Applicazione su Larga Scala della Spettrometria di Massa in Medicina di Laboratorio

Piero Rinaldo, MD, PhD

Professor of Laboratory Medicine

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Mayo Clinic College of Medicine

Rochester, MN (USA)

Firenze, May 8th, 2009

CAD MIKES: A New Method for a Rapid and Unequivocal Structural Identification of Organic Acids in Biological Fluids

1984

A First Application to a Case of Methylmalonic Aciduria

BIOMEDICAL MASS SPECTROMETRY VOL 11 NO 12 1984 643

Piero Rinaldo,† Lino Chiandetti and Franco Zacchello
Dipartimento di Pediatria, Via Giustiniani, 3, I-35128 PADOVA, Italy

Sergio Daolio and Pietro Traldi
Istituto di Polarografia ed Elettrochimica Preparativa del CNR, Corso Stati Uniti 4, I-35100 PADOVA, Italy

Hydroxyl Negative Chemical Ionization Mass Spectrometry Linked with Collisionally Activated Decomposition. A Modern Analytical Tool in Inborn Errors of Metabolism

570 BIOMEDICAL MASS SPECTROMETRY, VOL. 12, NO. 9, 1985

P. Rinaldo,† G. Miolo, L. Chiandetti and F. Zacchello
Dipartimento di Pediatria, Universitá di Padova, viale Giustiniani 3, I-35128 Padova, Italy

S. Daolio and P. Traldi
Istituto di Polarografia ed Elettrochimica del CNR, corso Stati Uniti 4, I-35100 Padova, Italy

1985

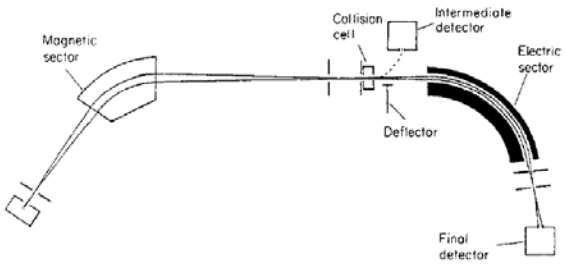


Figure 1. Schematic representation of the ZAB-2F mass spectrometer.

John H. Beynon (RSRU 1981)

REVIEW PAPER ORGANIC MASS SPECTROMETRY, VOL. 16, NO. 3, 1981 101

The Modern Mass Spectrometer— A Complete Chemical Laboratory

C. J. Porter, J. H. Beynon and T. Ast†
Royal Society Research Unit, University College of Swansea, Singleton Park, Swansea SA2 8PP, UK

The Modern Mass Spectrometer: A Complete Chemical Laboratory

- Can be coupled with many separation techniques
- Can handle solids, liquids, and gases
- Can be used with different ionization techniques to highlight particular properties of the sample
- Can produce positive and negative ions
- Can produce ions carrying more than one charge
- Can separate ions according to their mass, momentum or kinetic energy
- Can study specific properties of separated ions
- Can be computer controlled

(OMS 16:101,1981)

RAPID COMMUNICATIONS IN MASS SPECTROMETRY
Rapid Commun. Mass Spectrom. 2004; **18**: 13–20
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/rcm.1301

John H. Beynon: the Swansea years 1974–1986

Gareth Brenton
University of Wales, Swansea, UK

In the meantime John had a major grant application in collaboration with Dr. Dudley Williams (Cambridge University) to purchase a ZAB mass spectrometer. As the story goes, VG Micromass (Manchester, UK) were developing a brand new high-resolution double-focusing mass spectrometer. The designers of the spectrometer at VG, Robert Bateman and Brian Green and their team, were wooed by John to deliver the first instrument to him and Dudley and also to construct the instrument as 'revered geometry' so that mass-analysed ion kinetic energy spectrometry (MIKES) could be undertaken. This enhancement of IKES had first been achieved by John and his group at Purdue University. After IKES this was the next important development of tandem mass spectrometry.

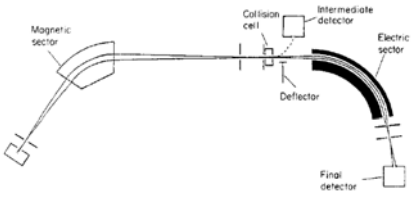
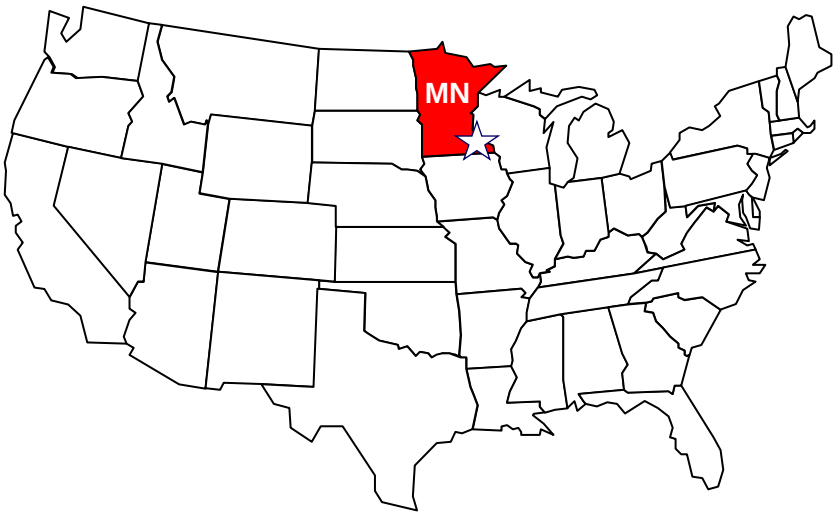


Figure 1. Schematic representation of the ZAB-2F mass spectrometer.

The Tandem Mass Spectrometer: A Complete CLINICAL Laboratory

- Can be coupled with many separation techniques
- Can handle solids and liquids
- Can be used with different ionization techniques to highlight particular properties of the sample
- Can produce positive and negative ions
- Can produce ions carrying more than one charge
- Can separate ions according to their mass
- Can study specific properties of separated ions
- Can provide high-throughput clinical testing
- Can improve quality and reduce cost (= VALUE)

Rochester, MN



mayo

Three shields

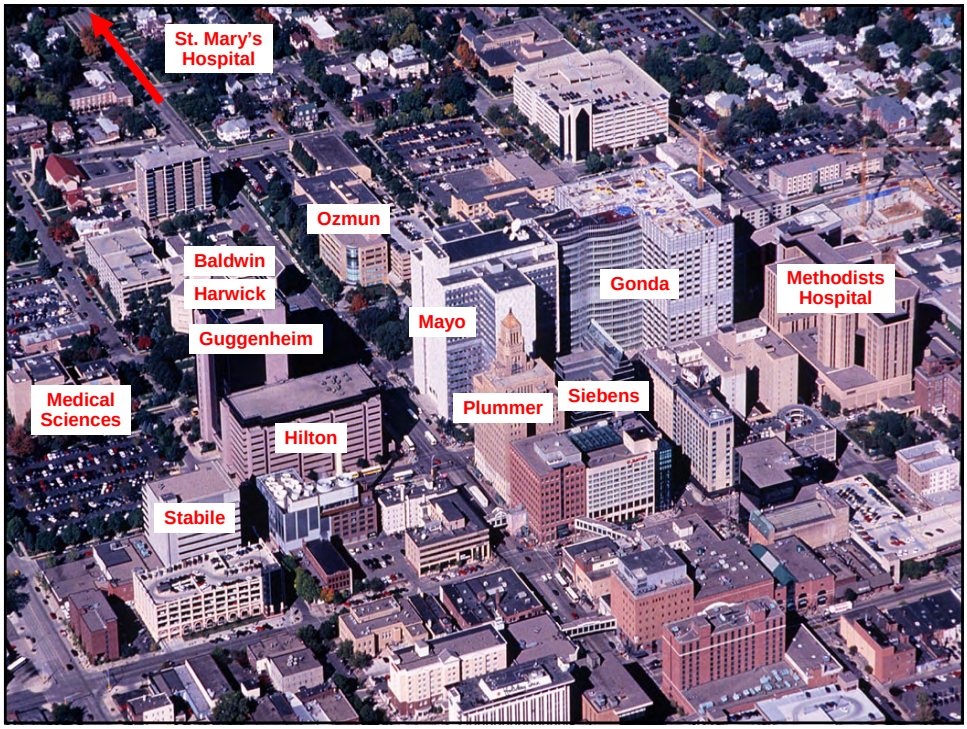
Patient care

Education

Research

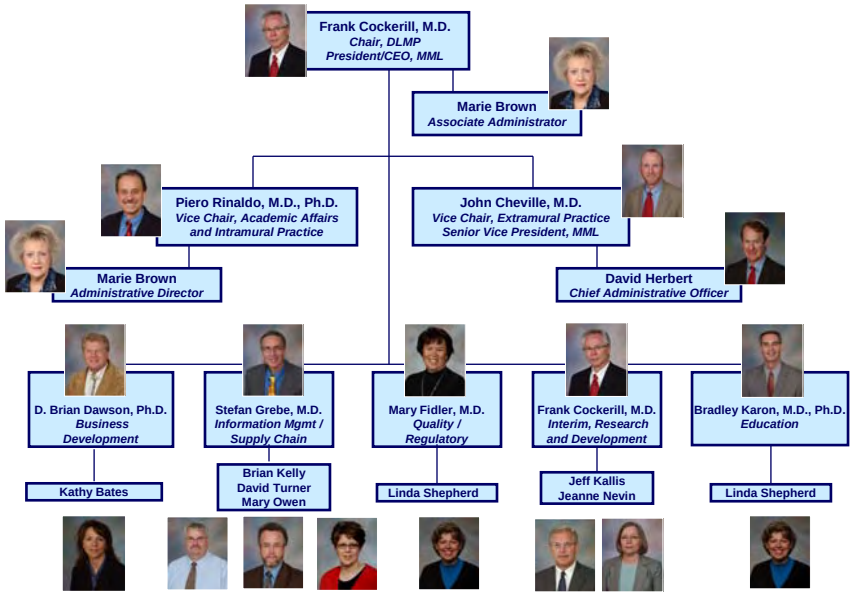
Core values

- **The needs of the patient come first**
- **The best interest of the patient is the only interest to be considered**


St. Mary's Hospital
Ozmun
Baldwin
Harwick
Guggenheim
Mayo
Gonda
Methodists Hospital
Medical Sciences
Plummer
Siebens
Hilton
Stabile


Department of Laboratory Medicine & Pathology

DLMP Governance (2008)



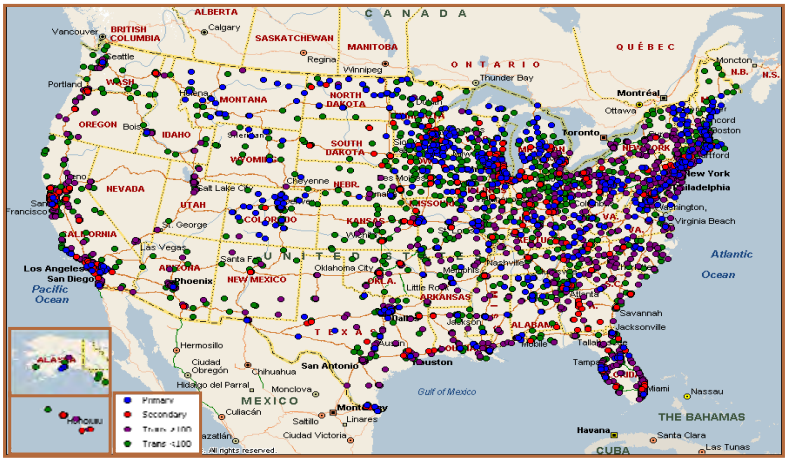
DLMP Consists of 9 Divisions

- Anatomic Pathology
- Clinical Biochemistry & Immunology
- Clinical Core Laboratory Services
- Clinical Microbiology
- Experimental Pathology & Lab Medicine
- Hematopathology
- Laboratory Genetics
- Transfusion Medicine
- Mayo Medical Laboratories

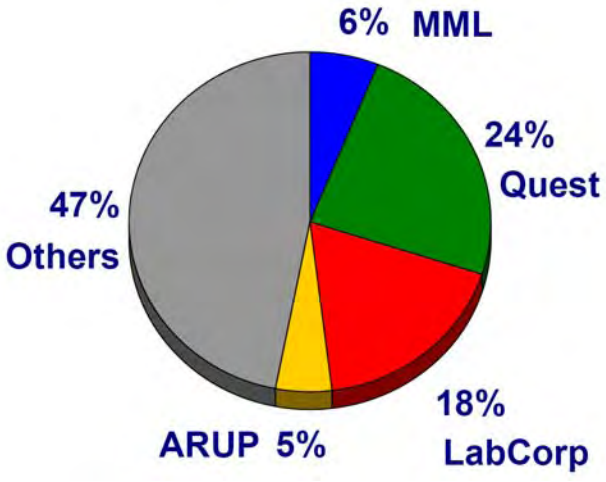
3,800 employees
2,800+ tests
150 MD & PhD
58 labs
4 sites

Mayo Medical Laboratories

MML has approximately 4,000 clients in the U.S.

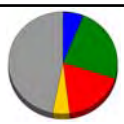


Esoteric Testing Market Share in the USA



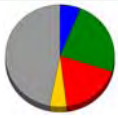
Source: MarketData Enterprises: *The U.S. Medical Laboratory Industry – 8th edition*, SEC Filings

Different Business Models

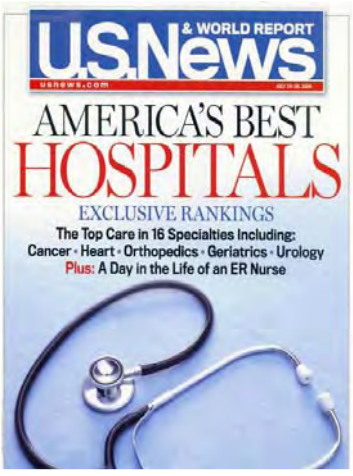


- MML does not compete with hospitals in the local physician office market
- Partners with clients to provide services to facilitate the development of community based lab outreach programs
- Provides services to community hospitals that optimize patient retention
- Quest & LabCorp have a different business model
- Compete with local hospital lab practice for physician office lab testing
- Offer high volume, automated routine as well as esoteric tests

U.S. News & World Report's Top U.S. Medical Centers

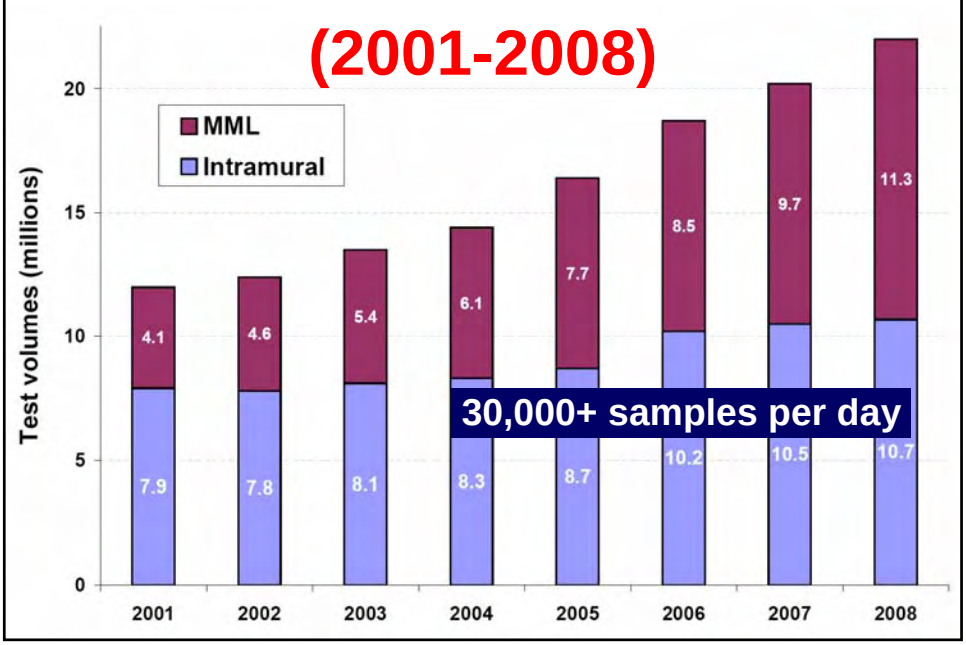


All are MML Clients



1. Johns Hopkins Hospital, Baltimore, MD
2. Mayo Clinic, Rochester, MN
3. Ronald Reagan UCLA Medical Center, Los Angeles, CA
4. Cleveland Clinic, Cleveland, OH
5. Massachusetts General Hospital, Boston, MA
6. New York-Presbyterian Univ. Hosp. Of Columbia and Cornell, NY
7. University of California, San Francisco Medical Center, CA
8. Brigham and Women's Hospital, Boston, MA
9. Duke University Medical Center, Durham, NC
10. Hospital of the University of Pennsylvania, Philadelphia, PA
10. University of Washington Medical Center, Seattle, WA
12. Barnes-Jewish Hospital, Washington University, St. Louis, MO
13. Univ. of Michigan Hospitals and Health Centers, Ann Arbor, MI
14. UPMC-University of Pittsburgh Medical Center, PA
15. Vanderbilt University Medical Center, Nashville, TN
16. Stanford Hospital and Clinics, Stanford, CA
17. University of Chicago Medical Center, IL
18. Cedars-Sinai Medical Center, Los Angeles, LA
19. Yale-New Haven Hospital, New Haven, CT

DLMP Test Volumes (2001-2008)



Clinical Applications of MS/MS

- Develop new methods
- Replace existing methods
  - Lack of positive identification
  - Difficult / cumbersome
  - Prone to interference
  - Expensive reagents
  - Time consuming
  - Outdated technology

The Value Equation

$$\text{Value} = \frac{\text{Quality}^*}{\text{Cost}}$$

MS/MS is BETTER (Q) FASTER and CHEAPER

* Safety
Services
Outcomes

MS/MS Presence in DLMP

DLMP laboratory	2008 Test Volume	MS/MS Test Volume	MS/MS Test %	No. of tests	No. of units
Anatomic Pathology	785,000	456	0.1%	1	1
Biochemical Genetics	563,268	229,721	41%	28	15
Cardiovascular (CVLM)	157,805	34,001	22%	3	2
Dev & Validation Center	n/a	n/a	n/a	n/a	4
Endocrinology	1,544,654	1,058,768	69%	28	16
Mayo Jacksonville	184,000	3,526	2%	3	3
MML New England	697,000	133,307	19%	3	4
Toxicology	461,985	155,829	34%	15	13
DLMP (total) (not sum of above)	22,000,000	1,615,608	7%	81	58

Number of MS/MS Instruments (April 2009)

	Agilent		Applied Biosystems							Thermo		Waters	
DLMP Laboratory	6410	6460	150	2000 *	3000	3200	4000	5000	5500	Quantum	LTQ Orbitrap XL	QTOF Premium	Total
Anatomic Pathology											1		1
Biochemical Genetics			1	2	5	4	1	2					15
Cardiovascular (CVLM)						1		1					2
Dev. & Validation Center	1							1		1		1	4
Endocrinology	1						6	8	1				16
Mayo Jacksonville (FL)							2	1					3
MML New England (MA)								4					4
Toxicology & Drug Monitoring		1		2		5	3	2					13
DLMP (total)	2	1	1	4	5	10	12	19	1	1	1	1	58

API 2000 Triple Quadrupole

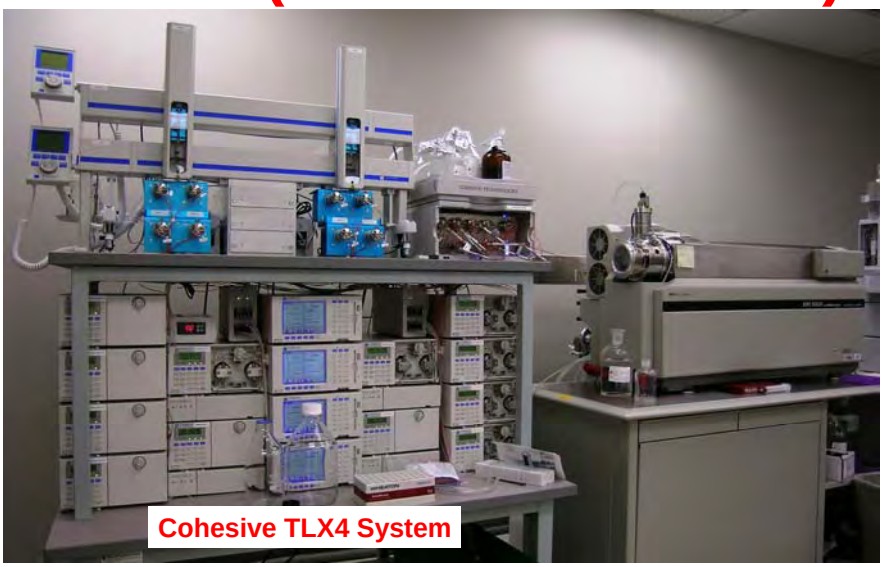
First MS/MS at Mayo (12/98)

Impact of MS/MS in Laboratory Medicine (Mayo)

Platform	1998	2009
HPLC	>400	<100
GC/MS	>50	<30
MS/MS	0	58 (52 ABI)

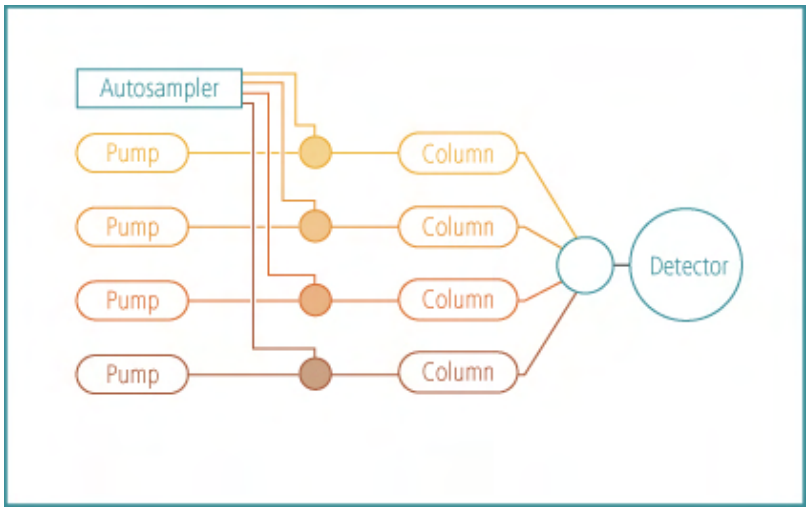
"High Density" MS/MS

High Throughput Front-End Device (Cohesive/Thermo)

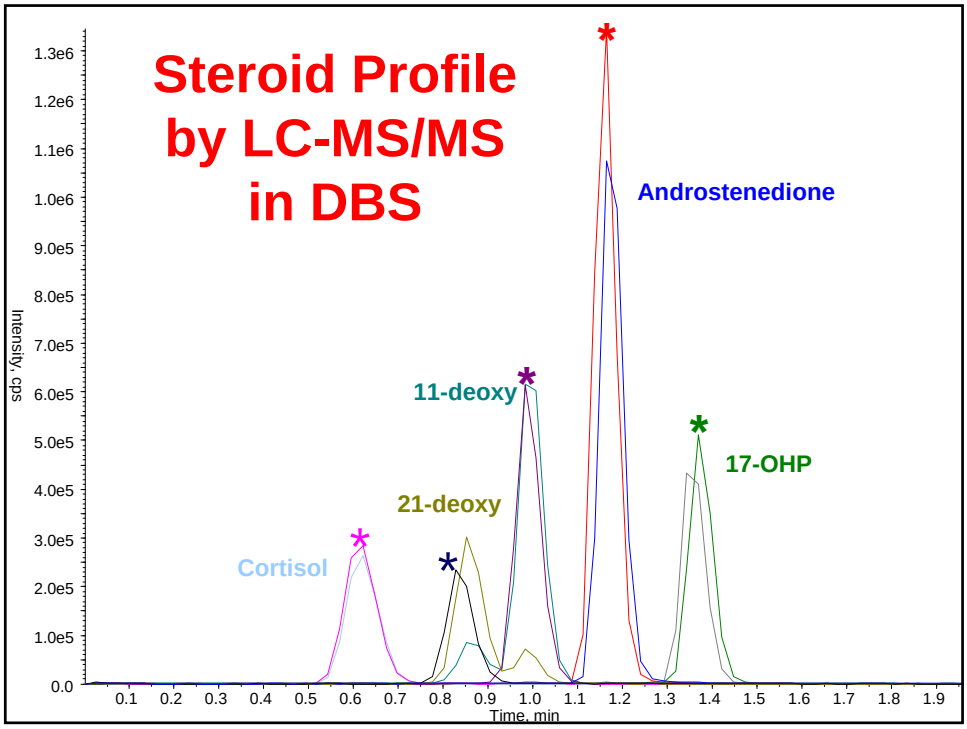


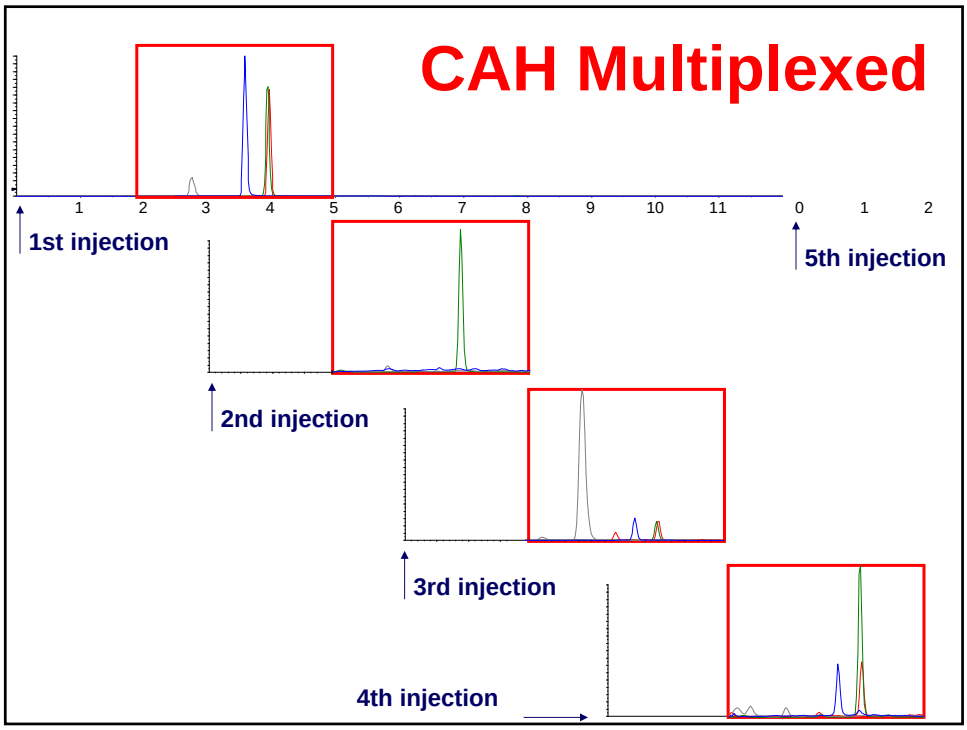
Cohesive TLX4 System

System Design



from www.CohesiveTech.com


Steroid Profile
by LC-MS/MS
in DBS
Androstenedione
11-deoxy
17-OHP
21-deoxy
Cortisol
Intensity, cps
Time, min

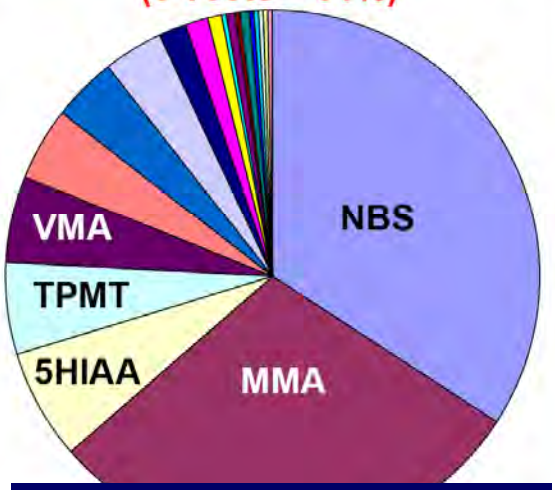

CAH Multiplexed
1st injection
2nd injection
3rd injection
4th injection
5th injection

Biochemical Genetics Laboratory

BGL 2008

Dimitar Gravrilov, MD, PhD
Devin Oglesbee, PhD
Dietrich Matern, MD (head)
Kimiyo Raymond, MD
Piero Rinaldo, MD, PhD
Silvia Tortorelli, MD, PhD

Biochemical Genetics
2008 MS/MS Volume = 229,721
(5 Tests = 81%)

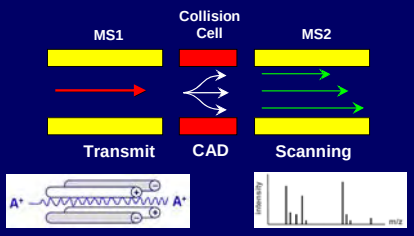


Not included:
Homocysteine (2008: 33,057 tests)

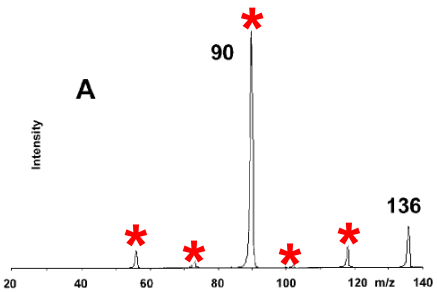
- Newborn Screening, DBS
- Methylmalonic Acid, S
- 5-Hydroxyindoleacetic Acid, U
- Thiopurine Methyltransferase, RBC
- Vanillylmandelic Acid, U
- Carnitine, S
- Acylcarnitines, Quantitative, P
- Carbohydrate Deficient Transferrin, S
- Porphyrins, U
- CAH 2nd tier test, DBS
- VMA and HVA, U
- Postmortem Screening, DBS/Bile
- Porphobilinogen, U
- PUPY Panel, U
- Homovanillic Acid, U
- Methylmalonic Acid, U
- Carnitine, U
- Creatine Disorders Panel, U
- Tryptophan, PL
- Succinylacetone, DBS
- FAO Probe Assay, fibroblast culture
- Familial Amyloidosis Reflex, S
- C4, Acylcarnitine, U
- C5-DC, Acylcarnitine, U
- C5-OH, Acylcarnitine, U
- Xanthine and Hypoxanthine, U
- Allo-isoleucine, DBS
- Tryptophan, U
- Methylmalonic Acid, AF
- HCMM, DBS

MS/MS Experiments

Product Ion Scan

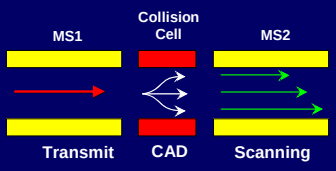


Homocysteine (*m/z* 136)

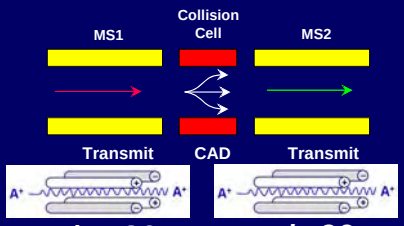


MS/MS Experiments

Product Ion Scan

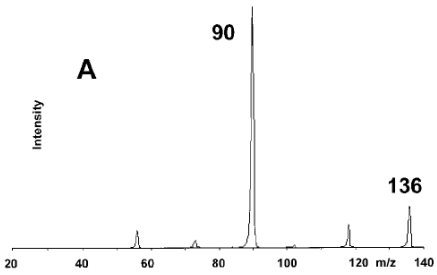


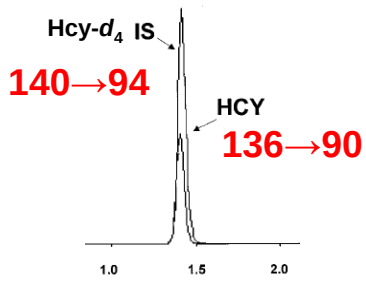
Selected Reaction Monitoring



m/z 136 m/z 90

Homocysteine (*m/z* 136)





Why Changing Existing Methods to MS/MS?

The Homocysteine Evidence

Clinical Chemistry 45:9
1517–1522 (1999)

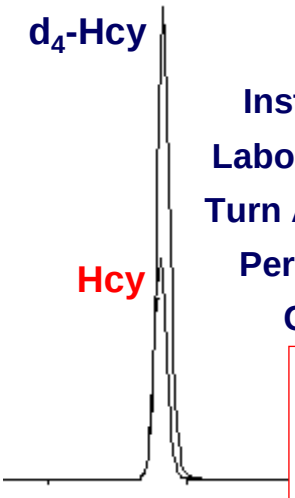
Endocrinology and Metabolism

Method for the Determination of Total Homocysteine in Plasma and Urine by Stable Isotope Dilution and Electrospray Tandem Mass Spectrometry

Mark J. Magera,[1] Jean M. Lacey,[1] Bruno Casetta,[2] and Piero Rinaldo[1*]

Why Changing Existing Methods to MS/MS?

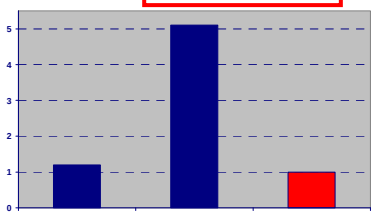
The Homocysteine Evidence



Run time: 2 min.

METHOD	HPLC	IMX	MS/MS
Instrumentation	5	2	1
Laboratory Space	4.9	1.3	1
Turn Around Time	21	1.1	1
Personnel (FTE)	1.4	0.6	1
Cost per Test	1.2	5.1	1

Requirements Expressed As Relative to MS/MS



Clinical Chemistry 49, No. 5, 2003

Clinical Chemistry 47:3
513–518 (2001)

Enzymes and Protein Markers

Rapid Determination of Transferrin Isoforms by Immunoaffinity Liquid Chromatography and Electrospray Mass Spectrometry

Jean M. Lacey,[1] H. Robert Bergen,[2] Mark J. Magera,[1] Stephen Naylor,[2,3] and John F. O'Brien[1*]

author at: Biochemical Genetics Laboratory, Hilton 360, Department of Laboratory Medicine & Pathology, Mayo Clinic and Foundation, 200 First St. SW, Rochester, MN 55905; fax 507-266-2888, e-mail rinaldo@mayo.edu)

Glycosylation Defects Identified

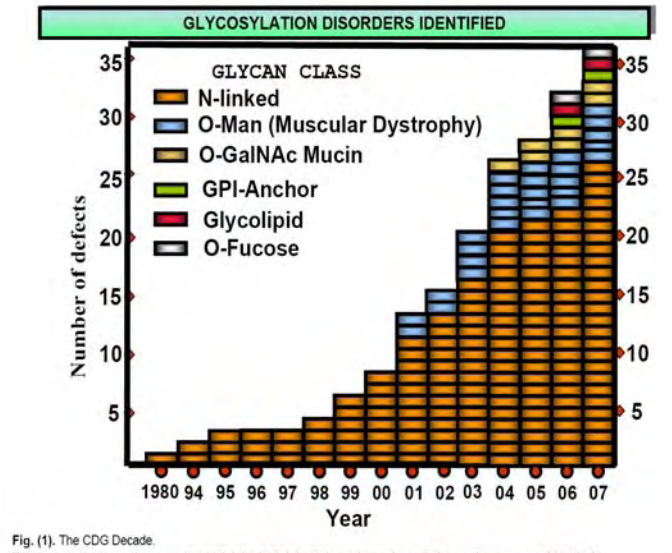


Fig. (1). The CDG Decade.
This bar graph shows the years in which inherited defects in various glycosylation pathways were identified.

CLINICAL FEATURES OF CDG PATIENTS

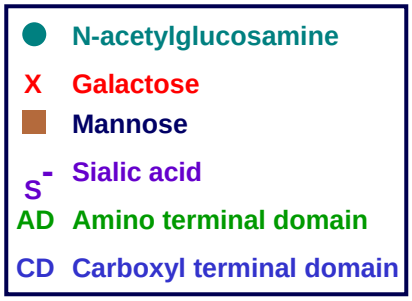
Neurology	Hypotonia, hyporeflexia, developmental delay, seizures, stroke-like events
GI/Hepatology	Failure to thrive, diarrhea, protein-losing enteropathy, liver dysfunction, vomiting, hepatomegaly, cholangitis
Neonatology	Ascites, hydrops, multiorgan failure
Hematology	Thrombocytosis, thrombocytopenia, coagulopathy, thrombosis
Endocrinology	Hyperinsulinemic hypoglycemia, hypothyroidism, hypogonadism
Clin. Genetics	Dysmorphic features, microcephaly
Orthopedics	Osteopenia, joint contractures, kyphosis/scoliosis
Ophthalmology	Abnormal eye movements, squint, cataract, retinitis pigmentosa, iris coloboma, nystagmus, cortical blindness
Radiology	Cerebellar hypoplasia, calcification of white matter, micropolygyria, delayed myelinization, cystic kidneys, renal hyperechogenicity
Histology	Liver fibrosis, liver cirrhosis, intestinal villus atrophy
Dermatology	Ichthyosis
Nephrology	Nephrotic syndrome, tubulopathy, cystic kidneys
Immunology	Recurrent infections, hypogammaglobulinemia
Cardiology	Cardiomyopathy, pericardial effusions
Laboratory	Hypoalbuminemia, elevated transaminases, low triglycerides, decreased AT-III, decreased F-VIII & F-XI, decreased protein C & S

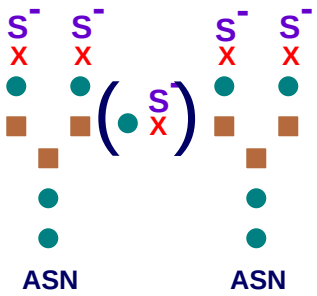
(Leonard JV et al. Diversity of congenital disorders of glycosylation. *Lancet* 357:1382, 2001)

"In view of the extreme diversity of clinical problems the transferrin pattern should be examined in a wide range of patients that may present to many specialists"

(*Lancet*, 357:1382, 2001)

Transferrin Structure

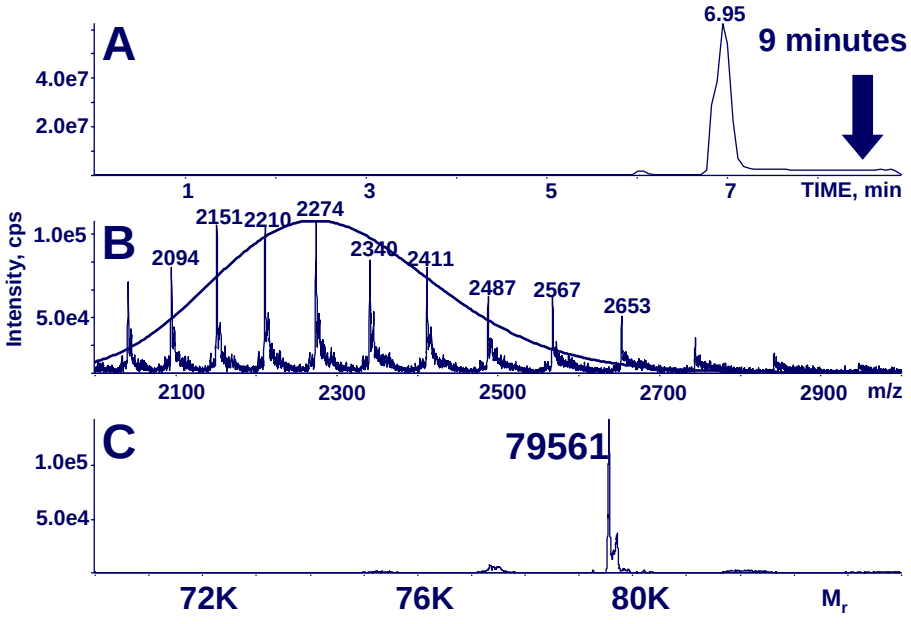


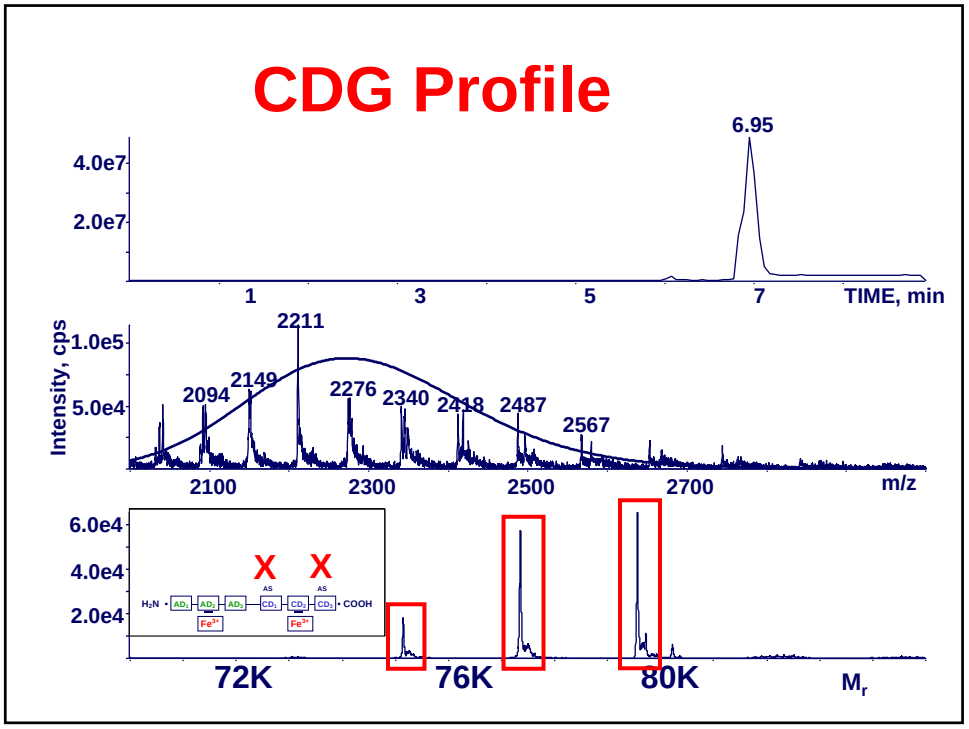


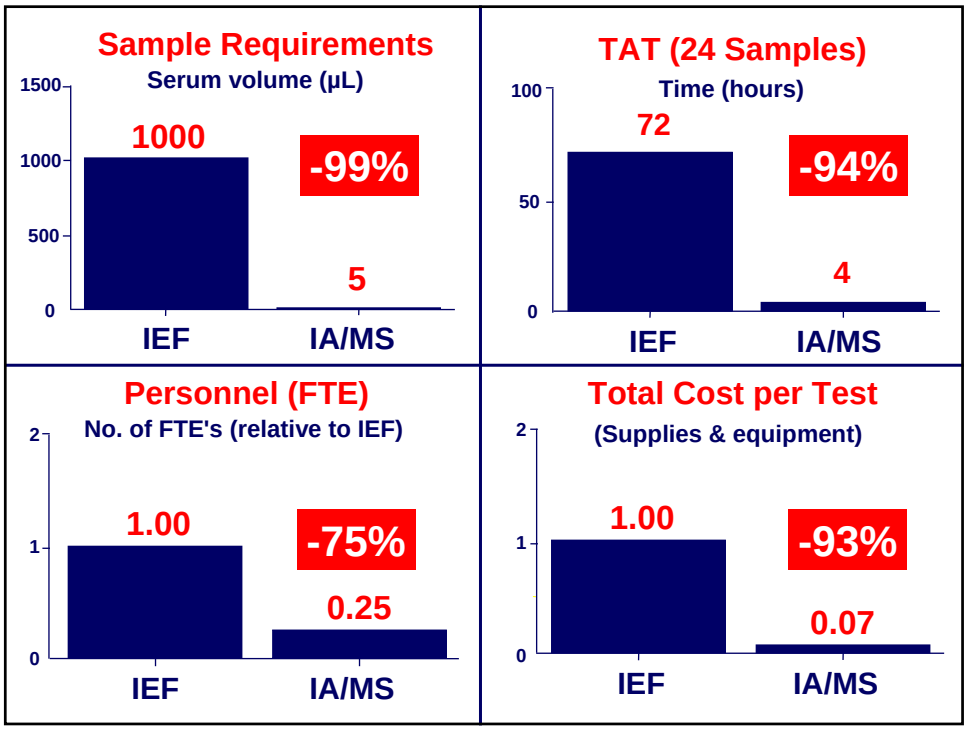
H_2N • AD_1 AD_2 AD_3 CD_1 CD_2 CD_3 • COOH

Fe^{3+} Fe^{3+}

Control Profile



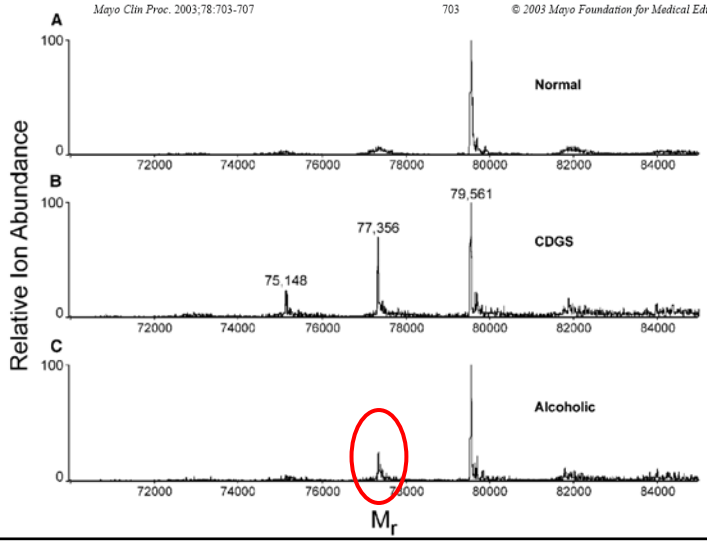

CDG Profile
6.95
4.0e7
2.0e7
1
3
5
7
TIME, min
Intensity, cps
1.0e5
5.0e4
2211
2094
2149
2276
2340
2418
2487
2567
2100
2300
2500
2700
m/z
6.0e4
4.0e4
2.0e4
X X
72K
76K
80K
Mr


Sample Requirements
Serum volume (µL)
1500
1000
500
0
1000
-99%
5
IEF
IA/MS
TAT (24 Samples)
Time (hours)
100
50
0
72
-94%
4
IEF
IA/MS
Personnel (FTE)
No. of FTE's (relative to IEF)
2
1
0
1.00
-75%
0.25
IEF
IA/MS
Total Cost per Test
(Supplies & equipment)
2
1
0
1.00
-93%
0.07
IEF
IA/MS

Value of Determining Carbohydrate-Deficient Transferrin Isoforms in the Diagnosis of Alcoholic Liver Disease

LINDA M. STADHEIM, RN; JOHN F. O'BRIEN, PHD; KEITH D. LINDOR, MD; GREGORY J. GORES, MD; AND DOUGLAS B. MCGILL, MD

Mayo Clin Proc. 2003;78:703-707



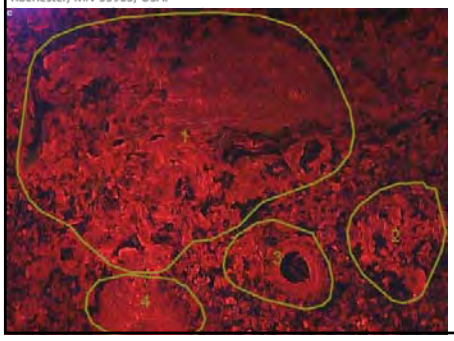
Laboratory Investigation (2008) 88, 1024–1037

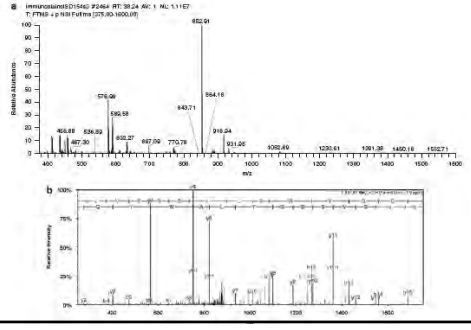
Immunoglobulin derived depositions in the nervous system: novel mass spectrometry application for protein characterization in formalin-fixed tissues

Fausto J Rodriguez[1], Jeffrey D Gamez[1], Julie A Vrana[1], Jason D Theis[1], Caterina Giannini[1], Bernd W Scheithauer[1], Joseph E Parisi[1], Claudia F Lucchinetti[2], William W Pendlebury[3], H Robert Bergen III[4] and Ahmet Dogan[1]

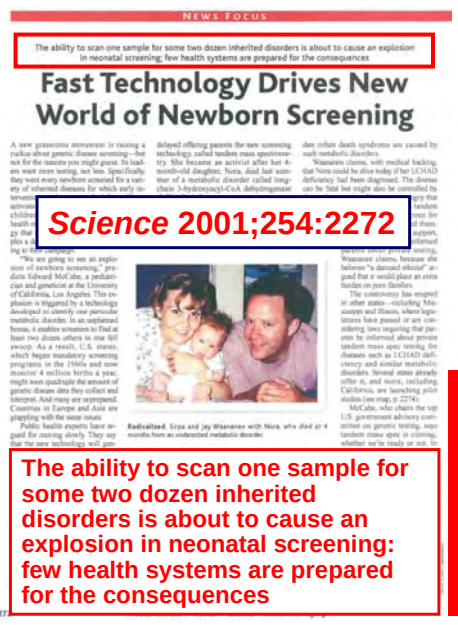
[1]Department of Laboratory Medicine and Pathology, Research Center, Mayo Clinic, Rochester, MN, USA; [2]Department of Neurology, Research Center, Mayo Clinic, Rochester, MN, USA; [3]Department of Pathology, University of Vermont College of Medicine, Burlington, VT, USA and [4]Department of Proteomics, Research Center, Mayo Clinic, Rochester, MN, USA

Correspondence: Dr FJ Rodriguez, MD, Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, 200 First Street SW, Mayo Clinic, Rochester, MN 55905, USA.





Newborn Screening by MS/MS



NEWS FOCUS

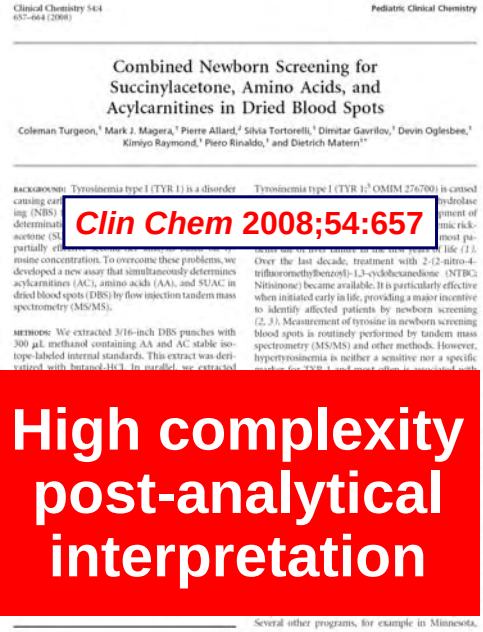
The ability to scan one sample for some two dozen inherited disorders is about to cause an explosion in neonatal screening; few health systems are prepared for the consequences

Fast Technology Drives New World of Newborn Screening

Science 2001;254:2272

Radicalized. Sirpa and Jay Waenanen with Nora, who died at 4 months from an undetected metabolic disorder.

The ability to scan one sample for some two dozen inherited disorders is about to cause an explosion in neonatal screening: few health systems are prepared for the consequences



Clinical Chemistry 54:4 657–664 (2008) Pediatric Clinical Chemistry

Combined Newborn Screening for Succinylacetone, Amino Acids, and Acylcarnitines in Dried Blood Spots

Coleman Turgeon,[1] Mark J. Magera,[1] Pierre Allard,[2] Silvia Tortorelli,[1] Dimitar Gavrilov,[1] Devin Oglesbee,[1] Kimiyo Raymond,[1] Piero Rinaldo,[1] and Dietrich Matern[1*]

Clin Chem 2008;54:657

BACKGROUND: Tyrosinemia type I (TYR I) is a disorder causing ear... (NBS) ... determinati... acetone (SU... partially c...

Tyrosinemia type I (TYR I;[3] OMIM 276700) is caused ... hydrolase ... ment of ... mic rick- ... most pa- ... life (*1*). Over the last decade, treatment with 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC; Nitisinone) became available. It is particularly effective when initiated early in life, providing a major incentive to identify affected patients by newborn screening (*2, 3*). Measurement of tyrosine in newborn screening blood spots is routinely performed by tandem mass spectrometry (MS/MS) and other methods. However, hypertyrosinemia is neither a sensitive nor a specific ...

METHODS: We extracted 3/16-inch DBS punches with 300 μL methanol containing AA and AC stable isotope-labeled internal standards. This extract was deri- ... vatized with butanol-HCl. In parallel, we extracted ...

Several other programs, for example in Minnesota,

High complexity post-analytical interpretation

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

REGIONAL COLLABORATIVE PROJECT - PRIORITY 1

TRAINING PROGRAM IN NEWBORN SCREENING BY MS/MS

Biochemical Genetics Laboratory, Mayo Clinic College of Medicine - Rochester (MN), April 27 - May 1, 2009

Personnel	Title
Dimitar Gavrilov, Dietrich Matern, Devan Oglesbee, Kimyo Raymond, Piero Rinaldo, Silvia Tortorelli	BGL directors
Ruben Bonilla, Emily Smith	BGL fellow
Amy Barthel	BGL supervisor (MS/MS)
Mark Magera	BGL development coordinator
Ned Maffit	Programmer
David McHugh	Project coordinator

PARTICIPANTS

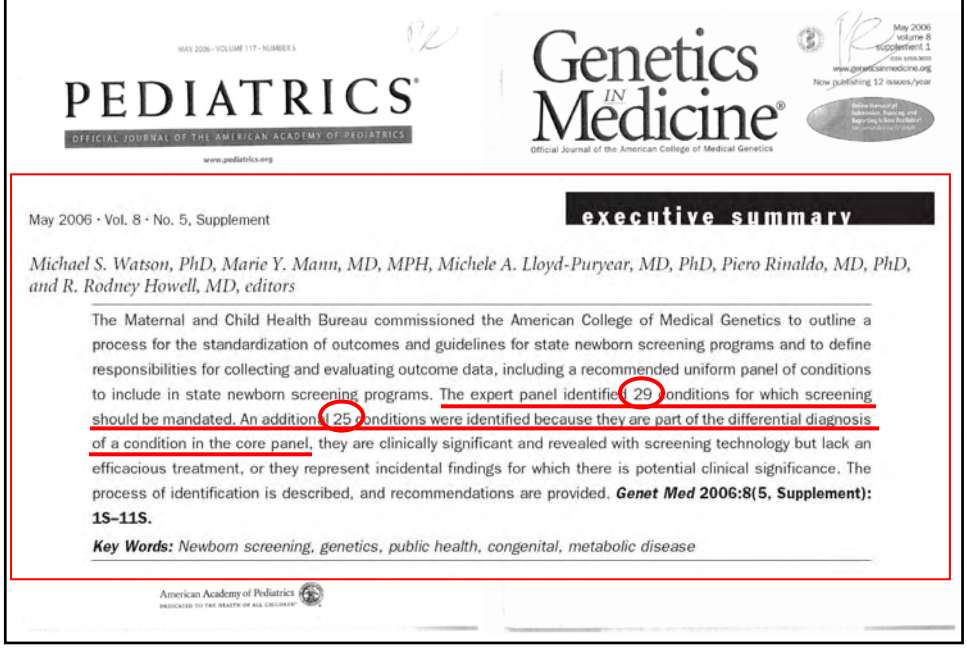
Name	State (Reg)	Name	State (Reg)
Sarah Eroh	(AR, R5)	Partha Neogi	(CA, R7)
Pat Garrod	(MI, R4)	Beth Tarini	(MI, R4)
Michael Kayser	(OK, R5)	Stuart Graham	(UAE, R6)
Mark Mcinsey	(NY, R2)		

Practical | Presentation | LEGEND | Flex time | Conferences

TIME	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY	TIME
8:00	Group assembly, tour of BGL, transfer to Stabile 3-03	2nd tier tests (Dieter Matern)	REVIEW of results and REPORTING	REVIEW of results and REPORTING	REVIEW of results and REPORTING	8:00
8:30						8:30
9:00	Introduction to MS/MS analysis (Mark Magera)	REVIEW of results and REPORTING (including 2nd tier tests)				9:00
9:30						9:30
10:00	Introduction to amino acids and acylcarnitines					10:00
10:30						10:30
11:00	Examples of profile interpretation (I)					11:00
11:30						11:30
12:00		Break (lunch)	Break (lunch)	Break (lunch)	Capabilities and limitations of NBS piloting and expansion	12:00
12:30	Lunch in conference room					12:30
13:00		Overview & Status of Collaborative Project	MN NBS conference call	Discussion participants comparison tools	Lunch in conference room	13:00
13:30	Examples of profile interpretation (II)					13:30
14:00		Region 4 Stork (R4S) Project Tools	FLEX TIME		FLEX TIME	14:00
14:30	Planning of FLEX time			VLCAD & OTC tools		14:30
15:00						15:00
15:30	How things look like at Mayo					15:30
16:00		Project SOPs	Short term follow up (TP cases) (I)	Short term follow up (TP cases) (II)	REVIEW of results and REPORTING	16:00
16:30	REVIEW of results and REPORTING					16:30
17:00		REVIEW of results and REPORTING	REVIEW of results and REPORTING	REVIEW of results and REPORTING	Final remarks, end of course	17:00
17:30				Group Dinner		17:30

FLEX TIME OPTIONS			
	2nd tier tests (CAH)	2nd tier tests (MMA)	SUAC (sample prep)
	MS/MS calibration, tuning	QC/QA process & procedures	MS/MS troubleshooting

The HRSA/ACMG Uniform Panel



PEDIATRICS
OFFICIAL JOURNAL OF THE AMERICAN ACADEMY OF PEDIATRICS
www.pediatrics.org

Genetics IN Medicine
Official Journal of the American College of Medical Genetics
May 2006 volume 8 Supplement 5
www.geneticsinmedicine.org
Now publishing 12 issues/year

May 2006 · Vol. 8 · No. 5, Supplement

executive summary

Michael S. Watson, PhD, Marie Y. Mann, MD, MPH, Michele A. Lloyd-Puryear, MD, PhD, Piero Rinaldo, MD, PhD, and R. Rodney Howell, MD, editors

The Maternal and Child Health Bureau commissioned the American College of Medical Genetics to outline a process for the standardization of outcomes and guidelines for state newborn screening programs and to define responsibilities for collecting and evaluating outcome data, including a recommended uniform panel of conditions to include in state newborn screening programs. The expert panel identified 29 conditions for which screening should be mandated. An additional 25 conditions were identified because they are part of the differential diagnosis of a condition in the core panel, they are clinically significant and revealed with screening technology but lack an efficacious treatment, or they represent incidental findings for which there is potential clinical significance. The process of identification is described, and recommendations are provided. ***Genet Med*** **2006:8(5, Supplement): 1S–11S.**

Key Words: *Newborn screening, genetics, public health, congenital, metabolic disease*

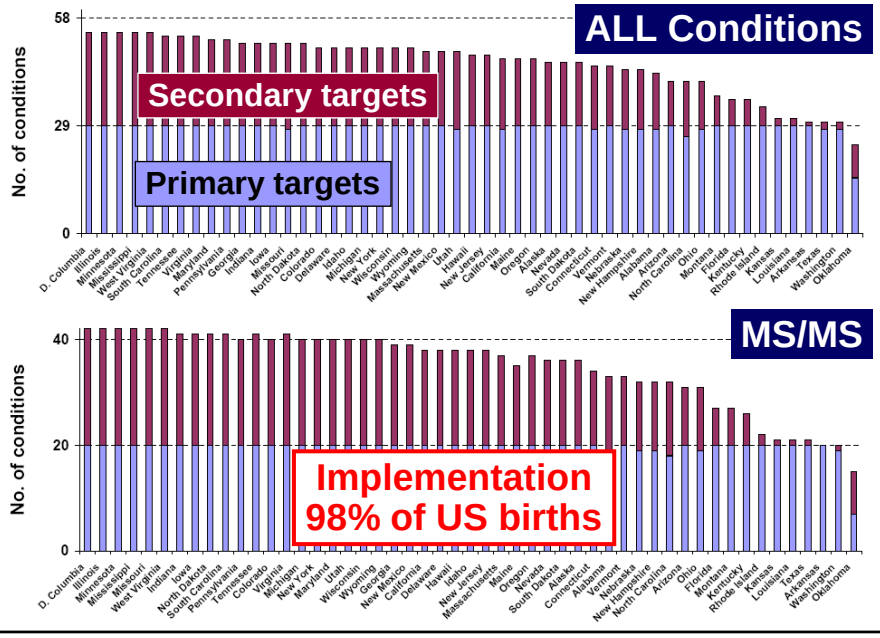
American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN™

Uniform Screening Panel

- 29 primary conditions
  - 20 detected by MS/MS (AA, FAO, OA)
  - 3 Hb-pathies (S/S, S/βThal, S/C)
  - 6 others (BIOT, CAH, CF, CH, GALT, HEAR)
- 25 secondary targets *
  - 22 detected by MS/MS (AA, FAO, OA)
  - 1 Hb-pathy (many variants counted as one)
  - 2 others (GAL-epimerase, GAL-kinase)

* At least 20 more conditions could be detected

Implementation of Uniform Panel (UP)



What About Europe?

J Inherit Metab Dis (2007) 30:423–429
DOI 10.1007/s10545-007-0647-2

NEWBORN SCREENING

Introducing new screens: Why are we all doing different things?

R. J. Pollitt

Table 2 Disorders recommended for newborn screening using MS/MS

Disorder					
Phenylketonuria	P	+	+	+	+
Maple syrup urine disease	P	+	+	+	
Homocystinuria	P			+	
Tyrosinaemia type I	P			+	
Citrullinaemia	P	+			
Argininosuccinic aciduria	P	+			
Argininaemia	S	+			
HHH syndrome		+			
Very long-chain acyl-CoA dehydrogenase deficiency	P	+	+	+	
Long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency[b]	P	+	+	+	
Medium-chain acyl-CoA dehydrogenase deficiency	P	+	+	+	+
Short-chain acyl-CoA dehydrogenase deficiency	S	+			
Multiple acyl-CoA DD (glutaric aciduria type II)	S	+			
Carnitine palmitoyltransferase deficiency type I	S	+	+		
Carnitine palmitoyltransferase deficiency type II	S	+	+		
Carnitine-acylcarnitine translocase deficiency	S	+	+		
Carnitine uptake (OCTN2) deficiency	P				
Propionic acidaemia	P	+			
Methylmalonic acidaemia	P[c]	+			
Isovaleric acidaemia	P		+	+	
Glutaryl-CoA dehydrogenase deficiency	P	+	+	+	
Multiple carboxylase (holocarboxylase synthase) deficiency	P			+	
3-Hydroxy-3-methylglutaryl-CoA lyase deficiency	P	+		+	
Beta-ketothiolase (T2) deficiency	P	+			
3-Methylcrotonyl-CoA carboxylase deficiency	P	+		+	
Plus 17 other conditions	S				
	42	**20**	**10**	**12**	**2**

Data sources as for Table 1.
[a] P denotes a primary target, S a secondary target.
[b] Includes trifunctional protein deficiency.
[c] Mut^0, CblA and CblB. CblC and CblD were secondary targets.

What Europe Thinks of Us

ACMG Expert Group recommendations

Mandatory

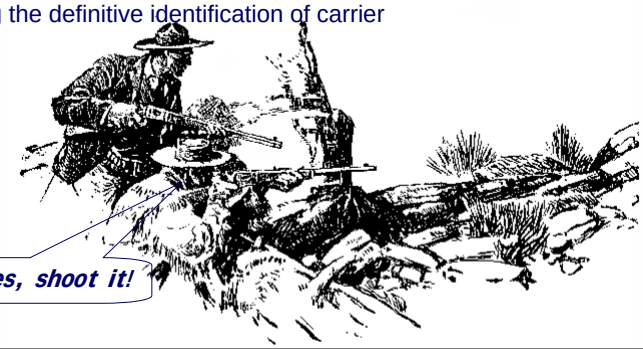
- **screening** for primary target conditions
- **reporting** of all secondary target conditions
- **reporting** of any abnormal results that may be associated with clinically significant conditions, including the definitive identification of carrier status

J Inherit Metab Dis (2007) 30:423–429
DOI 10.1007/s10545-007-0647-2

NEWBORN SCREENING

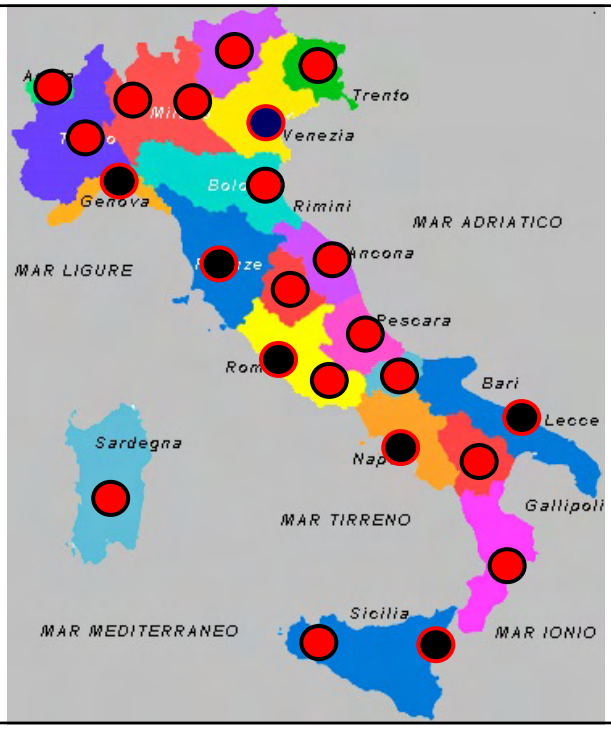
Introducing new screens: Why are we all doing different things?

R. J. Pollitt



If it moves, shoot it!

Centri Italiani dotati di NBS by MS/MS



Padova

Genova

Firenze

Roma

Napoli

Foggia

Catania

Further Expansion of Newborn Screening Using MS/MS

- Continue implementation of uniform panel worldwide
- 2nd tier tests (FPR reduction)
- New conditions

2nd Tier Tests


J Inherit Metab Dis
DOI 10.1007/s10545-007-0691-y

NEWBORN SCREENING

Reduction of the false-positive rate in newborn screening by implementation of MS/MS-based second-tier tests: The Mayo Clinic experience (2004–2007)

D. Matern · S. Tortorelli · D. Oglesbee · D. Gavrilov · P. Rinaldo

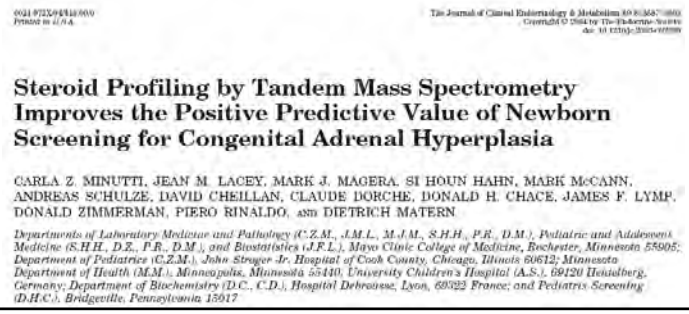
- A cost effective mean to implement clinically defined cutoffs when normal population and disease range overlap (poor specificity)
- Performed in 1-2 batches weekly (except CAH)
- Same specimen, no additional patient contact
- Normal result overrules primary screening
- Reporting of primary screening is not delayed

Clinical Chemistry 50:3
621–625 (2004)

Endocrinology and Metabolism

Improved Specificity of Newborn Screening for Congenital Adrenal Hyperplasia by Second-Tier Steroid Profiling Using Tandem Mass Spectrometry

Jean M. Lacey,[1] Carla Z. Minutti,[1,2] Mark J. Magera,[1] Angela L. Tauscher,[1] Bruno Casetta,[6] Mark McCann,[7] James Lymp,[5] Si Houn Hahn,[1,3,4] Piero Rinaldo,[1,3,4] and Dietrich Matern[1,3,4*]

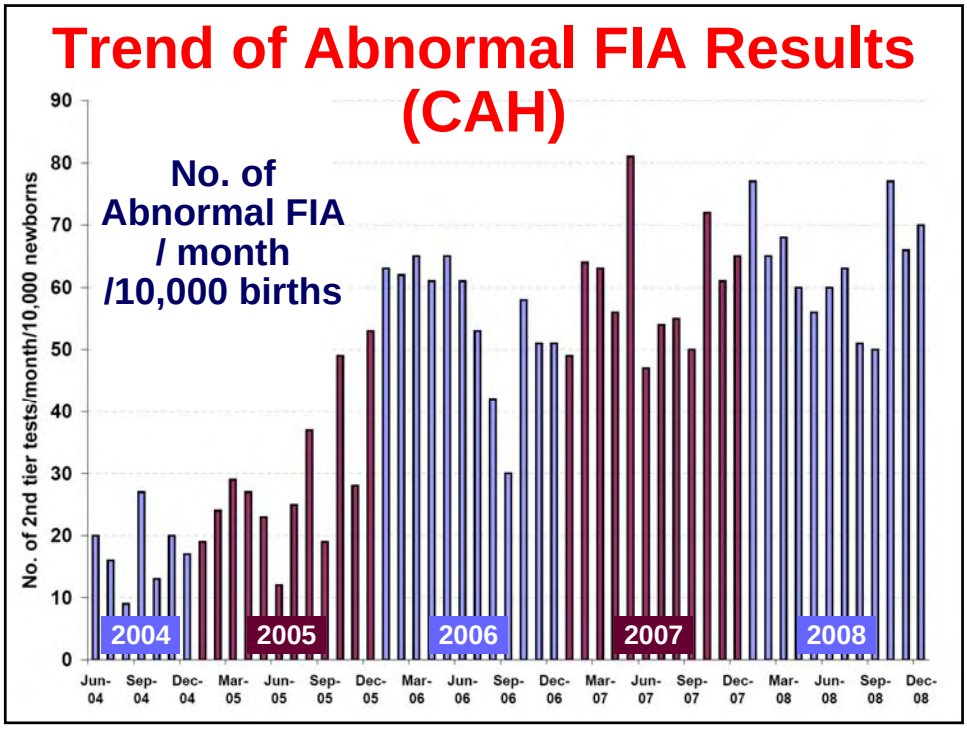


Steroid Profiling by Tandem Mass Spectrometry Improves the Positive Predictive Value of Newborn Screening for Congenital Adrenal Hyperplasia

CARLA Z. MINUTTI, JEAN M. LACEY, MARK J. MAGERA, SI HOUN HAHN, MARK McCANN, ANDREAS SCHULZE, DAVID CHEILLAN, CLAUDE DORCHE, DONALD H. CHACE, JAMES F. LYMP, DONALD ZIMMERMAN, PIERO RINALDO, AND DIETRICH MATERN

CAH Screening in MN

- **Cutoff (FIA) based on birth weight**
  - **<1500g 80 ng/mL**
  - **1500-2500g 65 ng/mL**
  - **>2500g 50 ng/mL**
- **Period June 2004 - December 2008**
- **Volume 329,033**
- **Abnormal FIA results 2,712 (0.82%)**


Trend of Abnormal FIA Results (CAH)
No. of Abnormal FIA / month /10,000 births
No. of 2nd tier tests/month/10,000 newborns
90
80
70
60
50
40
30
20
10
0
2004
2005
2006
2007
2008
Jun-04
Sep-04
Dec-04
Mar-05
Jun-05
Sep-05
Dec-05
Mar-06
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Sep-06
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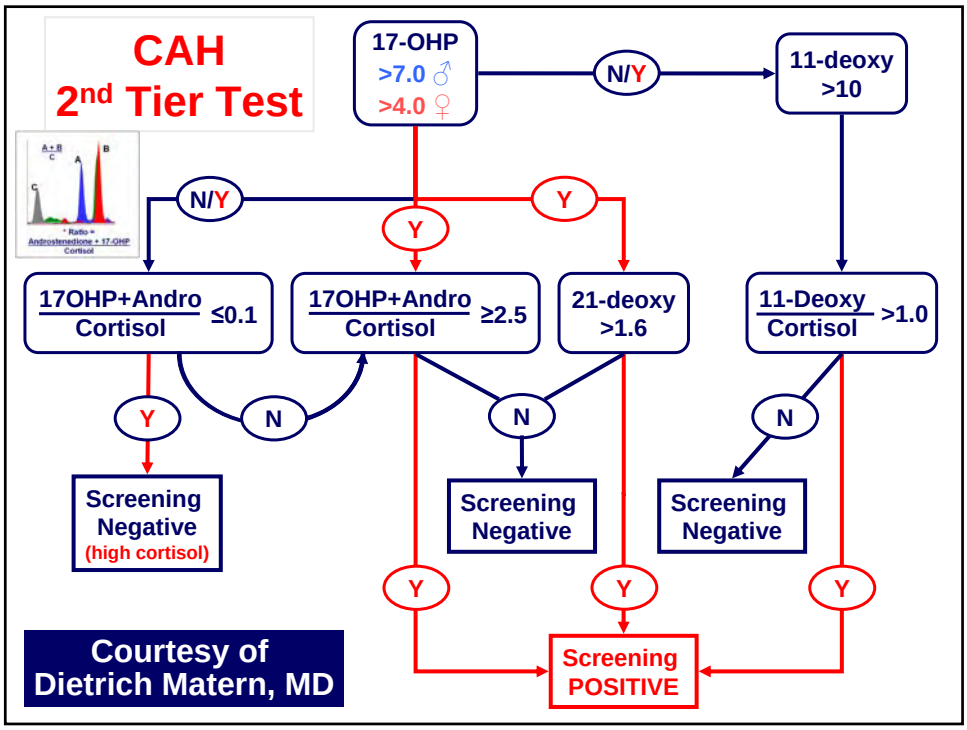
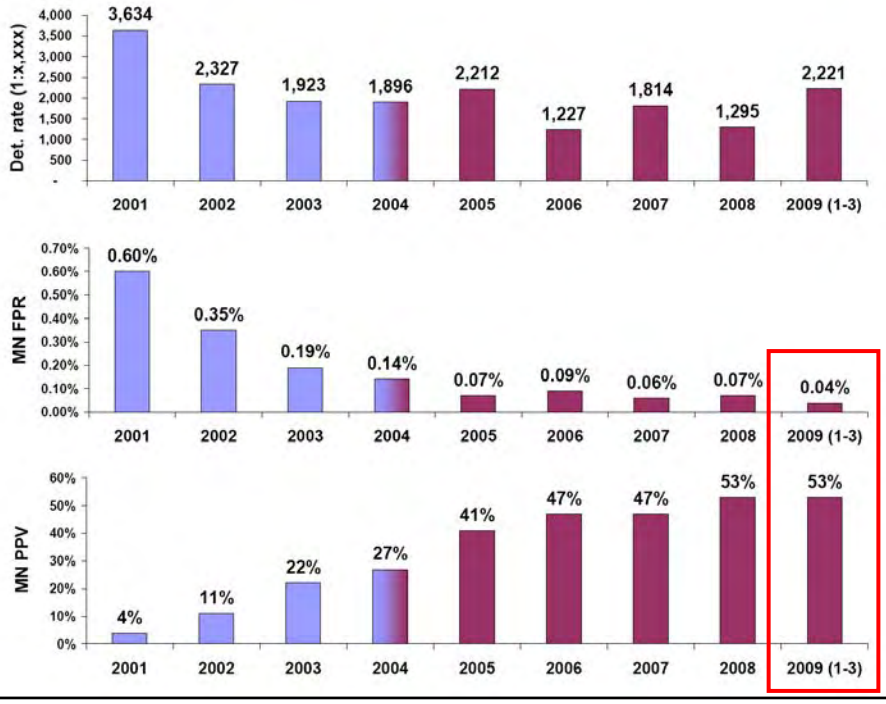
Mar-07
Jun-07
Sep-07
Dec-07
Mar-08
Jun-08
Sep-08
Dec-08

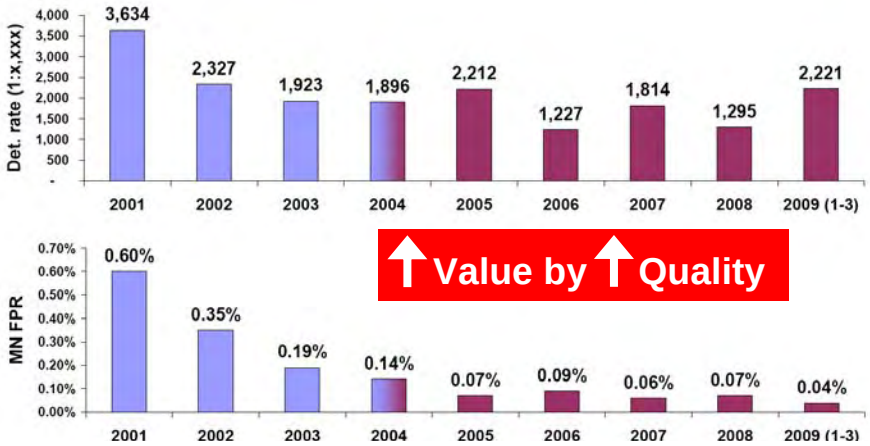

CAH
2nd Tier Test
17-OHP
>7.0 ♂
>4.0 ♀
N/Y
11-deoxy
>10
N/Y
Y
Y
17OHP+Andro / Cortisol ≤0.1
17OHP+Andro / Cortisol ≥2.5
21-deoxy
>1.6
11-Deoxy / Cortisol >1.0
Y
N
N
N
Screening Negative (high cortisol)
Screening Negative
Screening Negative
Y
Y
Y
Screening POSITIVE
Courtesy of Dietrich Matern, MD

Changing CAH Screening in MN

↑Value by ↓Cost	w/o 2nd Tier	w/ 2nd Tier
• False positives	710	41
• False (+) rate	0.97% →	0.06%
• Cost clinical F/U	$601,370	$38,115
• Cost 2nd tier test	$0	$24,850
• Total F/U cost	$601,370 →	$62,965
• Cost difference (savings)		(89.5%)

Cost clinical F/U	$847	per case
Cost 2nd tier test	$35	per test





Value by Quality

Addressing the Fallout Of Newborn Screening

Government and Researchers Seek to Reduce False Positives, Improve Physician Education and Follow-Up for Families

By Shirley S. Wang

Unintended Impact

help families find treatment. And researchers and health offi-

Wall Street Journal
10-30-07, D1

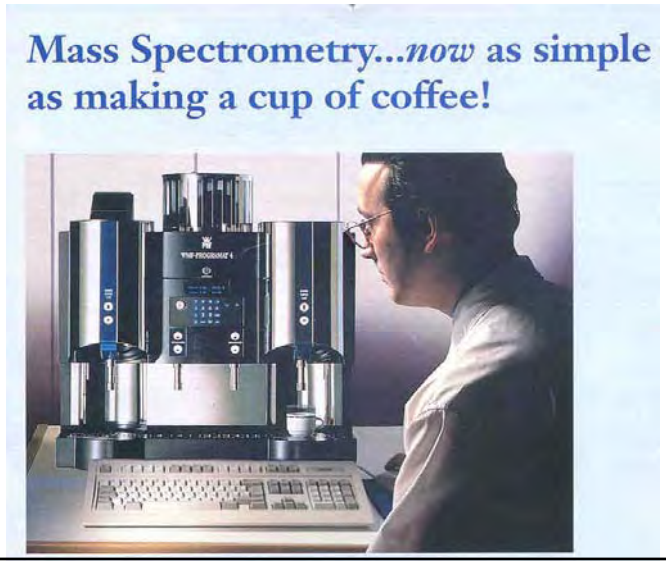
Partial List of Candidate Conditions for Expansion of Uniform Panel (in alphabetical order)

- ALD (X-linked)
- CDG Ib
- CMV
- Creatine defects
- Duchenne
- G6PD
- Gaucher *(LSD)*
- HIV
- MPS I/II/IIIa/VI *(LSD)*
- Fabry *(LSD)*
- Fam. Hypercholesterol.
- Fragile X
- Friedreich ataxia
- Krabbe *(LSD)*
- Niemann-Pick *(LSD)*
- Pompe *(LSD)*
- SCID
- SLO
- SMA
- Wilson disease

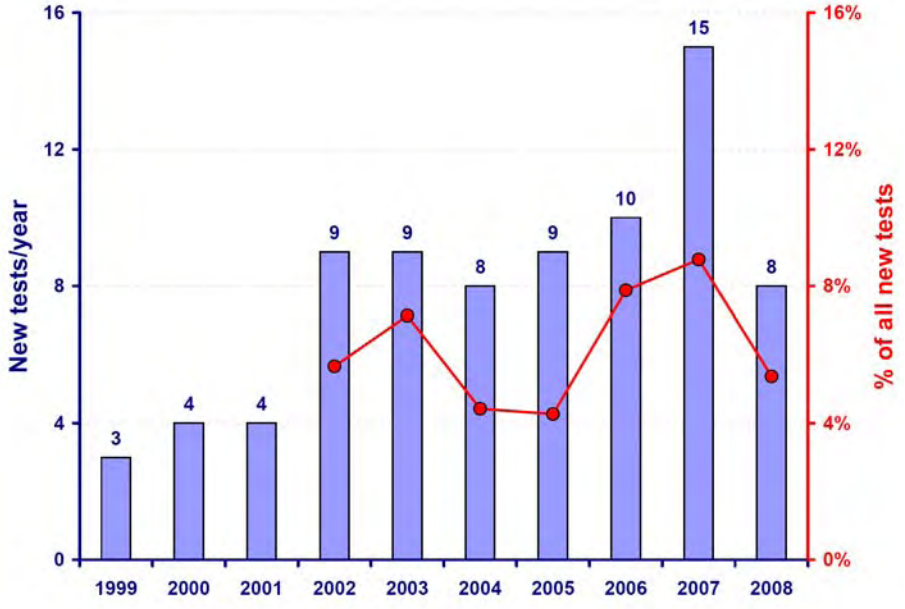
Partial List of Candidate Conditions for Expansion of Uniform Panel (MS/MS method)

- ALD (X-linked)
- CDG Ib
- Creatine defects
- Gaucher *(LSD)*
- MPS I/II/IIIa/VI *(LSD)*
- Fabry *(LSD)*
- Krabbe *(LSD)*
- Niemann-Pick *(LSD)*
- Pompe *(LSD)*
- SLO
- Wilson disease

Is MS/MS Really So "Simple"?



New MS/MSTests/Year (1999-2008)



Analytical Biochemistry 290, 122–129 (2001)
doi:10.1006/abio.2001.5232, available online at http://www.idealibrary.com on IDEAL®

Online Single-Step Analysis of Blood Proteins: The Transferrin Story

H. R. Bergen,* J. M. Lacey,† J. F. O'Brien,† and S. Naylor*,‡,1

*Biomedical Mass Spectrometry and Functional Proteomics Facility and Department of Biochemistry and Molecular Biology; †Biochemical Genetics Laboratory, Department of Laboratory Medicine and Pathology; and ‡Department of Molecular Pharmacology and Experimental Therapeutics, Clinical Pharmacology Unity and Division of Biomedical Engineering, Mayo Clinic/Foundation, Rochester, Minnesota 55905

Clinical Chemistry 47:3
513–518 (2001)

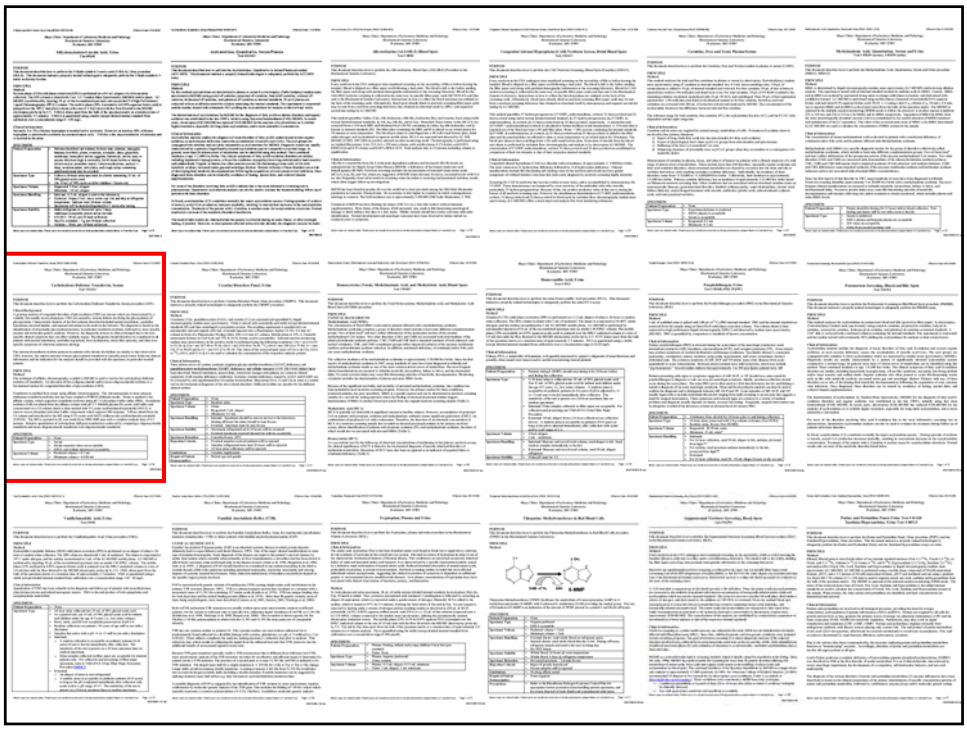
Enzymes and Protein Markers

Rapid Determination of Transferrin Isoforms by Immunoaffinity Liquid Chromatography and Electrospray Mass Spectrometry

Jean M. Lacey,[1] H. Robert Bergen,[2] Mark J. Magera,[1] Stephen Naylor,[2,3] and John F. O'Brien[1*]

Required Components of SOP

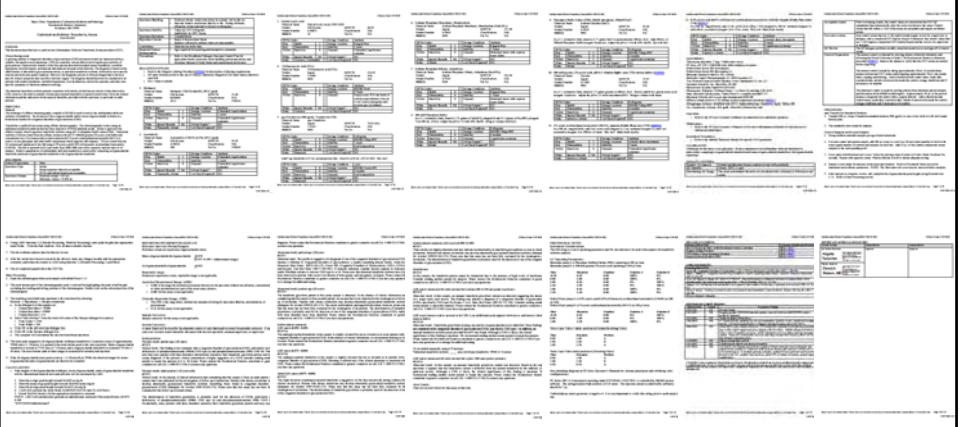
- Purpose
- Principle
- Specimens
- Reagents/Supplies
- Equipment
- Calibration
- Quality control
- Procedure
- Calculations
- Reporting
- Interpretation
- Related documents
- References
- Revisions
- Annual review
- Approval



Carbohydrate Deficient Transferrin, Serum [PROC 008810.008] Effective Date: 01/23/2009

Mayo Clinic: Department of Laboratory Medicine and Pathology
Biochemical Genetics Laboratory
Rochester, MN 55905

Carbohydrate Deficient Transferrin, Serum
Test # 82414



Clinical Requirements

- **Consistency (at all levels)**
- **Robustness (reproducibility)**

Development "super" tech

PhD clinical technologist

Technical specialist

Clinical technologist (5+ yr)

Clinical technologist (<1 yr)

Clinical Requirements

- Consistency (at all levels)
- Robustness (reproducibility)
- Documentation (inspections)
- Reproducibility (site harmonization)
- Monitoring (real time)
- Surveillance (clean up……)

Quest Says Nearly 10% Of Its Vitamin D Tests Were Inaccurate (Jan 2009)

Last October, Quest Diagnostics contacted "thousands of doctors" around the country to notify them that one or more of their patients might have received "questionable" results on vitamin D tests performed over the past two years. It's offering free retests to anyone who was affected.

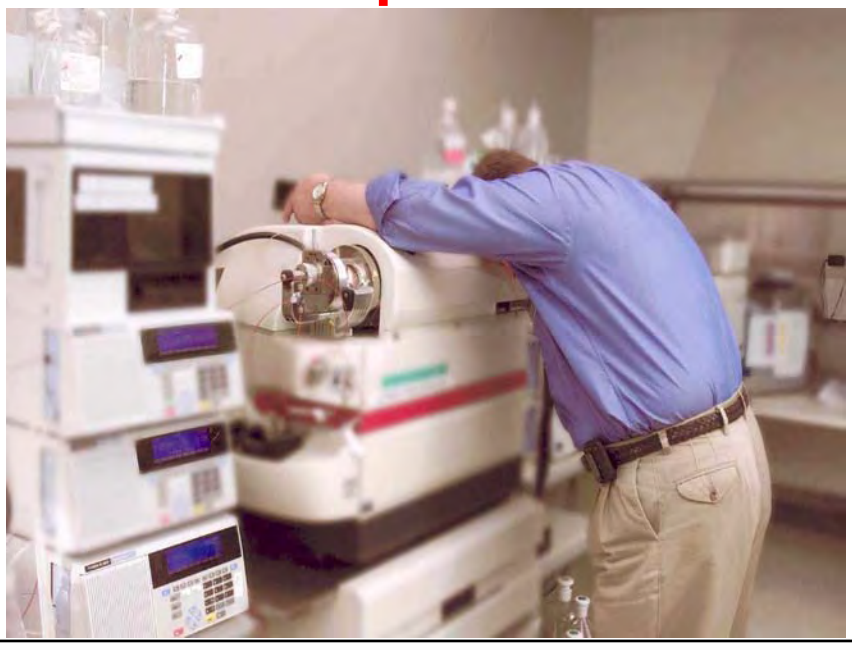
The errors came about when Quest switched from an FDA-approved test to "a new test of its own design," reports the New York Times.

Dr. Salameh, a medical director for Quest, says the mass spectrometers Quest uses weren't calibrated properly, and that 4 of the 7 labs didn't always follow proper procedure.

From Research to Clinical

- There is a huge difference between “proof of concept” and adequate test development plus clinical validation
- Must secure (and document) day-by-day test “robustness”, and performance
- Implementation must include QA/QC, proficiency testing, peer comparison
- Evidence of real clinical utility is needed
- (Pre)-acceptance by medical field is essential

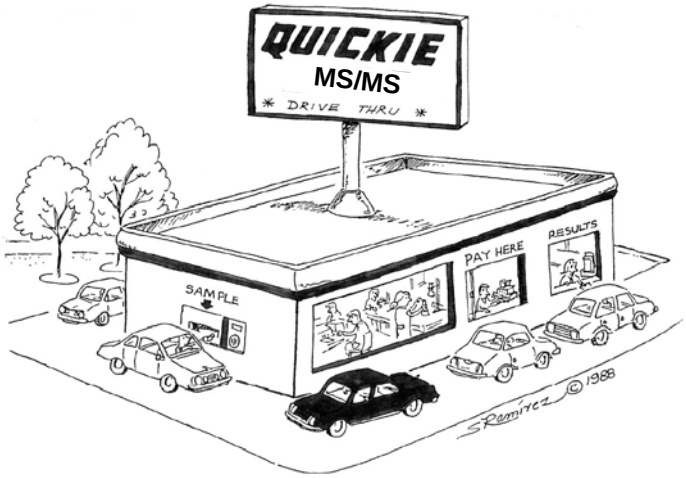
Not So Simple After All….



Conclusions

- MS/MS methods are increasingly popular in Laboratory Medicine because they are faster, better, and cheaper (↑ value)
- New applications are emerging in virtually all fields (Pathology, Infectious Diseases, biomarker discovery)
- "Simplicity" at the analytical level is no remedy for post-analytical complexity

Conclusions



- "Simplicity" at the analytical level is no remedy for post-analytical complexity