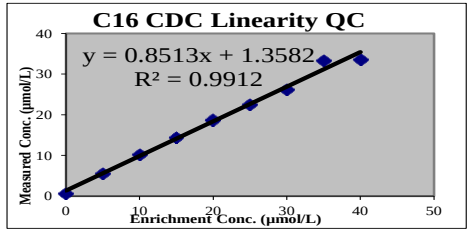


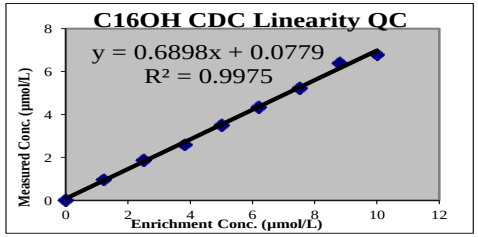


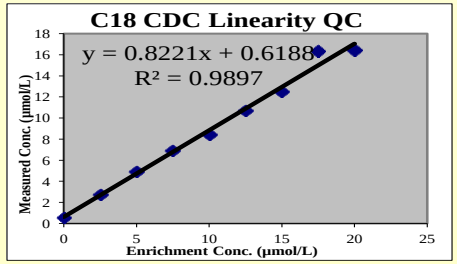


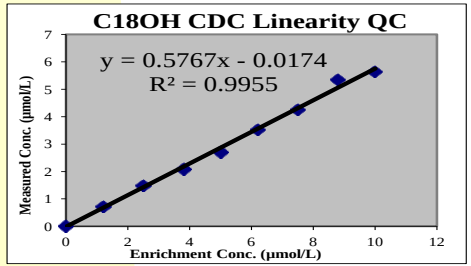












Connecticut NBS Timeline Cont.

9/1/2010 Additional MS/MS Disorders Detected	10/1/2011	7/1/2016	8/15/2016 Removal of Disorders	8/15/2016 Additional Disorder Markers
Medium/Short Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency (M/SCHAD)	Severe Combined Immunodeficiency Disease (SCID)	X-linked Adrenoleukodystrophy (ALD)	Removal of Hyperornithinemia (HHH)	Tyrosinemia Type I (TYR)
Isobutyryl-CoA dehydrogenase deficiency (IBG)	T-Cell Lymphopenia		Removal of Nonketotic Hyperglycinemia (NKH)	Arginosuccinic Aciduria (ASA)
Ethylmalonic Encephalopathy (EME)				Ornithine Transcarbamylase Deficiency (OTC)
Formiminoglutamic Acidemia, Glutamate Formiminotransferase Deficiency (FIGLU)				
2-Methyl Butyryl-CoA Dehydrogenase Deficiency (2MBG)				
2-Methyl 3 Hydroxy Butyric Aciduria (2M3HBA)				
3-Methylglutaconic Aciduria (3MGA)				
Carbamoyltransferase Deficiency, Carbamoyl Phosphate Synthetase I Deficiency (CPS)				
Pyruvate Carboxylase Deficiency (PC)				
Homocystinuria due to MTHFR (5,10-methylenetetrahydrofolate reductase (NADPH)) deficiency, Remethylation Defect (RMD)				
Hyperphenylalaninemia (PHE)				
Defects of Biopterin Cofactor Regeneration (BIOPT (REG))				
Defects of Biopterin Cofactor Biosynthesis (BIOPT (BS))				

Sixty-four (64) Disorders Screened for by 2016

Severe Combined Immunodeficiency (SCID) In Connecticut

► **National Level:** 2010 SACHDNC Recommends SCID Screening to be added to NBS core panel of disorders

► **Connecticut:** Mid-2010 to 2011: 6 laboratory staff

►**January 2011 SB543 "An Act Providing Newborn Screening for Severe Combined Immunodeficiency Disease"**

► July 2011 SCID mandated to start October 1, 2011 via Section 38 of Public Act (PA) 11-48

► CDC *In situ* method chosen

► Equipment requisitions using agency funding for capital equipment procurement placed in July 2011

► Method development and testing began July 2011

► August 2011 staff attend training at CDC for preparation of testing calibrator and control reference materials

► Validation began October 2011

► All infants born as of October 1, 2011 screened for SCID with official start date of January 1, 2012

Severe Combined Immunodeficiency (SCID) In Connecticut

- Intern from UCONN assisted with method validation process due to major **staffing shortages**
- Accuracy and precision batches in duplicate for 5 successful runs by two analysts (10/11/11-10/20/11)
- Randomized order with NTC (No Template Control, blank filter paper) widespread to identify possible cross-contamination issues
- Single calibration curve per analysis
- Positive and Negative QC material for TREC and RNase P analyzed in six replicates per batch per day per analyst
- Three QC levels for Precision calculations (results 20.6%-27.2%)
- Linearity (0.932-0.986 R^2, 85.3-111.9% Efficiency), Sensitivity (98.2-100%) and Specificity (98.3-100%) used for Accuracy calculations
- In-house stored sample identified as true positive SCID patient by clinical immunologist used in validation
- Pre-patient analysis meeting held with state clinical immunologist (information about who could fulfill this role obtained through **discussions with CDC and Dr. Lisa Kobrynski**) to set guidelines for follow-up for possible true abnormal findings with end result of a lower limit action TREC recovery limit set
- Patient sample population analysis commenced during later phase of accuracy and precision study (samples received 10/3/11 to 11/15/11, >4400 samples analyzed)
- Massachusetts (**New England Newborn Screening**) program assisted with second analysis of potentially abnormal results using their well-established and validated method
- Guidance available through **Massachusetts, CDC and Wisconsin** during the validation process
- Ongoing patient median and mean calculations carried out during patient population evaluation

Severe Combined Immunodeficiency (SCID) In Connecticut

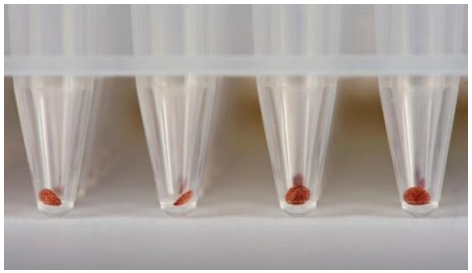
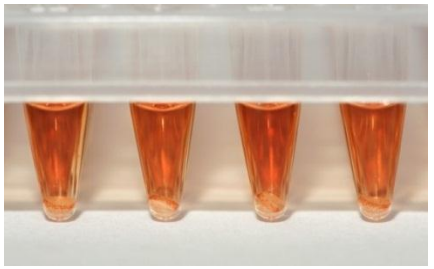
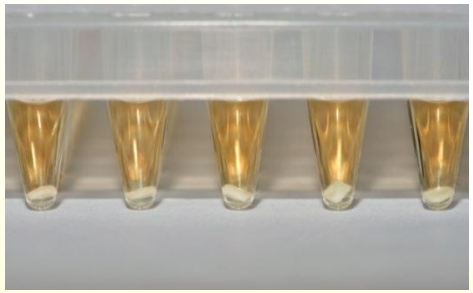
- 5 Full Term Patient samples sent to Massachusetts for analysis during validation patient population study (4 normal), 1 **CONFIRMED SCID** during validation
- First **two years** of SCID testing results:

Results	Full Term (EGA >= 37 weeks)	Preterm (EGA < 37 weeks)
ABN Initial (Post Validation)	47 (35 Normal)	62 (50 Normal, 3 Expired)
ABN Routine Retest	3 (Normal)	41 (31 Normal)
ABN Retest Initial UNSAT	2 (2 Normal)	3 (3 Normal)
Total ABN	52 (42 Normal)	106 (84 Normal, 3 Expired)
Flow Cytometry RESULTS	17 TOTAL	12 TOTAL
	3 TBD	1 Moderate T-cell Lymphopenia
	3 SCID	1 T-cell and B-cell Lymphopenia, deceased
	2 DiGeorge Syndrome	1 pancytopenia due to prematurity (Normal)
	3 T-cell Lymphopenia (1 ZAP70-heterozygous)	1 Followed; neonatal sepsis syndrome, ICU, ill (Normal)
	1 CLOVES SYNDROME	1 Followed; persistent thrombocytopenia, ill (Normal)

- CT Algorithm for reporting sample results:

Actual Gestation Age	TREC (copies/µL)	RNase P (Ct)	Action	Final result
Any	>/= 30	<28	NA	Normal
<37	>/= 25	<28	NA	Normal
Any	Any	>/=28	Rpt x 2	Invalid, Repeat Testing
>= 37	>=10, <30	<28	Rpt x 2	Abnormal, Repeat Testing 1X
Any	<10	<28	Rpt x 2	Abnormal, Immediate Referral
Any	=No Ct	<28	Rpt x 2	Abnormal, Immediate Referral
< 37	>=10, <25	<28	Rpt x 2	Abnormal, Repeat Testing 2X

Severe Combined Immunodeficiency Disease (SCID) (2011)

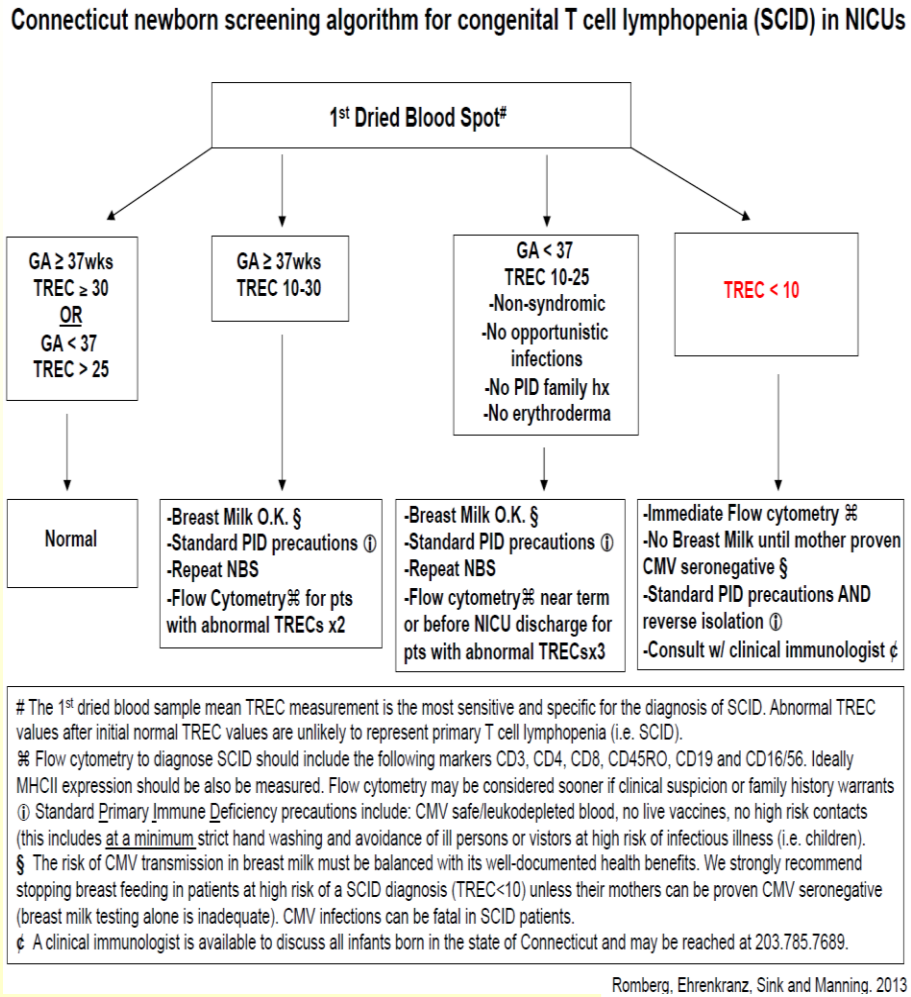








Severe Combined Immunodeficiency Disease (SCID) In Connecticut

Current CT SCID Testing Information

- CT NICU Algorithm:



Romberg, Ehrenkranz, Sink and Manning, 2013

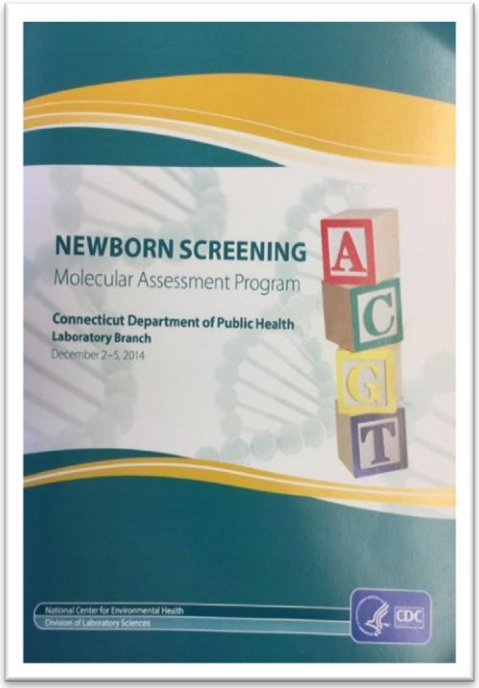
- CT Patient Results: Total Infants Screened 221,554 (10/1/2011 to 10/25/2017)

Patient #	Description
Patient 1	Moderate T-cell Lymphopenia
Patient 2	SCID
Patient 3	22q11; partial DiGeorge
Patient 4	SCID
Patient 5	SCID
Patient 6	T & B-cell lymphopenia
Patient 7	T-cell Lymphopenia
Patient 8	T-cell Lymphopenia
Patient 9	DiGeorge Syndrome
Patient 10	CLOVES Syndrome
Patient 11	T-cell Lymphopenia
Patient 12	T-cell Lymphopenia
Patient 13	T-cell Lymphopenia
Patient 14	T and B cell lymphopenia
Patient 15	T-cell Lymphopenia; 7q32 deletion including TCR beta gene
Patient 16	Moderate T-cell Lymphopenia
Patient 17	T-cell Lymphopenia
Patient 18	Sepsis, prematurity
Patient 19	Chronic Lymphopenia
Patient 20	T-cell Lymphopenia due to prematurity
Patient 21	T-cell Lymphopenia due gastroschisis and prematurity
Patient 22	DiGeorge Syndrome
Patient 23	DiGeorge Syndrome
Patient 24	T-cell Lymphopenia due to prematurity. Bronchopulmonary dysplasia, chromosomal abnormalities with duplication at 19q13.33 and 8q13.3
Patient 25	DiGeorge Syndrome with CCHD
Patient 26	Lost to f/u
Patient 27	Sepsis, prematurity

Severe Combined Immunodeficiency Disease (SCID) In Connecticut

FURTHER IMPROVEMENTS

- New Laboratory Space (as of 2012)
- Additional Instrumentation
- Additional Staff

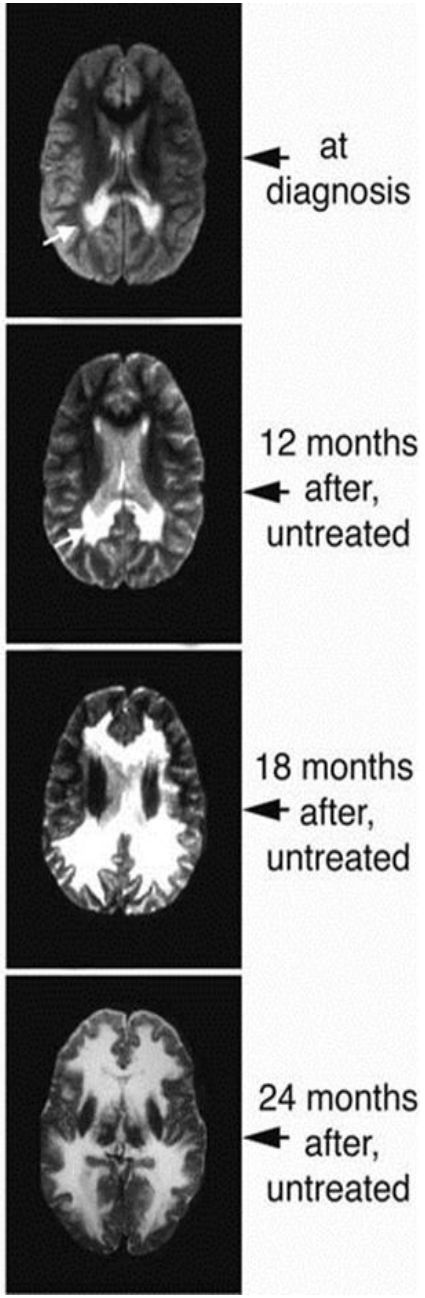


MOLECULAR ASSESSMENT PROGRAM

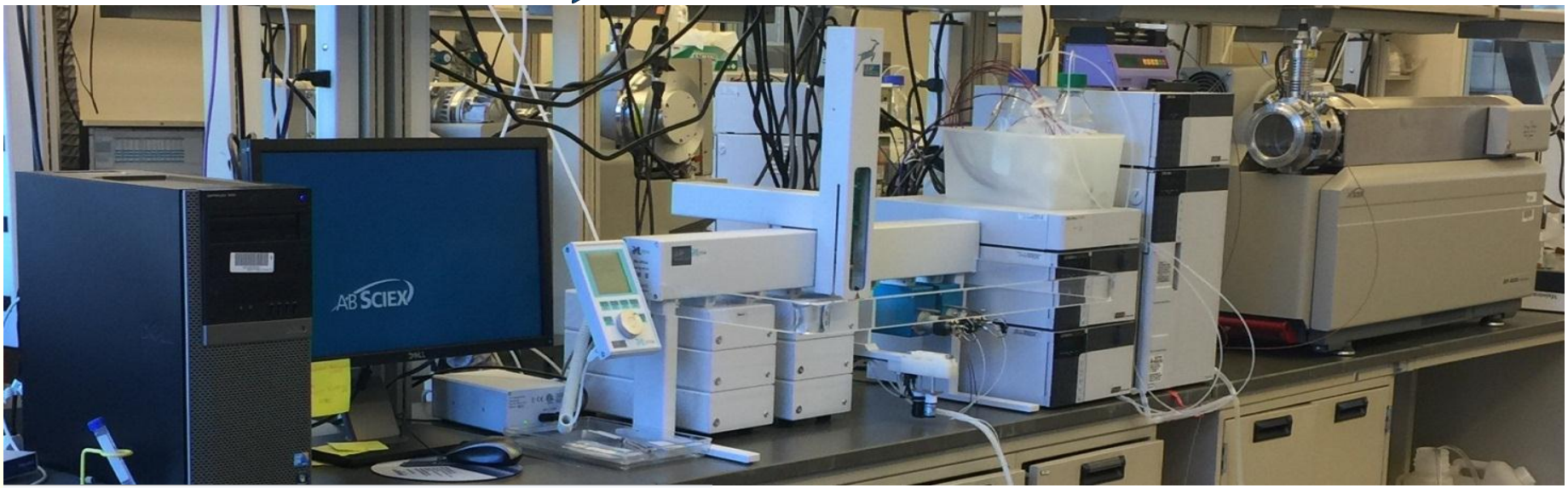
- Reconfiguration of laboratory SCID testing setup/space

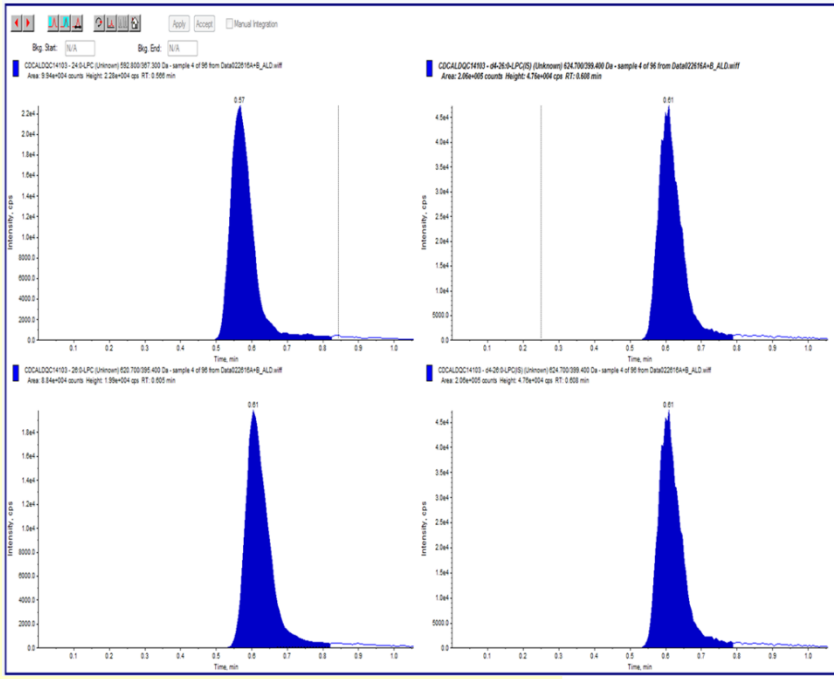
X-Linked Adrenoleukodystrophy (X-ALD) (2016)

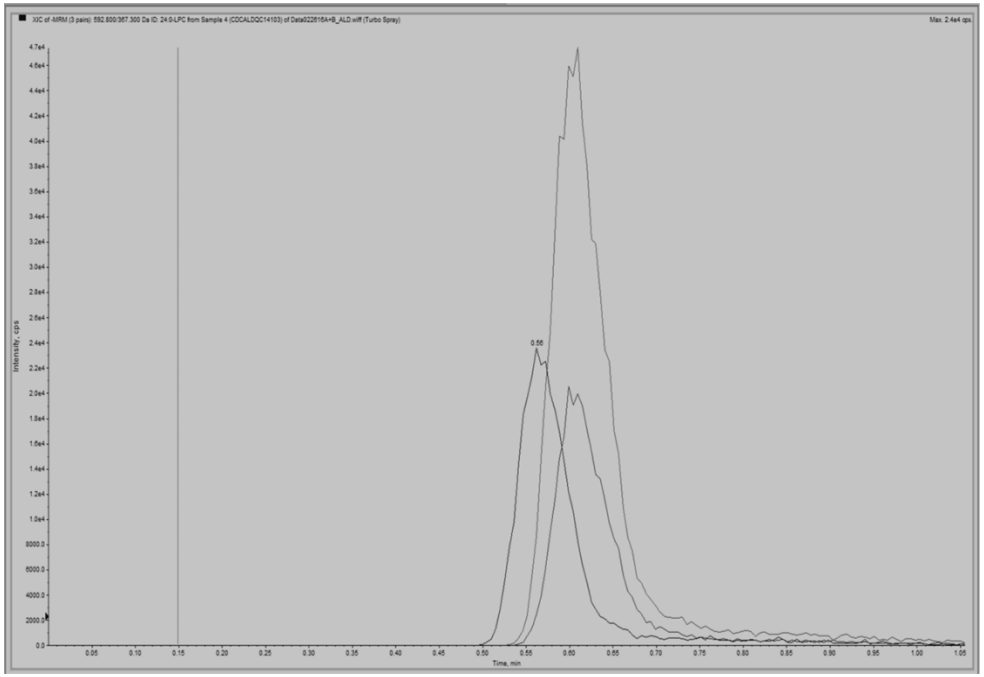
- X-ALD is the most common peroxisomal disorder, estimated incidence of 1:17,000
- This disorder is caused by mutations in the ALD peroxisomal transmembrane protein, ALDP, and the ABCD1 gene
- The severity of this mutation varies from childhood cerebral ALD (C-CALD), generally lethal with onset between ages 4 and 10, to adult-onset adrenomyeloneuropathy (AMN)
- Caused by accumulation of C26:0-lysophosphatidylcholine (C26:0-LPC), resulting in inflammatory demyelination of nerve cells within the brain and lesions that can be seen using an MRI
- X-ALD often also causes adrenal insufficiency or Addison's disease.
- The childhood form of the disease often leads to rapid degeneration, loss of cognitive ability, vegetative state and death



X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut







X-Linked Adrenoleukodystrophy (X-ALD) (2016)

Number of infants analyzed for X-ALD as of 02/26/18 (10/1/2015-02/26/2018)	87797
Total Screen Positive	31
Samples reported with 2nd request	16
Samples normal on second sample analysis	13
False Positive 2016	1
False Positive 2017	1
Pending	1
Confirmed ALD carrier diagnosis newborn infant results (negative molecular testing results, positive biochemical testing results)	1 female
Confirmed ALD diagnosis newborn infant results	14 (8 male, 6 female)
Siblings Identified (and confirmed at Treatment Center) with ALD	2 (1 male, 1 female)
Other	1 Zellweger 2017 (female)
Approximate Detection Incidence Overall	~1:6271

X-Linked Adrenoleukodystrophy (X-ALD) In Connecticut

Challenges for Endocrine Screening

Endocrine disorder testing and reporting is dependent upon time of collection and, for CAH, birth weight/gestational age of infant. In early **2015** calculations were carried out for refining the cutoffs for CH and CAH. All samples collected at less than 24 hours of age were invalidated.

Below are the calculations for CH (TSH):

CH 2012-2014

Age at Collection	Median	99.1 percentile	99.8 percentile	n
<24hr	18.6	106.2	153.8	4712
≥24hr	9.6	30.0	38.2	90517
All	9.8	46.6	79.4	95229

CH 12/31/14-8/7/15

Age at Collection	Median	99.1 percentile	99.8 percentile	n
<24hr	34.4	130.0	147.4	427
≥24hr	10.8	30.0	39.5	21433
All	10.9	43.6	85.6	21860

CT TSH

Age at Collection	TSH Cutoff (uIU/mL)	Interpretation
< 24 hours	Inconclusive	Inconclusive
≥ 24 hours	< 30 uIU/mL	Normal
≥ 24 hours	≥ 30 uIU/mL	Borderline
≥ 24 hours	≥ 38 uIU/mL	Presumptive Positive

Confirmed Cases by Year

CH	2011	2012	2013	2014	2015	2016	2017*
	17	6	10	12	28	29	12

*** as of October 2017; more cases will likely be added**

Challenges for Endocrine Screening

Below are the calculations for CAH (17OHP):

CAH 2012-2014

All Birth Weights

Age at Collection	Median	99.8 percentile	99.9 percentile	n
<24hr	19.1	153	217	4551
≥24hr	7.4	61.7	80.4	83899
all	7.6	76.8	96.3	88450

≥2500g

Age at Collection	Median	99.8 percentile	99.9 percentile	n
<24hr	15.1	85.0	93.5	2668
≥24hr	7.3	38.3	46.2	79150
all	7.4	51.2	59.1	81818

≥1500-2500g

Age at Collection	Median	99.8 percentile	99.9 percentile	n
<24hr	23.7	145	167	1156
≥24hr	11.7	98.9	107	4382
all	13.7	109	119	5538

<1500g

Age at Collection	Median	99.8 percentile	99.9 percentile	n
<24hr	24.5	279	329	727
≥24hr	32.8	297	384	367
all	26.3	301	359	1094

CAH 12/31/14-8/7/15

All Birth Weights

Age at Collection	Median	99.8 percentile	99.9 percentile	n
≥24hr	7.1	77.5	99.9	21020

≥2500g

Age at Collection	Median	99.8 percentile	99.9 percentile	n
≥24hr	7.0	35.1	42.7	19534

≥1500-2500g

Age at Collection	Median	99.8 percentile	99.9 percentile	n
≥24hr	11.6	85.5	90.5	1243

<1500g

Age at Collection	Median	99.8 percentile	99.9 percentile	n
≥24hr	37	214	214	243

As observed above, 17OHP concentrations for the detection of CAH is dependent upon both age at collection and birth weight.

Challenges for Endocrine Screening

Below are the AutoDELFIA Cutoffs for 17-OHP for CAH Screening:

CT 17-OHP

Birth Weight	Age at Collection	17-OHP Cutoff (ng/mL)	Interpretation
ALL	< 24 hours	Inconclusive	Inconclusive
≤ 1500 grams	≥ 24 hours	< 100ng/ml	Normal
≤ 1500 grams	≥ 24 hours	≥ 100 ng/ml	Presumptive Positive
1500-2499 grams	≥ 24 hours	< 75 ng/ml	Normal
1500-2499 grams	≥ 24 hours	≥ 75ng/ml	Presumptive Positive
>2499 grams	≥ 24 hours	< 38.3 ng/ml	Normal
>2499 grams	≥ 24 hours	≥ 38.3 ng/ml	Borderline
>2499 grams	≥ 24 hours	≥ 46.2 ng/ml	Presumptive Positive

Confirmed Cases by Year

CAH	2011	2012	2013	2014	2015	2016	2017*
	1 (SW)	5 (3SV, 1SW, 1 unspecified)	1 (SW)	2 (1SW, 1SV)	0	3 (1SW, 2 unspecified)	2 (1SW, 1 unspecified)

* as of October 2017; more cases will likely be added

Endocrine Screening Changes 2017

Summer of **2017** instrument upgrade for the analysis of 17OHP and TSH. Benefits include a temperature controlled instrument requiring less maintenance and less analyst time to set up. Also will be integrated within a software package that will allow for the upload of demographic parameters which may affect the results obtained allowing for better refinement of cutoffs.

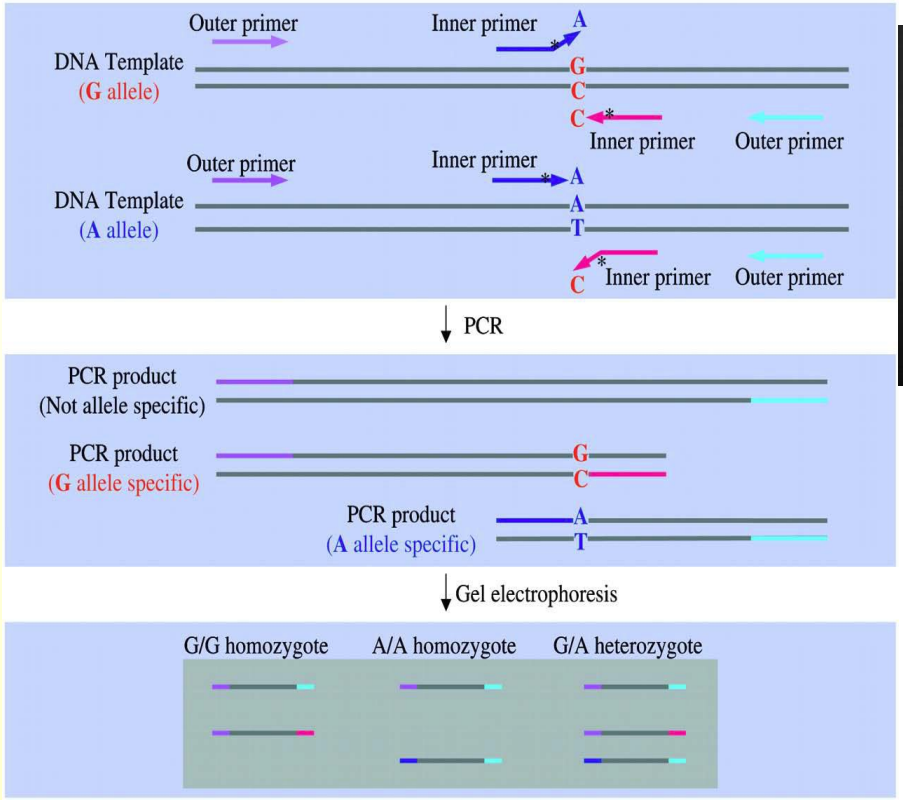
August 7, 2017 Endocrine Testing Cutoff Changes

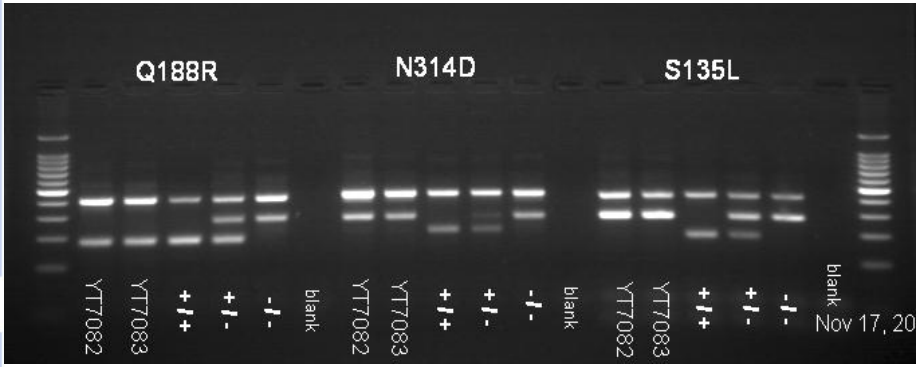
	Age at Collection	TSH Cutoff (µIU/mL)	Interpretation
GSP CT TSH	< 24 hours	Inconclusive	Inconclusive
	≥ 24 hours	< 25.5 µIU/mL	Normal
	≥ 24 hours	≥ 25.5 µIU/mL	Borderline
	≥ 24 hours	≥ 34.0 µIU/mL	Presumptive Positive

	Birth Weight	Age at Collection	17-OHP Cutoff (ng/mL)	Interpretation
GSP CT 17-OHP	ALL	< 24 hours	Inconclusive	Inconclusive
	< 1500	≥ 24 hours	< 100ng/ml	Normal
	< 1500 grams	≥ 24 hours	≥ 100 ng/ml	Presumptive Positive
	1500-2499 grams	≥ 24 hours	< 75 ng/ml	Normal
	1500-2499 grams	≥ 24 hours	≥ 75ng/ml	Presumptive Positive
	>2499	≥ 24 hours	< 38.3 ng/ml	Normal
	>2499	≥ 24 hours	≥ 38.3 ng/ml	Borderline
	>2499 grams	≥ 24 hours	≥ 42.8 ng/ml	Presumptive Positive

Future of Connecticut Newborn Screening

Second-tier analyses for high false-positive rate first-tier tests: Galactosemia testing is adversely affected by heat and humidity, particularly in the summer months creating false positive results. There are well-established second-tier molecular methods that are inexpensive and quick such as Amplification Refractory Mutation System (ARMS) PCR which are designed for point mutations such as the mutation in the galactose-1-phosphate uridyltransferase gene that results in Classical Galactosemia.



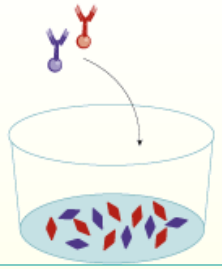


Notes:
Q188R—one of common galactosemia disease causing mutations.
N314D--Duarte (D2) variant, and reducing enzyme activity by 25%
S135L is associated with a mild phenotype.
Newborns who are G/D heterozygotes may have a positive newborn screen

Future of Connecticut Newborn Screening

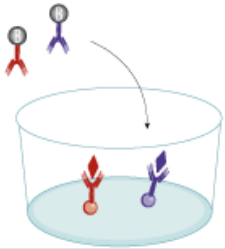
Other second-tier analyses can be added using technologies currently in use in other areas of molecular testing. For example, adding a Luminex assay for some of the metabolic disorders such as MCAD which has an incident rate of about 1:10,000 infants.

STEP 1



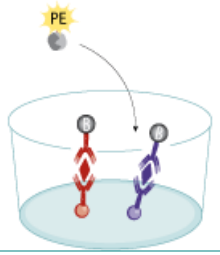
Color-coded analyte-specific are added. Multiple analytes can be simultaneously detected in the same sample.

STEP 2



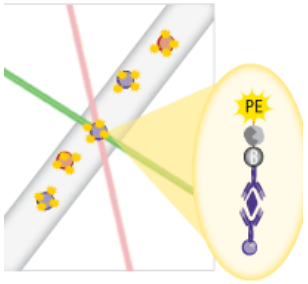
Specific antibodies capture the analyte of interest. Antibodies specific to analyte of interest are added and form antibody-antigen sandwich.

STEP 3

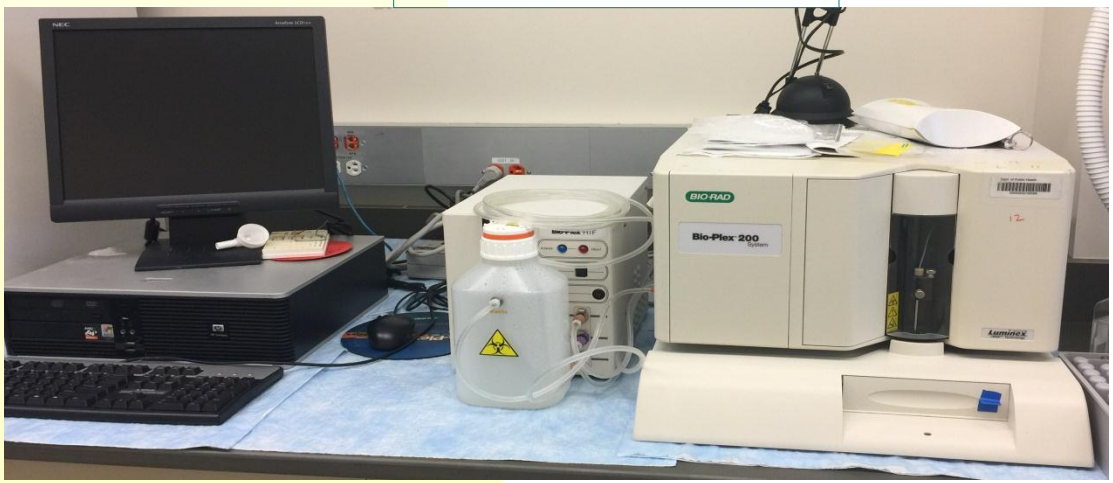


Phycoerythrin (PE)-conjugated Streptavidin is added.

STEP 4

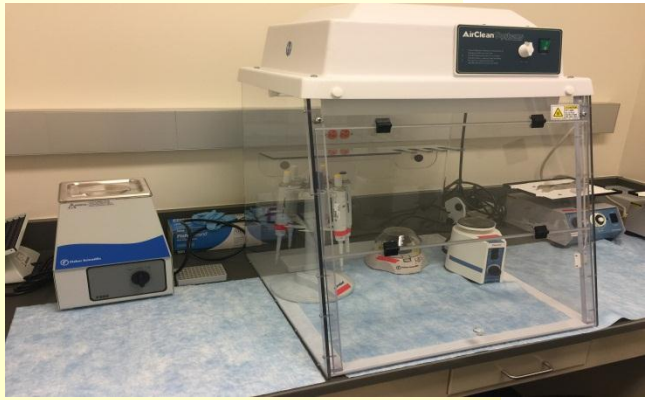


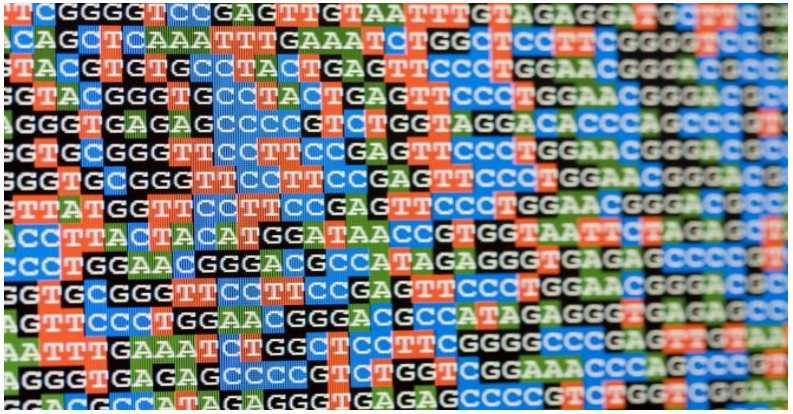
The beads are read on a dual-laser flow-based detection instrument, such as the Luminex 200™ or Bio-Rad® Bio-Plex® analyzer. One laser classifies the bead and determines the analyte that is being detected. The second laser determines the magnitude of the PE-derived signal, which is in direct proportion to the amount of bound analyte.



Future of Connecticut Newborn Screening

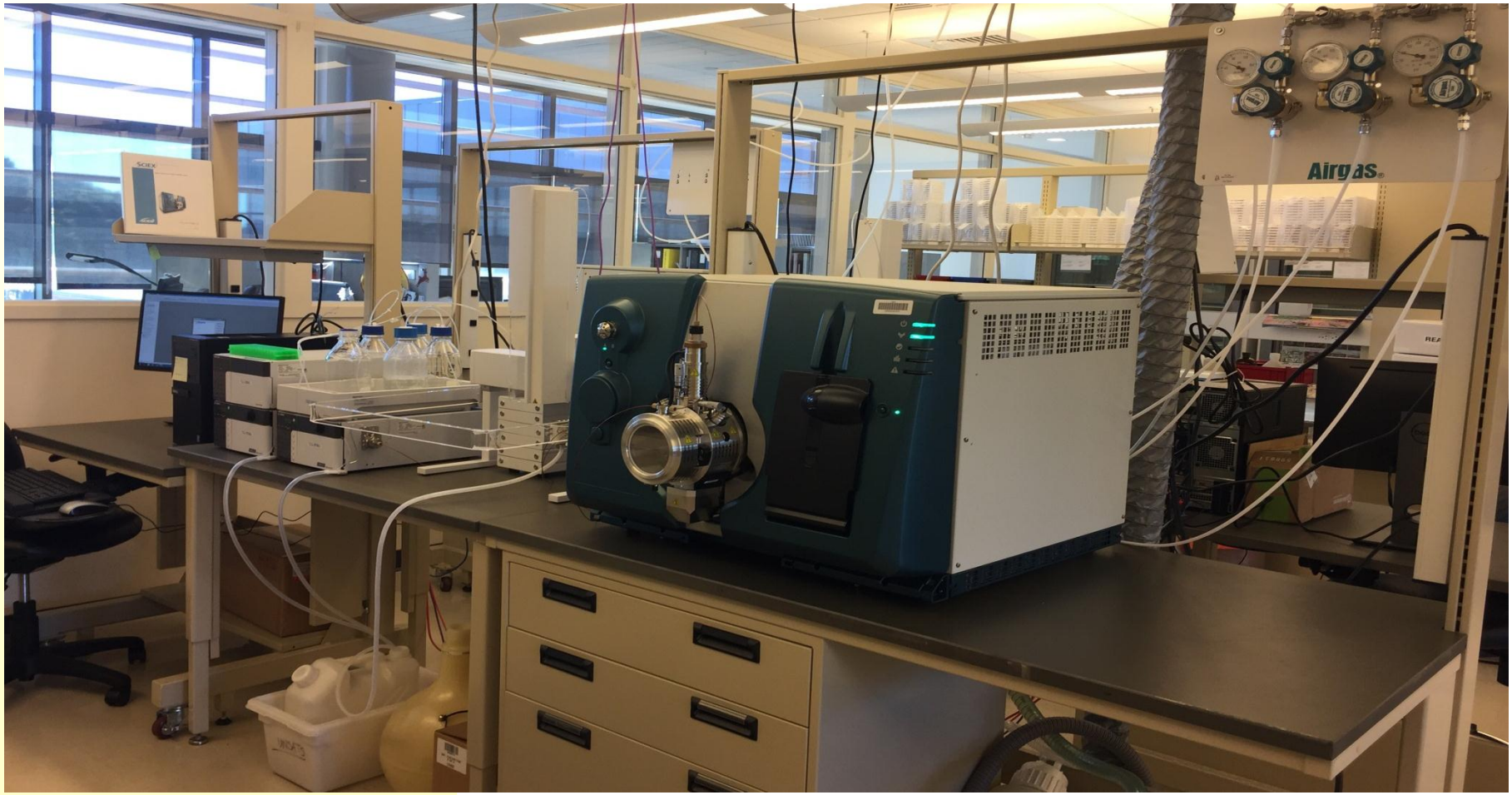
Whole Genome Sequencing: This has very promising applications for second or third-tier analysis for disorders that have multiple mutations such as Cystic Fibrosis. Currently this is being implemented in several of the laboratories around the country who currently carry out other molecular second-tier analyses.





Future of Connecticut Newborn Screening

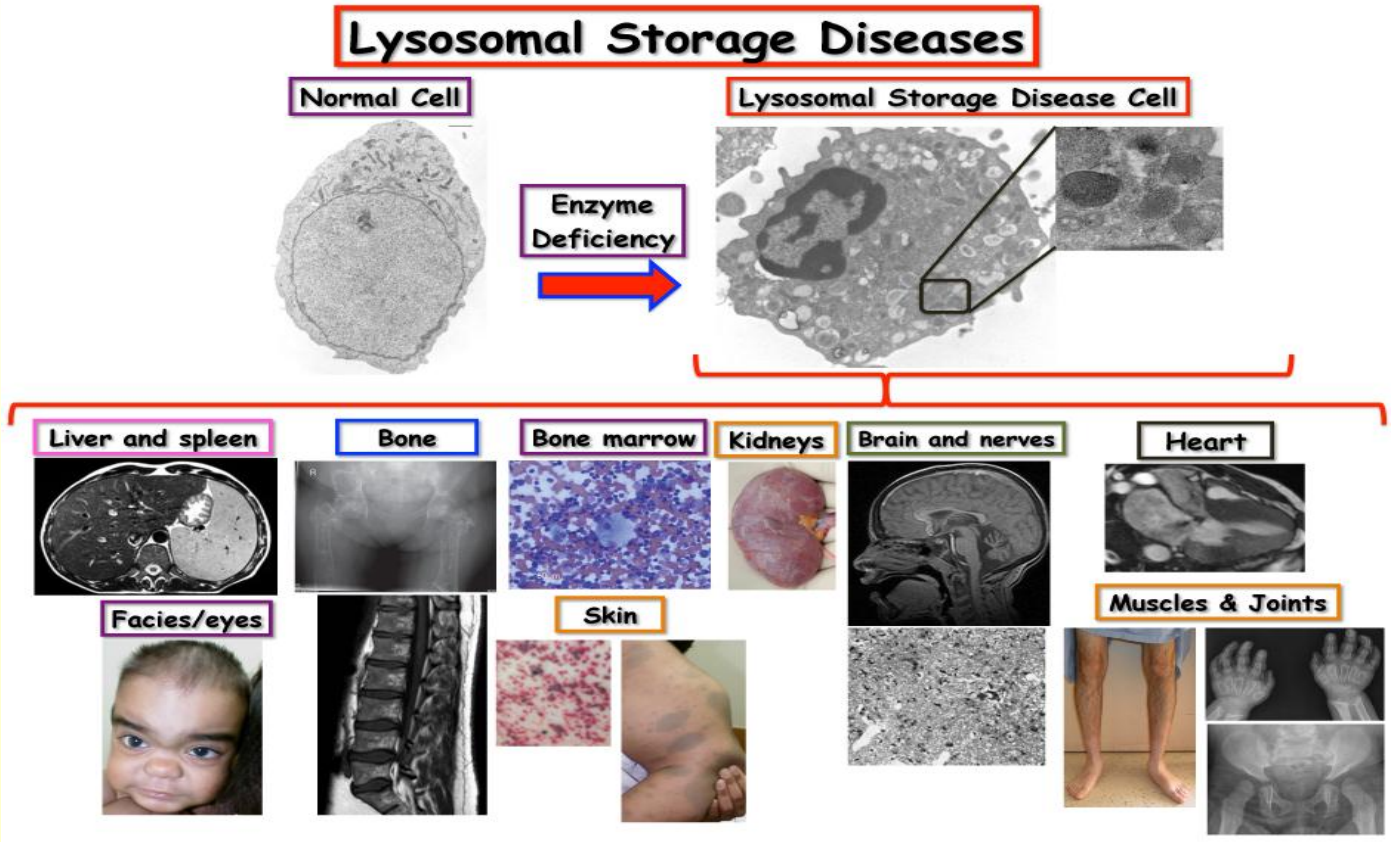
Also implemented in other laboratories are second-tier methods using LC-MS/MS analysis. There are laboratories who carry out LC-MS/MS second tier analyses for the steroid profile when an abnormal 17OHP result is obtained using the initial fluorescence assay. Additionally, second-tier LC-MS/MS assays can be used to reduce the number of false positive results for some of the aminoacidopathies and organic acidurias by specifically targeting the metabolite affected by the disorder rather than a surrogate biochemical marker (examples are Homocysteine specifically for any Methionine elevations or Methylmalonic Acid for any C3 acylcarnitine elevations).



Future of Connecticut Newborn Screening

New Disorder Screening: Two Lysosomal Storage Disorders have been added to the Recommended Uniform Screening Panel (RUSP) by the Secretary of Health and Human Services: Pompe and MPS-1.

Lysosomal storage disorders (LSDs) are mainly autosomal recessively inherited metabolic diseases characterized by an abnormal build-up of various toxic materials in the body's cells as a result of enzyme deficiencies. There are an estimated 50 of these disorders, and they may affect different parts of the body, including the skeleton, brain, skin, heart, and central nervous system caused by an absence or deficiency of an enzyme, leading to the inappropriate storage of material in various cells of the body. *

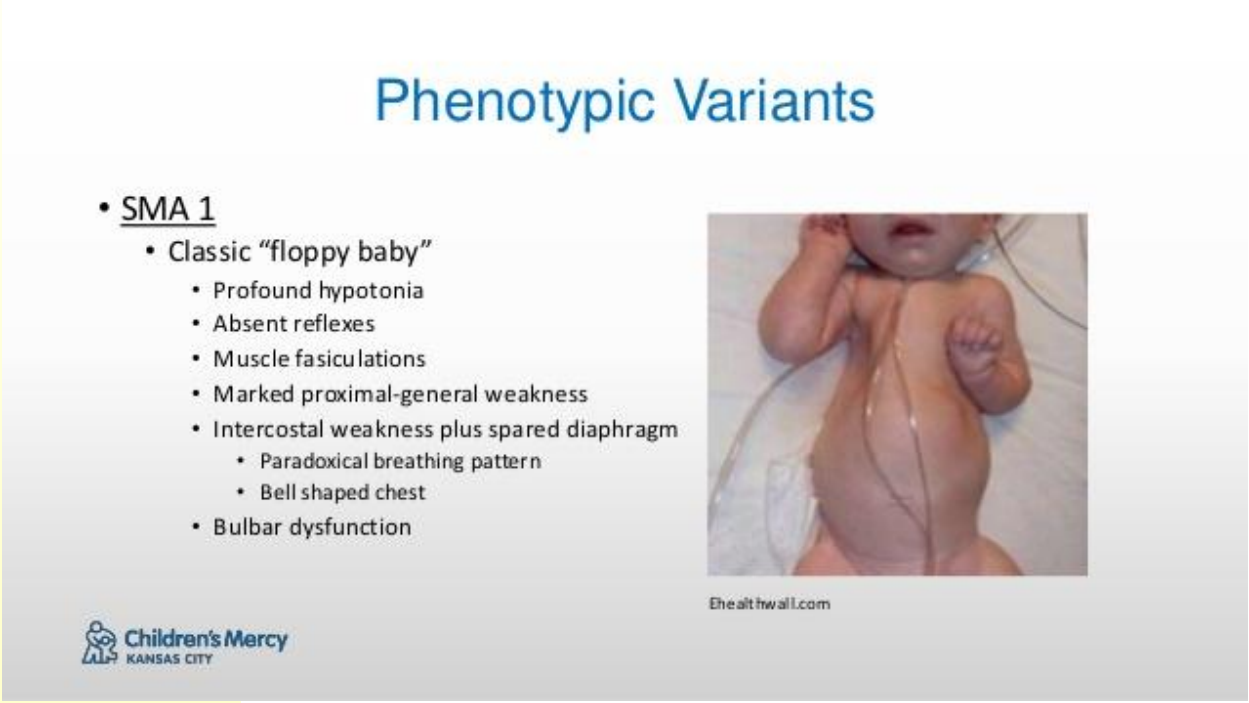


*https://rarediseases.org/rare-diseases/lysosomal-storage-disorders https://igm.jhmi.edu/sites/default/files/lsd-program/Fig1_LSD_940px.jpg

Future of Connecticut Newborn Screening

New Disorder Screening: Has been nominated to RUSP and awaiting approval by Secretary of Health and Human Services (FDA cleared treatment available through Biogen) **Spinal Muscular Atrophy (SMA)**

(SMA) is a rare, autosomal recessive neuromuscular disorder which is characterized by loss of motor neurons and progressive muscle wasting, often leading to early death. The disorder is caused by a defect in the *SMN1* gene, which encodes SMN, a protein that is necessary for survival of motor neurons. Lower levels of SMN results in loss of function of neuronal cells in areas of the spinal cord and system-wide muscle wasting (atrophy). SMA has various degrees of severity, but all result in progressive muscle wasting and impaired mobility. Proximal muscles of the arms and legs as well as lung muscles are affected first. Other body systems may be affected as well, particularly in early-onset forms (infantile form SMA1) of the disorder. SMA is the most common genetic cause of infant death.



https://en.wikipedia.org/wiki/Spinal_muscular_atrophy

Thank You!