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Obiettivi dello screening neonatale

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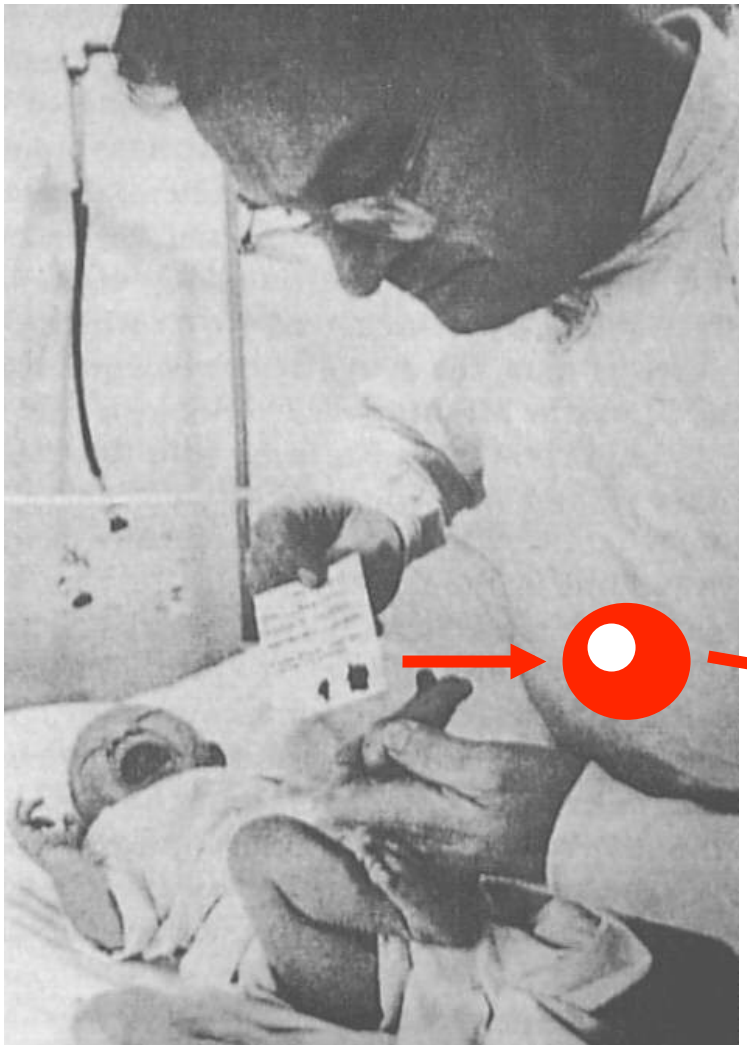
THE GOALS OF NBS

(a) ***Prediction:*** identifying patients before they manifest disease

(b) ***Prevention:*** initiation of therapeutic interventions to forestall the course of the disorders

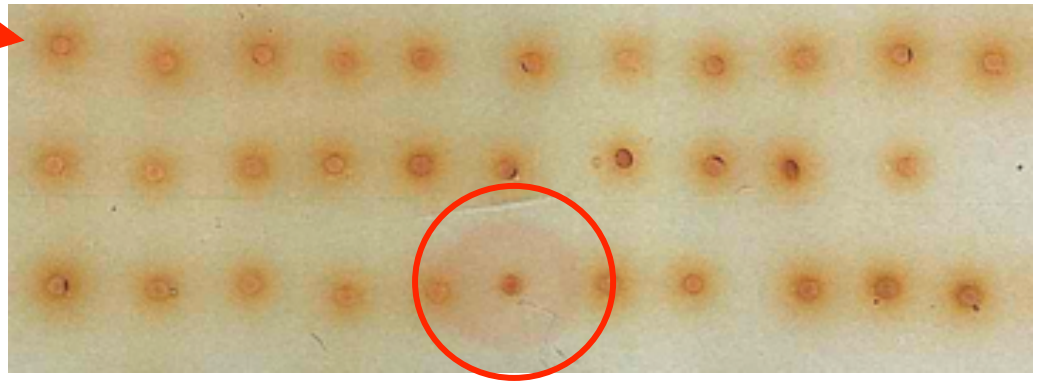
(c) ***Personalization:*** individualizing patients' therapies to optimize their outcomes.

THE EARLY DAYS OF NEWBORN SCREENING



1958: Bacterial inhibition assay (BIA) for PKU (bacterial growth activated by high Phenylalanine concentrations in serum)

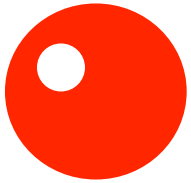
1961: Newborn screening for PKU started using the BIA and blood collected and dried on filter paper (Guthrie card)



THE TRADITIONAL NBS MODEL

(The same for 30+ years….)

- One disease
- One test
- One marker
- One cut-off (N/Abn)



PKU

↓

BIA

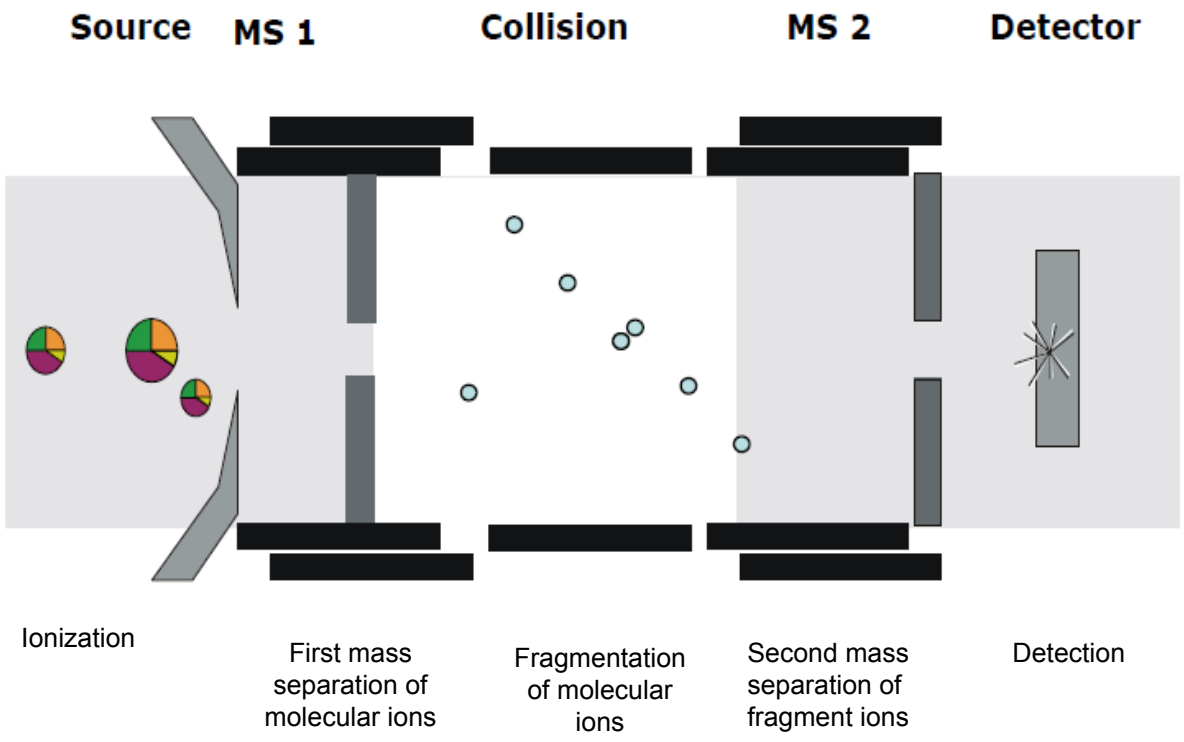
↓

Phe

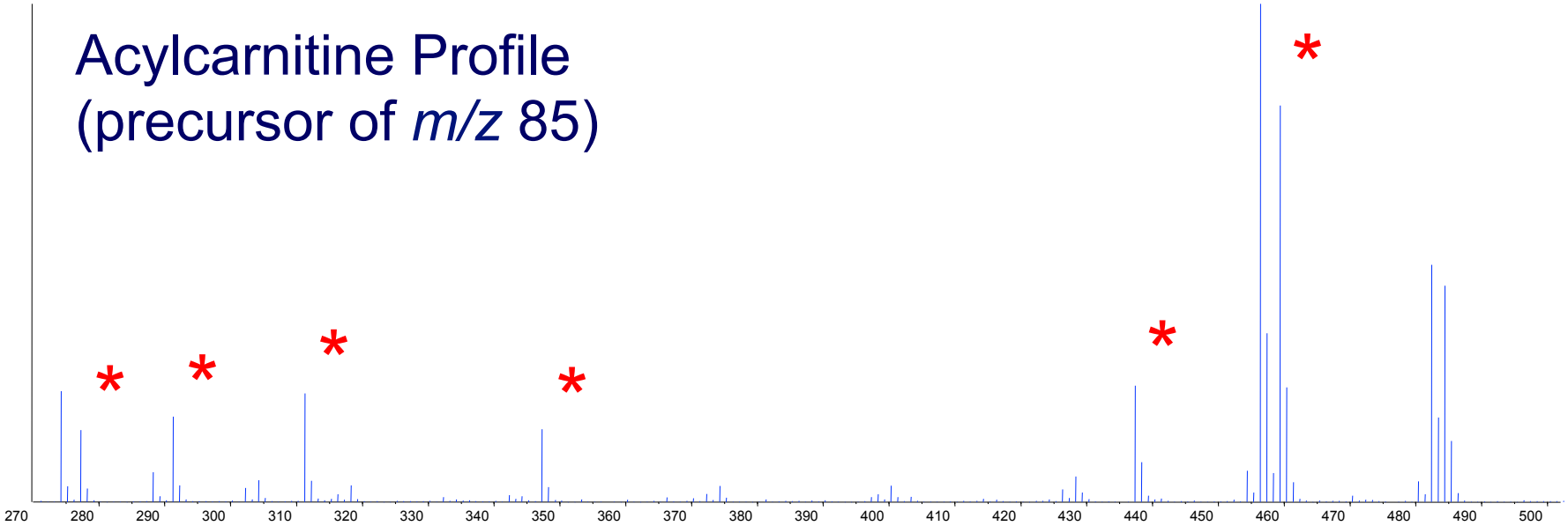
↓

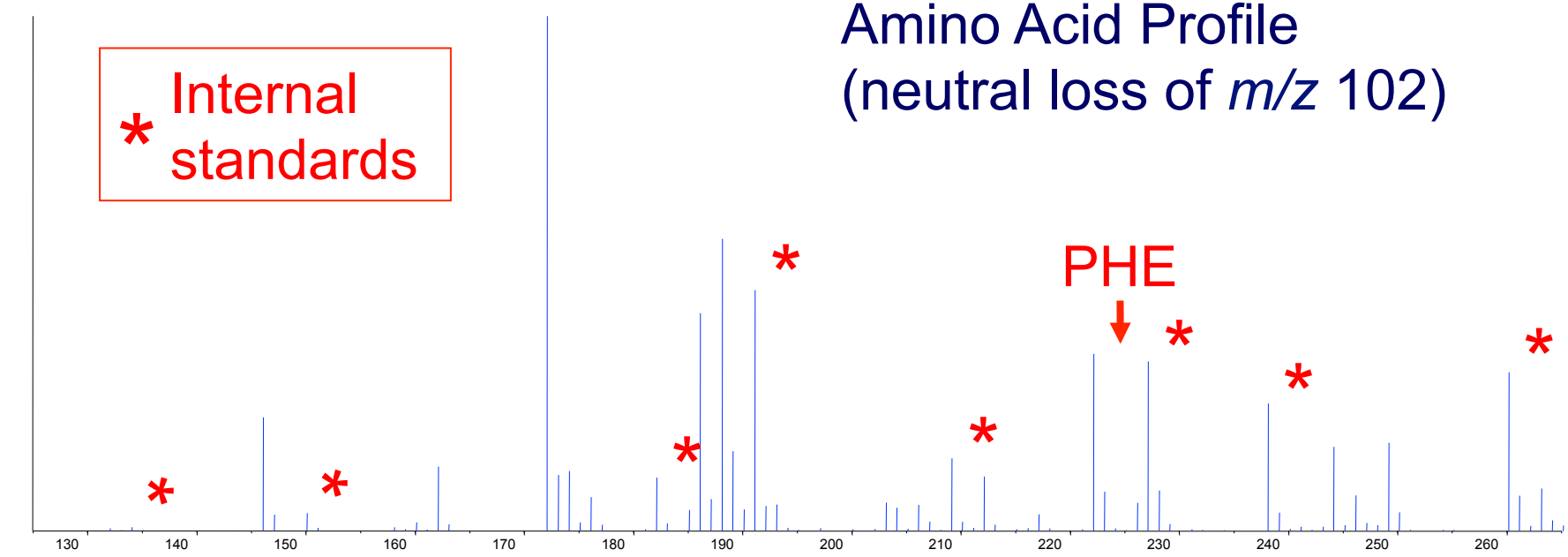
4 mg/dL

ELECTROSPRAY IONISATION TANDEM MASS SPECTROMETRY



NEWBORN SCREENING BY MS/MS





Clinical Chemistry 44, No. 12, 1998

Editorial

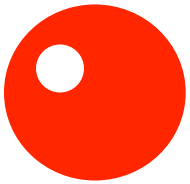
Newborn Screening by Tandem Mass Spectrometry: A New Era

✓ Technology now allows a “sea change” in newborn screening.

✓ In addition to PKU , *it can* identify at least 10 other amino acid disorders, and disorders of organic acid degradation and fatty acid oxidation . *These 20–25 disorders are screened in* the blood specimen, avoiding the need for an additional specimen.

NBS by MS/MS
(Multiplex Testing)

- Many conditions
- One test
- Many markers
- Many cut-offs



$(IEM)_n$

↓

MS/MS

↓

$(AA,AC)_n$

↓

0.1-1,000 µM

Table 1 (continued)

Disease	Methods	Relevance ranking	Screening programs	Test[a] available	Therapy available	Benefit from early detection	References	Remarks
Galactosemia	Substrate and/or enzyme assay	+ +	a	y	y	y	[119]	Long-term outcome not as favorable as initially thought in the 1970s
Glucose-6-phosphate dehydrogenase deficiency	Enzyme assay	?	e	?	y	y	[120]	High genetic variability
Disorders of creatine metabolism	TMS	?	p	?	y	?	[121–123]	Feasibility has been demonstrated, results of pilots not available so far
Lysosomal storage disorders	TMS	?	p	?	y	?	[124, 125]	Enzyme replacement therapy is available for M. Fabry, M. Gaucher, M. Krabbe, M. Niemann-Pick A/B, and M. Pompe
Cystic fibrosis	IRT/DNA	+ +	m	y	y	y	[126–129]	
Diabetes mellitus type I	DNA	?	p	?	?	?	[130]	"Genetic risk" screening
Other diseases								
Hearing deficiency	Otoacoustic	+ +	m	y	y	y	[131]	Decentralized
Congenital CMV infection	CMV viral load	+	m	y	y	y	[132–138]	Late-onset hearing loss is not detectable by the otoacoustic method in newborns
Congenital toxoplasmosis infection	Toxoplasmosis viral load	- -	mat	- -	- -	- -	[139]	Not recommended, (prenatal care)
Congenital syphilis infection	Nontreponemal antibodies	- -	mat/epd	- -	- -	- -	[140]	Not recommended, (prenatal care)
Neuroblastoma screening[b]	HPLC	- -	d	- -	- -	n	[141–143]	Not recommended
Duchenne muscular dystrophy	Creatine kinase activity	- -	p	y	n	n	[144, 145]	Disposition-screening; no effect on outcome
SCID	T-cell lymphopenia	?	pro	- -	- -	- -	[146]	Not recommended
HIV	Immunoassays	?	epd	- -	- -	- -	[147]	Not recommended
Hepatitis C	Immunoassays	?	epd	- -	- -	- -	[148]	Not recommended, (prenatal care)
Hepatitis B	HBsAg	?	epd	- -	- -	- -	[149]	Not recommended, (prenatal care)

CPT-I carnitine palmitoyl transferase I, *CPT-II* carnitine palmitoyl transferase II, *HBsAg* hepatitis B surface antigen, *HHH* hyperornithinemia–hyperammonemia–homocitrullinuria, *HPLC* high-performance liquid chromatography, *IEF* isoelectric focusing, *IRT* immunoreactive trypsin, *LCHAD* long-chain hydroxyacyl-CoA dehydrogenase, *MCAD* medium-chain acyl-CoA dehydrogenase, *3-MCC* 3-methylcrotonyl-CoA carboxylase, *NBS* newborn screening, *OAT* ornithine amino transferase, *SCAD* short-chain acyl-CoA dehydrogenase, *SCID* severe combined immunodeficiency, *TFP* trifunctional protein, *TLC* thin-layer chromatography, *TMS* tandem mass spectrometry, *VLCAD* very long chain acyl-CoA dehydrogenase, *a* all, *d* discontinued, *e* ethnic, *epd* epidemiologic, *m* most, *mat* recommended as a prenatal screening test, *n* no, *p* pilot, *pro* proposed, *y* yes, + + unquestioned, + favorable, ? questionable, - unfavorable, - - not recommended

[a] With sufficient sensitivity and specificity, economically justifiable

[b] Specimen for screening is urine dried on filter paper

Table 1 (continued)

Disease	Methods	Relevance ranking	Screening programs	Test[a] available	Therapy available	Benefit from early detection	References	Remarks
3-MCC deficiency	TMS	-	m	?	y	?	[95]	Low clinical expressivity and penetrance
3-Hydroxymethylglutaryl-CoA lyase deficiency	TMS	?	m	y	y	y	[96]	Reliable discrimination from 3-MCC not possible
Holocarboxylase synthase deficiency	TMS	?	m	y	y	y	[97]	Very rare, but easily treatable with biotin; reliable discrimination from 3-MCC not possible
β-Ketothiolase deficiency	TMS	+	m	?	y	y	[98]	Sensitivity and specificity presumably low
Disorders of glutathione metabolism	TMS	?	m	?	?	?	[99]	No prospective data
β-Oxidation defects/disorders of carnitine metabolism								
SCAD deficiency	TMS	- -	m	?	?	?	[100]	Causality between enzyme defect and clinical presentation is not proven
MCAD deficiency	TMS	+ +	a	y	y	y	[101, 102]	Positive effect unquestioned; however, patients that might never become symptomatic are also detected
VLCAD deficiency	TMS	+ +	m	y	y	y	[103]	Mild variants might be missed when the samples are taken under anabolic conditions
LCHAD/TFP deficiency	TMS	+	m	y	y	y/?	[104–106]	Information on long-term outcome is still pending; prognosis for TFP is rather bad
Carnitine transporter deficiency	TMS	+	m	?	y	y	[107]	Sensitivity unclear; free carnitine level can be normal postpartum, depending on maternal supply and renal loss
CPT-I deficiency	TMS	+ +	m	y	y	y	[108]	Ratio of free carnitine to the sum of palmitoylcarnitine and stearylcarnitine is sensitive and highly specific
CPT-II deficiency	TMS	+	m	?	?	?	[109]	Neonatal onset form with bad prognosis despite early diagnosis; in the late-onset form mainly skeletal muscle is involved, seems to have normal levels of acylcarnitines in the neonatal period
Translocase deficiency	TMS	+	m	y	?	?	[110]	Bad prognosis despite early diagnosis
Endocrinopathies								
Congenital hypothyroidism	ELISA	+ +	a	y	y	y	[111]	
Congenital adrenal hyperplasia	ELISA	+ +	a	y	y	y	[112, 113]	Sensitivity for the salt-wasting form is good, for simple virilizing congenital adrenal hyperplasia approximately 50%
Hemoglobinopathies								
Sickle cell anemia	IEF	+ +	e	y	y	y	[114–116]	
Hemoglobin S/β-thalassemia	IEF	+ +	e	y	y	y		
Hemoglobin SC disease	IEF	+ +	e	y	y	y		
Hemoglobin H	IEF	+ +	e	y	y	y		
Other inborn errors of metabolism								
Biotinidase deficiency	Enzyme assay	+ +	a	y	y	y	[117, 118]	

Table 1 Target diseases for newborn screening

Disease	Methods	Relevance ranking	Screening programs	Test[a] available	Therapy available	Benefit from early detection	References	Remarks
Amino acidopathies								
Phenylketonuria	TMS	+ +	a	y	y	y	[54–58]	Alternative therapies for mild phenylketonuria have been introduced recently
Maple syrup urine disease	TMS	+ +	m	y	y	y	[59–62]	Early blood collection is mandatory
Homocystinuria	TMS	+	m	n	y	y	[13, 63, 64]	Sensitivity and specificity low with methionine as a primary marker; determination of homocysteine could improve NBS
Tyrosinemia type I	TMS	+	m	y/?	y	y	[65–69]	Low sensitivity and low specificity with tyrosine as primary marker; determination of succinyl acetone could improve NBS
Citrullinemia	TMS	+	m	?	?	?	[70]	No positive effect on outcome; patients with a mild biochemical phenotype might never develop symptoms
Argininosuccinic acidemia	TMS	+	m	?	?	?	[71]	No positive effect on outcome
Arginase deficiency	TMS	+	m	?	?	y	[72–74]	Very rare; the first results of NBS and early treatment seem promising
Hyperornithinemia (OAT deficiency and HHH syndrome)	TMS	?	m	n	?	?	[75]	Normal ornithine levels during the first weeks of life
Nonketotic hyperglycinemia	TMS	- -	m	n	n	n	[76]	No therapy available
Histidinemia	TLC	- -	d	- -	- -	n	[77–81]	Benign, does not require treatment
Hydroxyprolinemia	TLC	- -	d	- -	- -	n	[82–84]	Benign, does not require treatment
Organic acidurias								
Glutaric aciduria type I	TMS	+ +	m	y	y	y	[85, 86]	
Isovaleric academia	TMS	+ +	m	y	y	y	[87, 88]	Screening also detects unaffected patients with mild variants
Propionic academia	TMS	+	m	y	y	?	[89, 90]	Acylcarnitine profile indistinguishable from methylmalonic academia profile in newborns
Methylmalonic academia (mutase)	TMS	+	m	y	y	?	[90]	Acylcarnitine profile indistinguishable from propionic academia profile in newborns
Methylmalonic academia (disorders of cobalamine metabolism types A–D, F)	TMS	+	m	y	y	?	[91]	Sensitivity unclear; propionylcarnitine level is often only slightly elevated
Cobalamin EG defect	TMS	- -	?	?	?	?	[92]	Low methionine level is the only marker; sensitivity and specificity unknown, but presumably low; determination of homocysteine in dried blood spots could improve NBS
Malonyl-CoA decarboxylase deficiency	TMS	+	m	y	y	y	[93, 94]	Very rare; no prospective data

UP TO 60 DISEASES

TANDEM – MS DISEASE PANELS IN EUROPEAN COUNTRIES

Table 2 Comparison of European Countries (***excluding Scotland) regarding number of screening centres, total population (year 2001*), screened infants (year 2003**) and metabolic disorders included in MS/MS screening; see Table 1 for abbreviations. The numbers for screening centres, total population and screened infants are partially based on a questionnaire that was initiated by ISNS in 2003. Details can be found on www.isns-neoscreening.org

Country	No of centres	Population*(millions)	Number of infants screened**(average sample number/ screening laboratory)	Disorders included in extended screening by MS/MS
Austria(including South Tyrol, Italy)	1	8.18	77 186(77 186)	PKU, MSUD, TyrI, Cit, ASLD, Homocyst, MCADD, LCHADD, VLCADD, CPT ID CPT IID/CACT, CTD, KTD, HMG-CoA LD, MMA, PA, IVA, GA 1, 3-MCCD
Belgium	6	10.29	105 335(17 555)	PKU, MSUD, TyrI, MCADD, LCHADD, VLCADD, CPT ID CPT IID/CACT, CTD, KTD, HMG-CoA LD, MMA, PA, IVA, GA I, 3-MCCD
Bulgaria	1	7.55	63 190	None
Croatia	1	4.43	No information	None
Czech Republic	4	10.25	93 685(23 421)	None
Denmark	1	5.38	66 657(66 657)	PKU, MSUD, Cit, ASLD, ArginaseD, MCADD, LCHADD, VLCADD, CPT ID CPT IID/CACT, CTD, KTD, HMG-CoA LD, MMA, PA, IVA, GA I, 3-MCCD (pilot study, not 100% population coverage)
Finland	40	5.19	56 000(1 400)	None
France	22	59.94	764 212(34 737)	None
Germany	13	83.43	725 125(60 427)	PKU, MSUD, MCADD, LCHADD, VLCADD, CPT ID, CPT IID/CACT, IVA, GA I
Great Britain***	20	54.78	625 749(32 287)	MCADD (pilot project, not 100% population coverage)
Hungary	4	10.05	approx. 100 000(25 000)	None
Iceland	1	0.308	4 000(4 000)	None
Ireland	1	3.92	62 000(62 000)	None
Italy	22	57.74	566 169(25 734)	No information
Netherlands	5	16.14	200 635(40 127)	PKU, MSUD, Homocyst, TyrI, MCADD, LCHADD, VLCADD, HMG-CoA LyaseD, IVA, GA I, 3-MCCD
Norway	1	4.54	56 846(56 846)	None
Poland	8	38.62	352 152(44 019)	PKU, MSUD, TyrI, MCADD, LCHADD, VLCADD, CPT ID, CPT IID/CACT, CTD, IVA, GA I; one centre screens for 30% of population
Portugal	1	10.10	112 557(112 557)	PKU, MSUD, MCADD, LCHADD, VLCADD, CPT ID, CPT IID/CACT, IVA, GA I, GAMTD
Romania	No information	22.28	No information	None
Serbia	1	10.50	57 354(57 354)	None
Slovakia	1	5.43	No information	None
Slovenia	1	1.94	14 000(14,000)	None
Spain	20	40.11	441 297(22,064)	PKU, MSUD, MCADD, LCHADD, VLCADD, CPT ID, CPT IID/CACT, IVA, GA I; one centre screens <10% of population)
Switzerland	1	7.32	74 450 74 450	PKU, MCADD
Total	176	478 418	4 618 599	

Netherlands
9 Metabolic disorders
PKU, MSUD, Hcys, Tyr I, MCAD, LCHAD, VLCAD,HMG-CoAL, MMA, PA, GA 1, IVA, 3-MCC

UK
2 Metabolic disorders
PKU, MCAD
Considering Hcys
GA 1, MSUD, IVA, LCHAD, Biotinidase

Germany
9 Metabolic disorders
PKU, MSUD, MCAD, LCHAD, VLCAD,CPT I, CPT II/CACT, GA 1, IVA,


Valle
d'Aosta
Trentino
Yes
Friuli
Lombardia
Yes
Veneto
Piemonte
Emilia Romagna
Yes
Yes
Yes
Marche
Liguria
Toscana
Yes
Umbria
Abruzzo
Molise
Yes
Lazio
Yes
Puglia
Campania
Sardegna
Basilicata
Calabria
Sicilia

Pompe Disease in Infants: Improving the Prognosis by Newborn Screening and Early Treatment

Pediatrics 2009;124:e1116–e1125

TABLE 1 Characteristics of Patients Whose Pompe Disease Was Detected by Screening

Characteristic	NBS1	NBS2	NBS3	NBS4	NBS5	NBS6
Gestational age, wk	38	39	40	39	37	38
Gender	Female	Female	Male	Male	Female	Male
Birth weight, kg	3.3	3.0	2.9	3.9	3.3	3.4
Age at diagnosis, d	40	19	22	9	33	7
Body weight at diagnosis, kg	4.4	3.3	3.2	3.5	4.6	3.3
Symptoms at diagnosis	—	—	Crying cyanosis	Feeding cyanosis	Feeding cyanosis	—
Age at first infusion	14 mo	26 d	29 d	17 d	34 d	12 d
GAA activity, nmol/mg per h[a]						
Lymphocyte	1.83	0.34	1.58	0.45	3.82	0.83
Fibroblast	0.64	0.06	—	0.11	0.14	0.06
CRIM status	+	+	+	+	+	+
GAA gene mutation						
Allele 1	c.424_440del (p.S142LfsX29)[b]	c.1935C→A (p.D645E)	c.1935C→A (p.D645E)	c.1935C→A (p.D645E)	c.1935C→A (p.D645E)	c.1935C→A (p.D645E)
Allele 2	c.811A→G (p.T271A)[b]	c.1411_1414delGAGA (p.E471fsX5)	c.2842insT (p.L948LfsX70)[b]	c.784G→A (p.E262K)	c.1935C→A (p.D645E)	c.1062C→G (p.Y354X)
Current age, mo	40	34	33	26	20	15
Gross motor status, walk at *n* mo	15	14	20	14	12.5	13
CK, U/L	101	922	1126	597	565	752
Echocardiography						
LVEF, %	70	56	70	66	73	77
LVMI, g/m^2	42.7	120.0	170.1	186.0	120.3	108.9

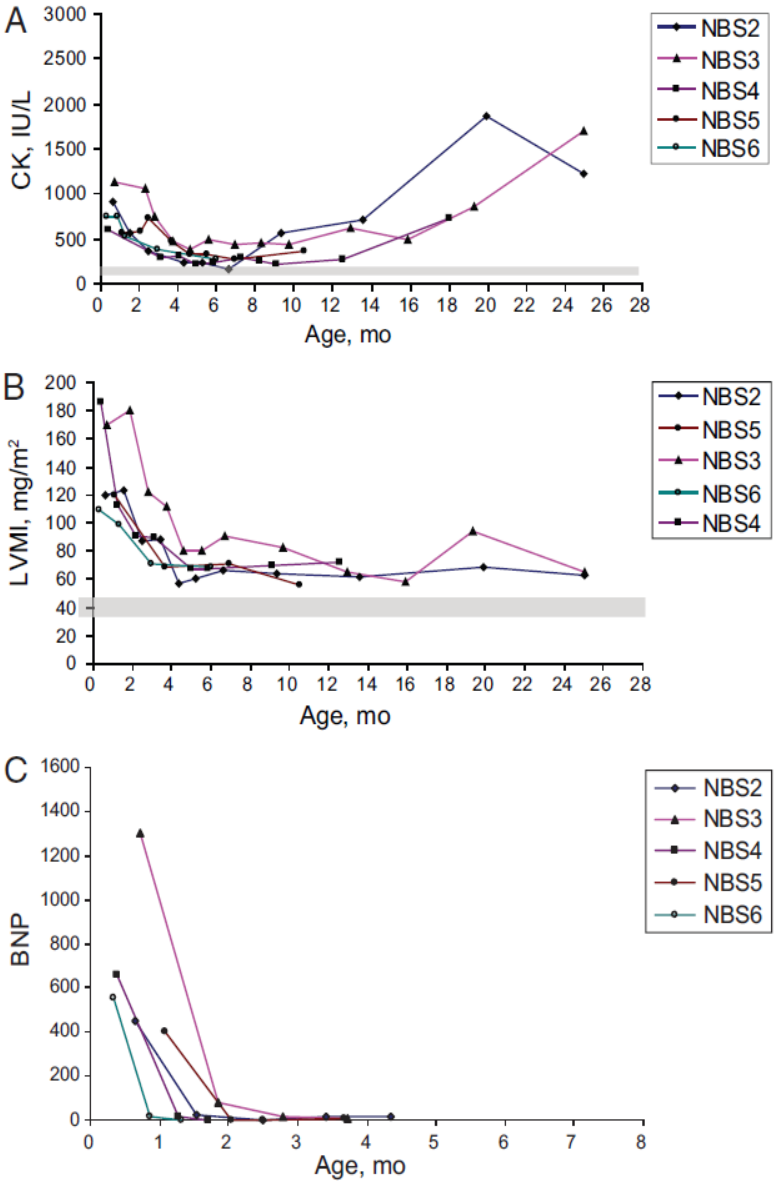
LVEF indicates left ventricular ejection fraction.

[a] Normal range for GAA activity in lymphocytes and fibroblasts is >60 nmol/mg per h.

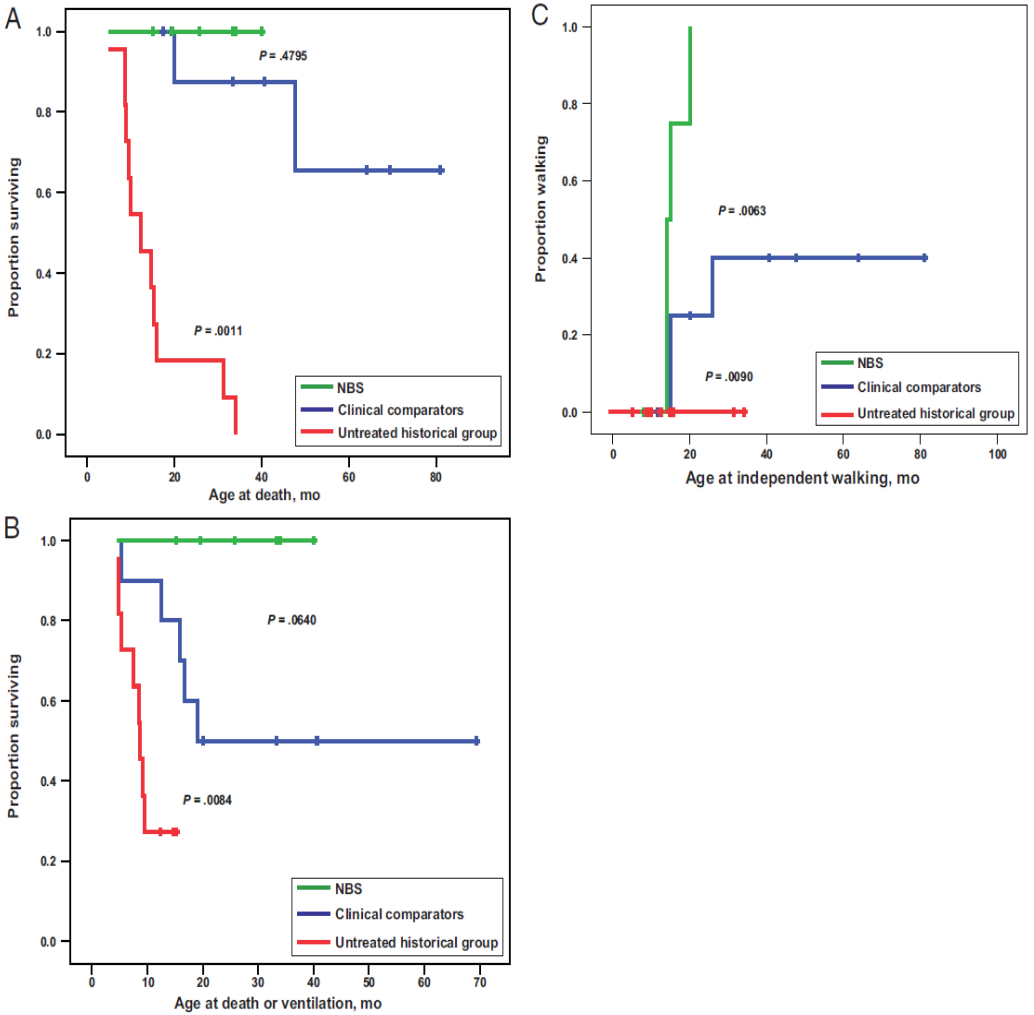
[b] Novel mutation.

INCIDENCE : 6/206088 IN TAIWAN (1/40 000 REST OF THE WORD)

CARDIAC PARAMETERS



SURVIVAL AND MOTOR OUTCOMES



LESSONS FROM EXPANDED NBS FOR IEM

✓ Through NBS we have created the “laboratory” for **personalized medicine.**

✓ Personalized medicine will be **predictive and preventive**. It will involve screening large populations to identify individual differences, to be able to predict disease predispositions, and to attempt to anticipate and prevent the consequences of these predispositions.

✓ **NBS** can be used as the **model for personalized medicine**.

Table 1. FPRs (Detection Rates in Parenthesis) as Reported From Various NBS Pilot Studies (IL, WA, Austria) and a NBS Program in Taiwan

	Illinois[a] (Digital Microfluidics)	Washington[b] (LC MS/MS)	Austria (LC MS/MS)	Taiwan (Fluorometry)
Fabry	0.05%[b] (1:1,144)	0.005%[b] (1:16,667)	0.055%[b] (1:3,859)	0.87%[b] (1:1,2410
Gaucher	0.25%[b] (1:4,006)	–	0.006%[b] (1:17,368)	–
Krabbe	–	–	–	–
MPS I	–	0.005%[b] (1:30,000)	–	–
NPA/B	–	–	0.003% (Not detected)	–
Pompe	0.025%	0.013%[b] (1:30,000)	0.006%[b] (1:8,6840	0.83%[b] (1:33,135)

[a]Burton et al. 2012; [b]Scott et al. 2012; [c]PPV, 64%; [d]PPV, 50%; [e]PPV, 32%; [f]Data combined from two separate studies (PPV, 5%) (Hwu et al., 2009; Lin et al., 2009); [g]PPV, 9%; [h]PPV, 50%; [i]PPV, 40%; [j]PPV, 20%.; [k]PPV, 80%; [l]PPV, 0.36%.

Table 2. Comparison of the Multiplexing Potential of Various NBS Assays Proposed for Specific LSDs, XALD, WD, and FRDA

Condition	FIA MS/MS	LC MS/MS[b]	LC MS/MS[c]	LC MS/MS[d]	Immuno capture	DM[e]
Fabry disease	+	+			+	+
Gaucher disease	+	+			+	+
Krabbe disease	+	+			+	
MLD					+	
MPS I	+	+			+	+
MPS II		+			+	
MPS IIIA					+	
MPS IIIB					+	
MPS IV		+				
MPS VI		+			+	
ML II/III					+	
NPA/B	+	+			+	+
Pompe disease	+	+			+	+
FRDA					+	
WD			+		+	
aCP[a]			+		+	
Menke disease[a]			+		+	
AT 1 deficiency[a]			+		+	
XALD	+			+		
ZSD[a]	+			+		
Acyl CoA oxidase def.[a]	+			+		
Bifunctional protein deficiency[a]	+			+		
Number of conditions	10	9	4	4	17	5

[a]Conditions not considered primary targets by proponents.
[b]Spacil et al. 2011.
[c]deWilde et al. 2008.
[d]Hubbard et al. 2009.
[e]Sista et al. 2011.
Abbreviations: aCP, aceruloplasminemia; AT-1, acetyl-CoA transporter; MLD, metachromatic leukodystrophy; MPS, mucopolysaccharidosis; ML, mucolipidosis; NPA/B, Niemann Pick A/B; FRDA, Friedreich ataxia; WD, Wilson disease; XALD, X-adrenoleukodystrophy; ZSD, Zellweger spectrum disorders.