Homocystinuria's Newborn Screening Problem

- Possible and Available Solutions -

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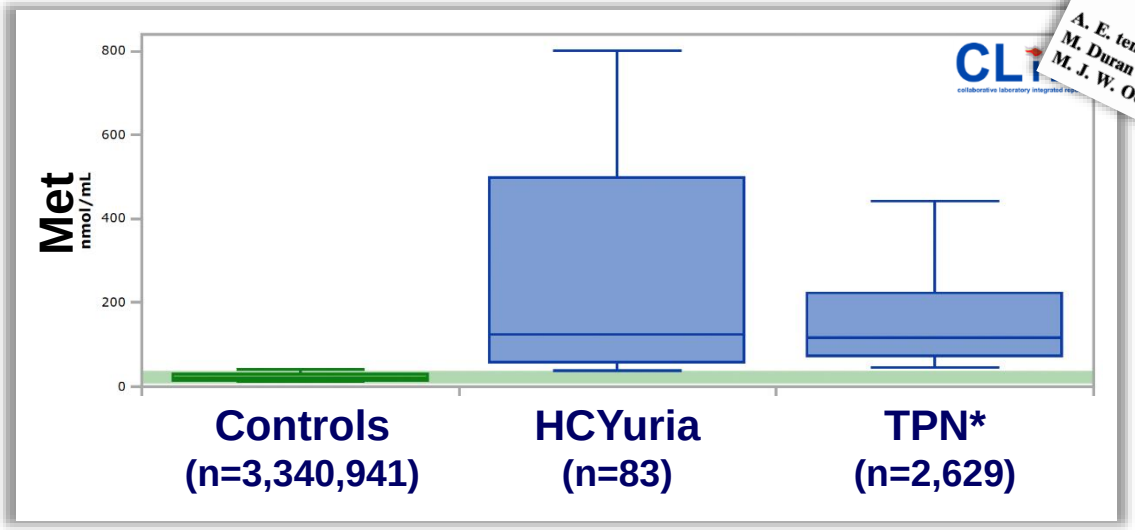
Homocystinuria's NBS Problem

Methionine:

- Easy to measure
- Not sensitive, even with low cutoff



*total parenteral nutrition


The New England Journal of Medicine

REDUCTION OF FALSE NEGATIVE RESULTS IN SCREENING OF NEWBORNS FOR HOMOCYSTINURIA

M. Judith Peterschmitt, M.D., Jane R. Simmons, B.S., and Harvey L. Levy, M.D.

J Inherit Metab Dis (2007) 30:978
DOI 10.1007/s10545-007-0701-0

SHORT REPORT

High incidence of hypermethioninaemia in a single neonatal intensive care unit detected by a newly introduced neonatal screening programme

A. E. ten Hoedt · A. A. van Kempen · A. Boelen · M. Duran · E. A. Kemper-Proper · F. A. Wijburg · A. M. Bosch · M. J. W. Oey-Spauwen

Conclusions A cutoff level for blood ... of 1 mg per deciliter in neonatal screening ... homocystinuria should identify affected infants ... have only slightly elevated concentrations of methionine and reduce the frequency of false negative results. (N Engl J Med 1999;341:1572-6.)

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HOMOCYSTINURIA due to a deficiency of cystathionine β-synthase, hereafter referred to simply as homocystinuria, is an inborn error of the metabolism of sulfur-containing amino acids that results in ectopia lentis, mental retardation, osteoporosis with bone deformities, thromboembolism, and psychiatric disturbances.[1] The major biochemical findings include high plasma homocyst(e)ine and methionine concentrations and low plasma cyst(e)ine concentrations. The gene for cystathionine β-synthase has been cloned and mapped to chromosome 21q22.3. Over 60 mutations associated with homocystinuria have been found.[2]

Cystathionine β-synthase is pyridoxal phosphate (vitamin B_6)–dependent (Fig. 1). There are two forms of homocystinuria, which are differentiated on the basis of the biochemical response to treatment with ... Infants with vitamin B_6–responsive ho... tend to be less severely affected and ... high doses of vitamin B_6. Infants ... monsive homocystinuria are ...icted diet supplement... ...osis after the first ...ding to the ...methyl ... B_6–non... after infancy. ...borns for this diso...

Persons with homocy... not identified during the neo... sion of screening for homocystinuria ... screening programs in which blood samp... by heel stick are used is a major reason for the ... identification of affected infants,[6] but the rate of fa...

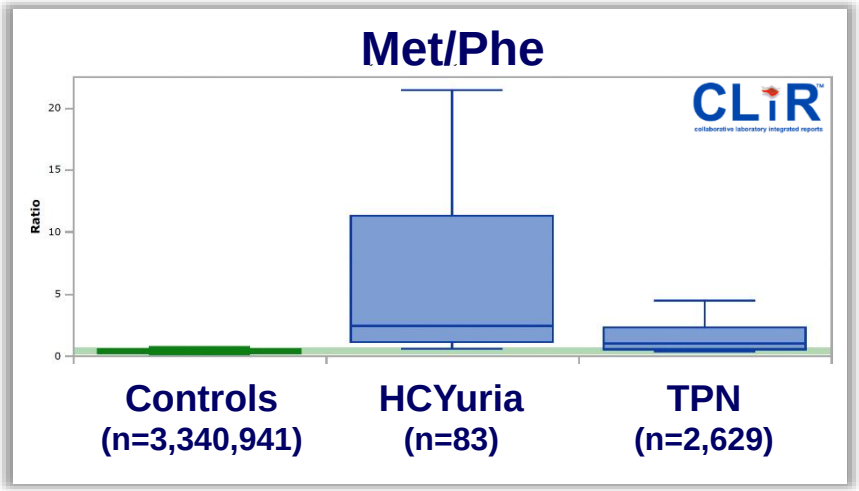
From the Division of Genetics, Children's Hospital, and the Department of Pediatrics, Harvard Medical School (M.J.P., H.L.L.), and the New England Newborn Screening Program, State Laboratory Institute (J.R.S.) — all in Boston. Address reprint requests to Dr. Levy at Children's Hospital, 300 Longwood Ave., IC-106, Boston, MA 02115, or at levy_h@al.tch.harvard.edu.

1572 · November 18, 1999

Homocystinuria's NBS Problem

Proposed solutions:

- **Methionine + Met/Phe ratio:**
  - **Easy to measure and calculate**
  - **Not sensitive, even with low cutoff**



Homocystinuria's NBS Problem

Proposed solutions:

- Methionine + Met/Phe ratio:
  - Easy to measure and calculate
  - Not sensitive, even with low cutoff

- **Molecular genetics of *CBS* gene:**

974 *CBS* variants in ClinVar (www.ncbi.nlm.nih.gov/clinvar; 5/8/2022):

- **Pathogenic (n=165)**
- **Benign (n=95)**

260 variants (27%) of known significance

- **Likely pathogenic (n=91), uncertain significance (n=245), likely benign (n=328), conflicting interpretations (n=50)**

714 variants (73%) of ??? significance

Homocystinuria's NBS Problem

Proposed solutions:

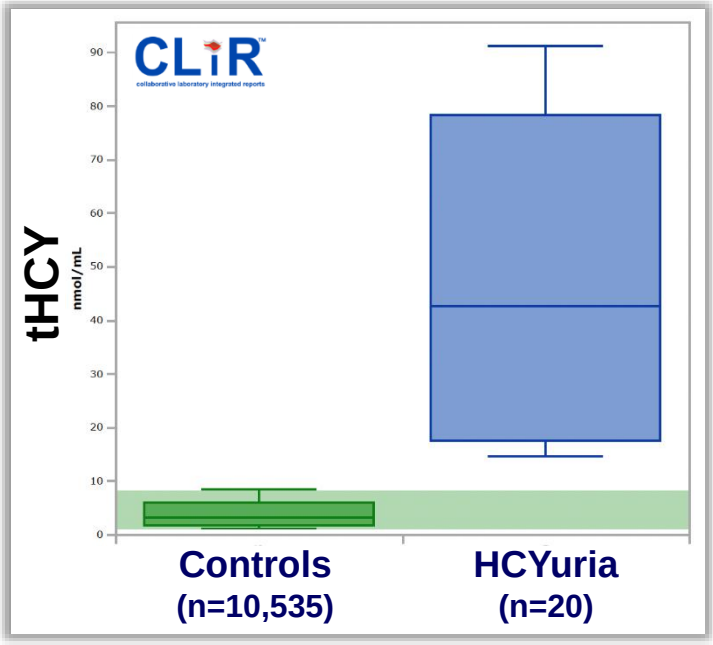
- **total homocysteine, primary screen:**
  - **Sensitive**
  - **Specific (esp. when combined with MMA)**

Clinical Chemistry 67:12
1709–1720 (2021)

General Clinical Chemistry

Combining First and Second-Tier Newborn Screening in a Single Assay Using High-Throughput Chip-Based Capillary Electrophoresis Coupled to High-Resolution Mass Spectrometry

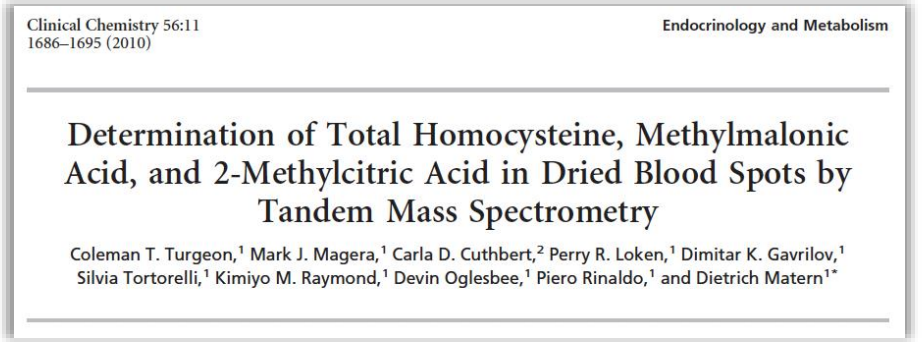
C. Austin Pickens, Samantha L. Isenberg, Carla Cuthbert, and Konstantinos Petritis *



Homocystinuria's NBS Problem

Currently available solution:

- **total homocysteine, 2nd tier test:**
  - **Sensitive**
  - **Requires LC-MS/MS**
  - **Regionalization is an option (Homocystinuria not a time critical condition)**
  - **Can be multiplexed with other markers**

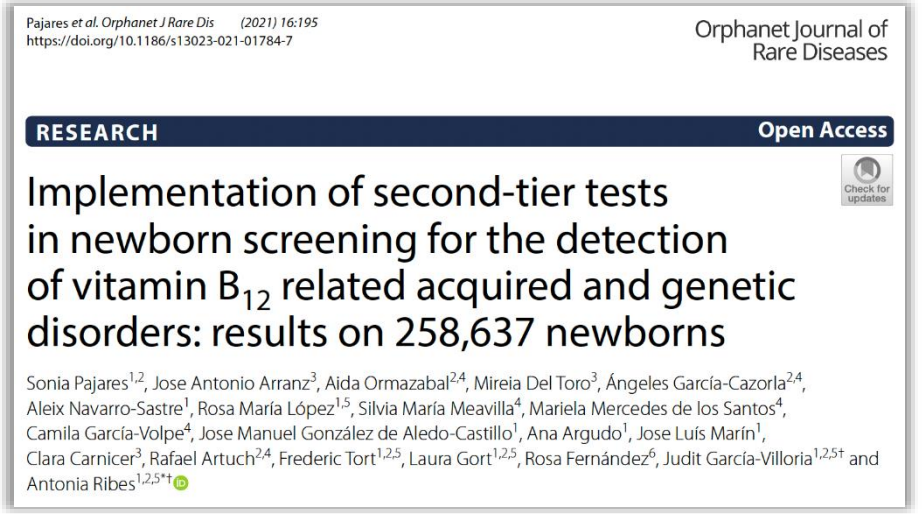


Clinical Chemistry 56:11
1686–1695 (2010)

Endocrinology and Metabolism

Determination of Total Homocysteine, Methylmalonic Acid, and 2-Methylcitric Acid in Dried Blood Spots by Tandem Mass Spectrometry

Coleman T. Turgeon,[1] Mark J. Magera,[1] Carla D. Cuthbert,[2] Perry R. Loken,[1] Dimitar K. Gavrilov,[1] Silvia Tortorelli,[1] Kimiyo M. Raymond,[1] Devin Oglesbee,[1] Piero Rinaldo,[1] and Dietrich Matern[1*]



Pajares *et al. Orphanet J Rare Dis* (2021) 16:195
https://doi.org/10.1186/s13023-021-01784-7

Orphanet Journal of Rare Diseases

RESEARCH

Open Access

Implementation of second-tier tests in newborn screening for the detection of vitamin B_{12} related acquired and genetic disorders: results on 258,637 newborns

Sonia Pajares[1,2], Jose Antonio Arranz[3], Aida Ormazabal[2,4], Mireia Del Toro[3], Ángeles García-Cazorla[2,4], Aleix Navarro-Sastre[1], Rosa María López[1,5], Silvia María Meavilla[4], Mariela Mercedes de los Santos[4], Camila García-Volpe[4], Jose Manuel González de Aledo-Castillo[1], Ana Argudo[1], Jose Luís Marín[1], Clara Carnicer[3], Rafael Artuch[2,4], Frederic Tort[1,2,5], Laura Gort[1,2,5], Rosa Fernández[6], Judit García-Villoria[1,2,5†] and Antonia Ribes[1,2,5*†]

Homocystinuria's NBS Problem

Currently available solution:

- 2nd tier test: total homocysteine, methylmalonic acid, and methylcitric acid
- Used when:
  - C_3-acylcarnitine elevated
  - Methionine elevated
  - Methionine reduced (!!!)

= ca. 1-2% of newborns

J Inherit Metab Dis (2011) 34:137–145
DOI 10.1007/s10545-010-9120-8

HOMOCYSTEINE AND B-VITAMIN METABOLISM

Isolated remethylation disorders: do our treatments benefit patients?

Manuel Schiff · Jean-François Benoist · Bogdana Tilea · Nicolas Royer · Stéphane Giraudier · Hélène Ogier de Baulny

Received: 15 February 2010 / Revised: 17 April 2010 / Accepted: 20 April 2010 / Published online: 21 May 2010

Concluding remarks and future prospects

One of the most important (and challenging) requirements for improving the outcome of isolated remethylation disorders is early recognition followed by aggressive treatment....

initially and then experience rapid neurological or behavioral deterioration. A few patients may have signs of

versible neuromotor impairments. Hematological abnormalities are easily corrected.

Homocystinuria's NBS Problem

Currently available solution:

- 2nd tier test: total homocysteine, methylmalonic acid, and methylcitric acid

International Journal of *Neonatal Screening*

MDPI

Article

The Combined Impact of CLIR Post-Analytical Tools and Second Tier Testing on the Performance of Newborn Screening for Disorders of Propionate, Methionine, and Cobalamin Metabolism

Dimitar K. Gavrilov *, Amy L. Piazza, Gisele Pino, Coleman Turgeon, Dietrich Matern, Devin Oglesbee, Kimiyo Raymond, Silvia Tortorelli and Piero Rinaldo

Biochemical Genetics Laboratory, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905, USA; Piazza.Amy@mayo.edu (A.L.P.); Pino.gisele@mayo.edu (G.P.); Turgeon.Coleman@mayo.edu (C.T.); Matern@mayo.edu (D.M.); Oglesbee.Devin@mayo.edu (D.O.); Raymond.Kimiyo@mayo.edu (K.R.); Tortorelli.Silvia@mayo.edu (S.T.); Rinaldo@mayo.edu (P.R.)
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check for updates

Table 1. Markers of inherited and acquired disorders of propionate, cobalamin, and methionine metabolism, count of cases, and Collaborative Laboratory Integrated Reports (CLIR), tools.

Disorder	Compl. Group	OMIM #	Gene	1st Tier Markers		CLIR		2nd Tier Markers		
				C3	Met	* No. Cases	MS/MS Tool	Hcy	MMA	MCA
Propionic acidemia	**n/a**	**606054**	**PCA, PCB**			136	PROP	N	N	High
Isolated Methylmalonic acidemia	mut^0 mut^-	251000	MCM		N	192	MUT/Cbl AB	N	High	High
	Cbl A	251100	MMAA							
	Cbl B	251100	MMAB							
Methylmalonic acidemia and Homocystinuria	Cbl C	277400	MMACHC			139	Cbl CD			
	Cbl D	277410	MMACHC							
	Cbl F	277380	LMBRD1	High	Low	3	Cbl F	High	High	N to High
	Cbl J	614857	ABCD4			-	-			
	Cbl X	309541	HCFC1			-	-			
Intrinsic factor deficiency	n/a	261000	GIF			-	-			
Megaloblastic anemia-1		261100	CUBN, AMN			-	-			
Transcobalamin II deficiency		275350	TCN2		N	-	-	High	High	N to High
Transcobalamin receptor defect		613646	CD320			11	TCblR			
Maternal Vitamin B12 deficiency		-	-		Low	138	B12 (mat)			
Homocystinuria (CBS deficiency)	n/a	236200	CBS		High	74	HCY			
Homocystinuria and megaloblastic anemia	Cbl G	250940	MTR		Low	11	RMD	High	N	N
	Cbl E	236270	MTRR							
MTHFR deficiency	n/a	236250	MTHFR	N						
Methionine adenosyltransferase def.		250850	MAT 1A							
Adenosine kinase deficiency		180960	ADK		High	112	H-MET	N	N	N
Glycine N-methyltransferase def.		606664	GNMT							
S-adenosylhomocysteine hydrolase def.		613752	AHCY							
FP C3	n/a	n/a	n/a	High	N	124	FP C3	N	N	N
TPN	n/a	n/a	n/a	N	High	2816	TPN	N	N	N

* Count of CLIR cases as of January 31, 2020. Abbreviations as follows: C3, propionylcarnitine; Cbl, cobalamin; CBS, cystathione β-synthase; CLIR, Collaborative Laboratory Integrated Reports (see text); FP, false positive; Hcy, total homocysteine; High, elevated concentration in dried blood spots in >50% of cases; Low, reduced concentration in dried blood spots in >50% of cases; Met, methionine; MCA, 2-methylcitric acid; MMA, methylmalonic acid; mut, mutase; MTHFR, (N)5,10-methylenetetrahydrofolate reductase; n/a, not applicable; N, normal concentration in dried blood spots; N to High, inconsistent elevation in <50% of cases; RMD, remethylation disorders, OMIM #—Online Mendelian Inheritance in Man symbol indicating a descriptive entry, usually phenotype.

Homocystinuria's NBS Problem

Currently available solution:

- 2nd tier test: total homocysteine, methylmalonic acid, and methylcitric acid

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The Combined Impact of CLIR Post-Analytical Tools and Second Tier Testing on the Performance of Newborn Screening for Disorders of Propionate, Methionine, and Cobalamin Metabolism

Dimitar K. Gavrilov *, Amy L. Piazza, Gisele Pino, Coleman Turgeon, Dietrich Matern, Devin Oglesbee, Kimiyo Raymond, Silvia Tortorelli and Piero Rinaldo

Biochemical Genetics Laboratory, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN 55905, USA; Piazza.Amy@mayo.edu (A.L.P.); Pino.gisele@mayo.edu (G.P.); Turgeon.Coleman@mayo.edu (C.T.); Matern@mayo.edu (D.M.); Oglesbee.Devin@mayo.edu (D.O.); Raymond.Kimiyo@mayo.edu (K.R.); Tortorelli.Silvia@mayo.edu (S.T.); Rinaldo@mayo.edu (P.R.)
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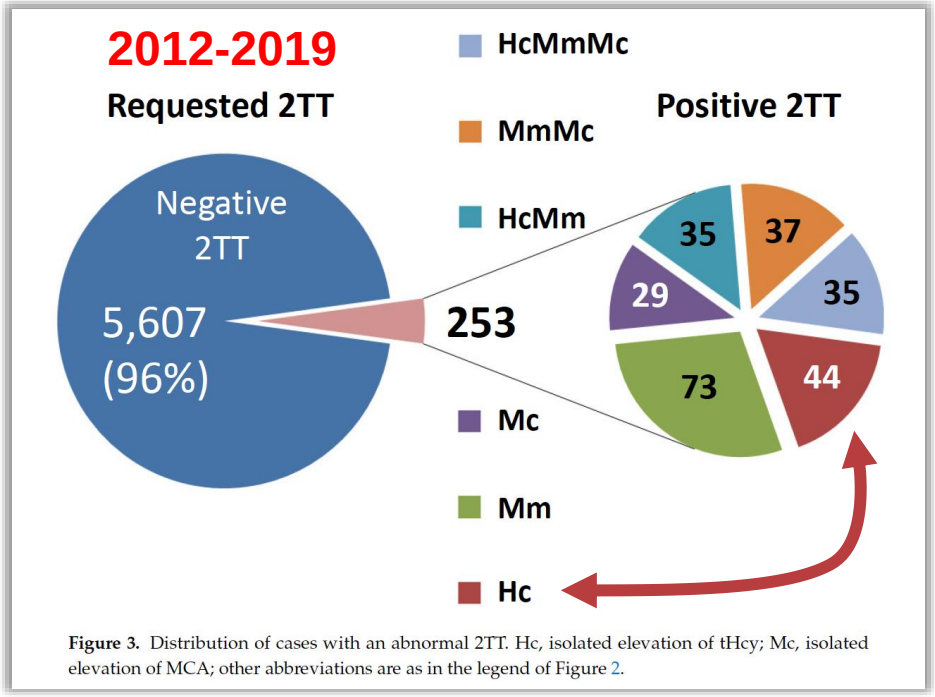
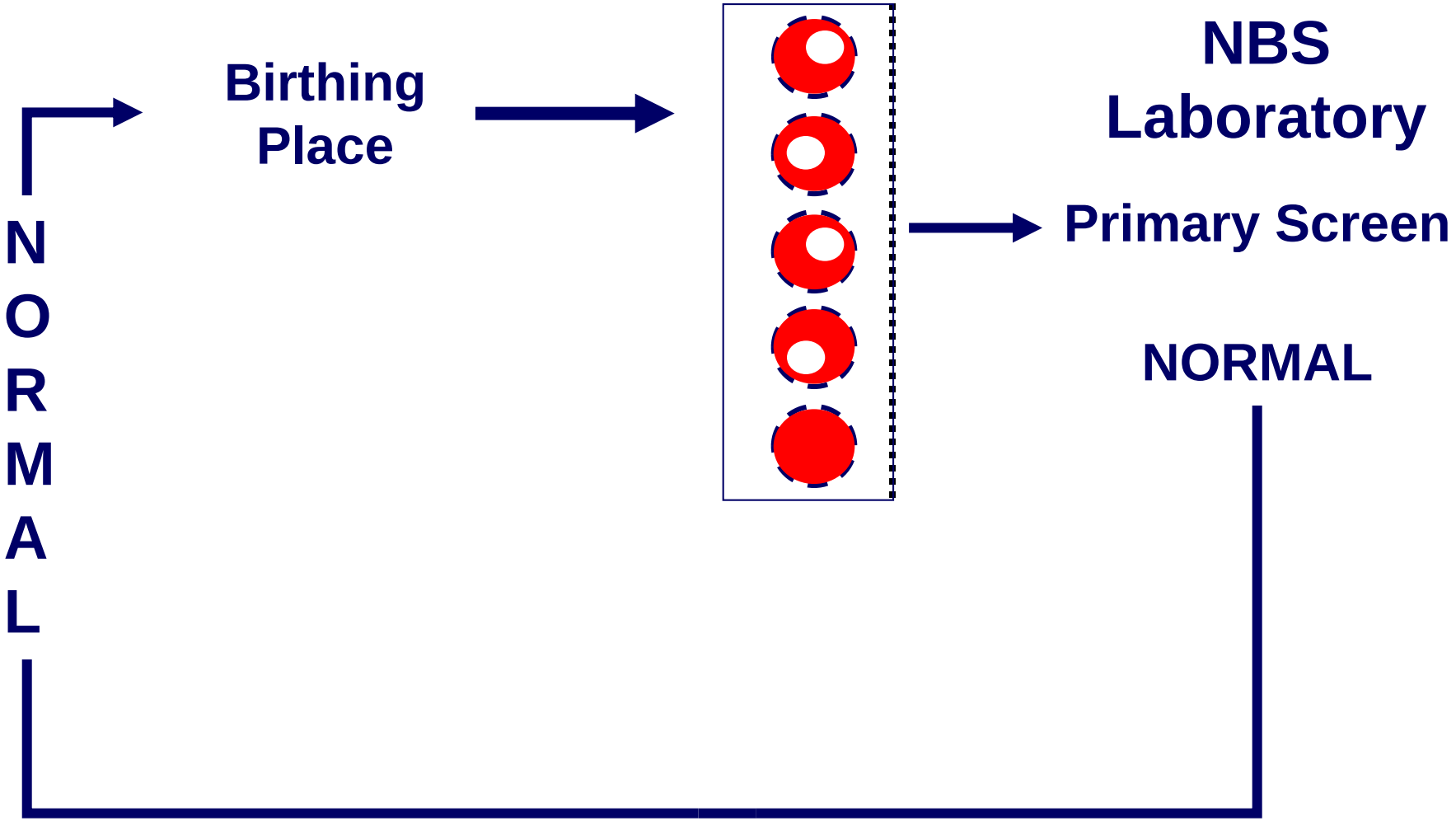


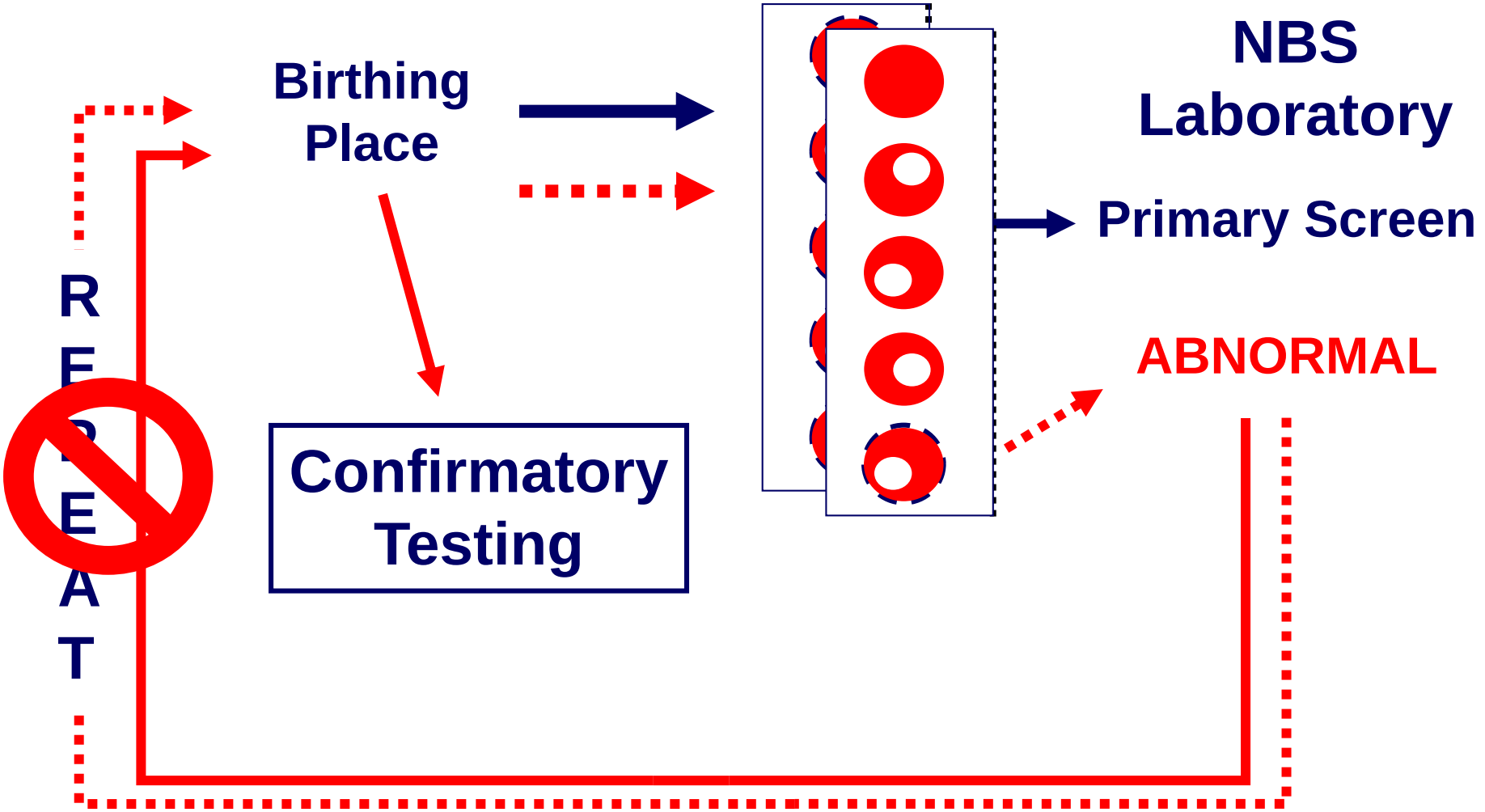
Figure 3. Distribution of cases with an abnormal 2TT. Hc, isolated elevation of tHcy; Mc, isolated elevation of MCA; other abbreviations are as in the legend of Figure 2.

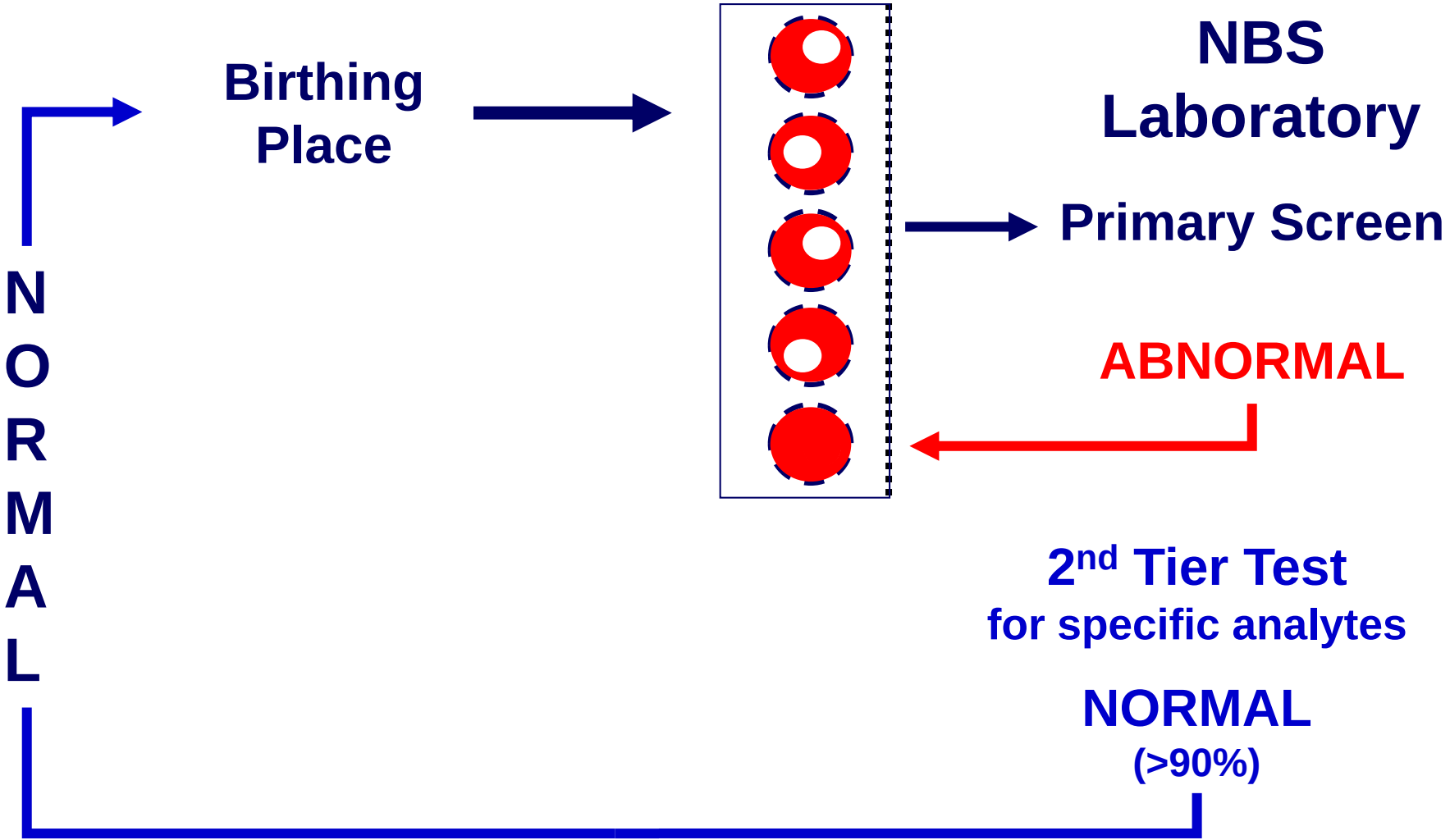
What are 2nd Tier Tests?

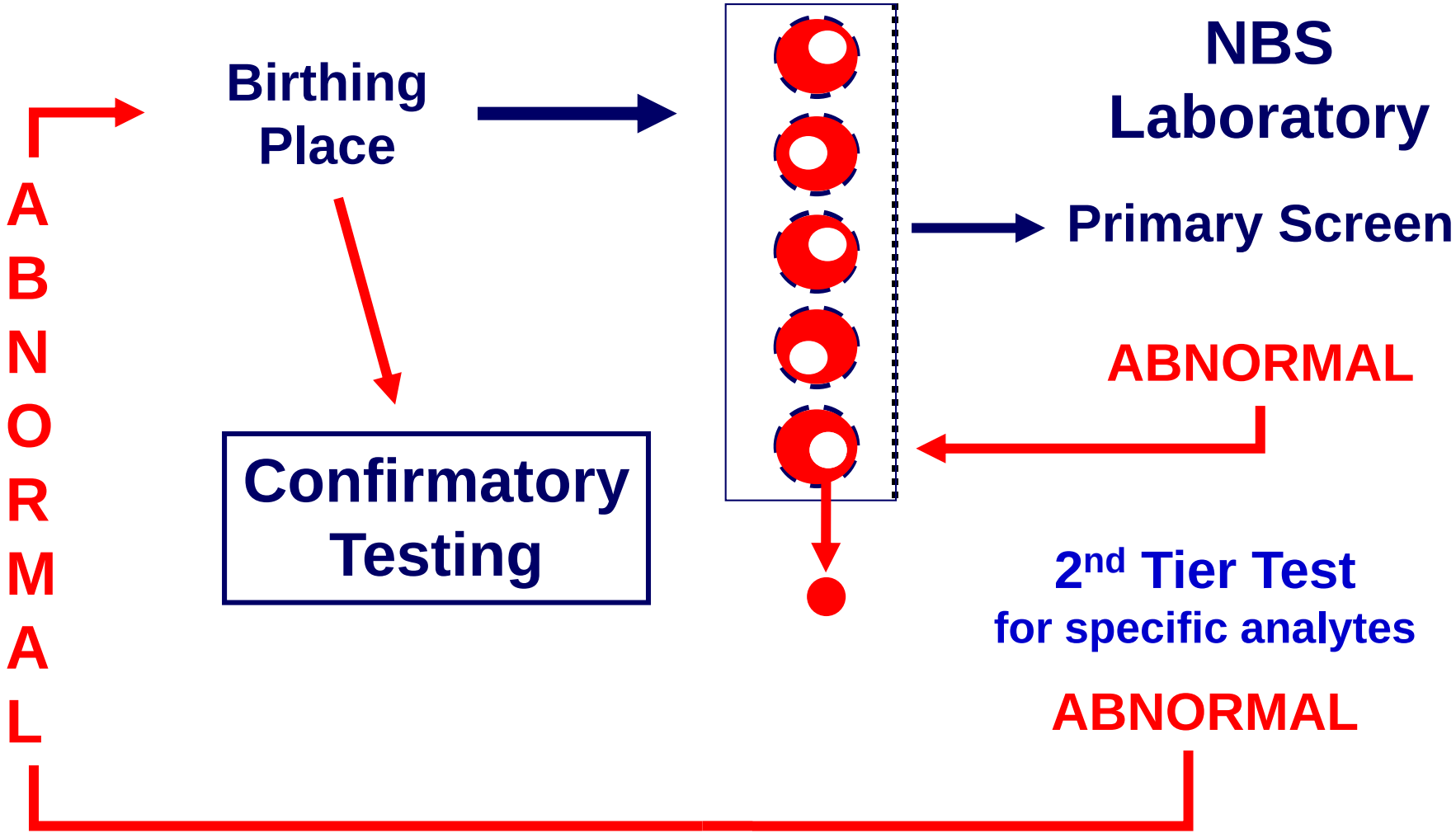
- A cost-effective approach to reduce false positive results when normal population and disease range overlap (poor specificity)
- After primary screen (based on CLIR score or cutoff)
- Same specimen, no additional patient contact
- Normal 2nd tier test result overrules primary screen

  ➔ reduction of false positive results
- Examples: biochemical (e.g. CAH), molecular (e.g. CF)


NORMAL
Birthing Place
NBS Laboratory
Primary Screen
NORMAL


Birthing Place
NBS Laboratory
Primary Screen
ABNORMAL
Confirmatory Testing
REPEAT


Birthing Place
NBS Laboratory
Primary Screen
ABNORMAL
2nd Tier Test
for specific analytes
NORMAL
(>90%)
NORMAL


Birthing Place
NBS Laboratory
Primary Screen
ABNORMAL
Confirmatory Testing
2nd Tier Test
for specific analytes
ABNORMAL
ABNORMAL

Improvement of NBS for C_3 & Met

(4/2005 – 12/2011: 502,978 newborns in MN)

	without 2nd Tier	with 2nd Tier
False Positive	10,900	31
False Positive Rate	2.17%	0.006%
F/U-Cost	$9,384,900	$26,691
Cost (2nd Tier Test)	$0	$370,600
Total F/U-Cost	$9,384,900	$397,291
Difference/Savings	$8,987,609 (96%)	

F/U Costs (physician, clinic, lab)*	$861/Patient
Cost for 2nd Tier Test*	$34/Test

*calculated in 2012 based on ACMG algorithm at the time

Improvement of NBS for C_3 & Met

US Annual Births: ca. 4 Million

	without 2nd Tier	with 2nd Tier
False Positive	86,800	240
False Positive Rate	2.17%	0.006%
F/U-Cost	$74,734,800	$206,640
Cost (2nd Tier Test)	$0	$2,951,200
Total F/U-Cost	$74,734,800	$3,157,840
Difference/Savings	$71,576,960 (96%)	

F/U Costs (physician, clinic, lab)*	$861/Patient
Cost for 2nd Tier Test*	$34/Test

*calculated in 2012 based on ACMG algorithm at the time

Summary

- Newborn screening for Homocystinuria is currently hampered by a marker (Methionine) with poor sensitivity and specificity.
- There is a solution (2nd tier tHCY) that is efficient, effective and accessible if identification of most cases with Homocystinuria was really desired.
- tHCY may be added to new primary screening assays in the future (Petritis K/CDC et al.).
- Reduction of unnecessary health care spending is possible if NBS was truly a “system” and not compartmentalized.

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