Newborn Screening of Lysosomal Storage Diseases

Michael H. Gelb (Dept. of Chemistry & Biochemistry, Univ. of Washington)
30 yrs of experience in enzymology, organic chemistry, mass spec.

Frank Turecek (Dept. of Chemistry, Univ. of Washington)
35 yrs experience in mass spec instrumentation and theory

C. Ron Scott (Dept. of Medicine, Univ. of Washington)
40 yrs of experience in metabolic diseases and newborn screening

NIH Grant DK67859 (Salvatore Sechi, Program Official)

Today's Slides:
Google me to find my home page.

Disclosures

We have received funding from and/or worked in collaboration the following companies:

1. Perkin Elmer (funding and consulting) *Mack Schermer, PE Life Sciecnes*
2. Genzyme (funding and consulting) *Joan Keutzer, Genzyme*
3. BioMarin (funding) *Nicole Miller, BioMarin*
4. Shire (funding) *David Whiteman, Shire*
5. Synageva (collaboration) *Dana Martin, Synageva*
6. Ultragenyx (collaboration) *Emil Kakkis, Ultragenyx*

Presentation Outline

1. Brief historical overview.
2. Worldwide NBS labs live or near-live for LSD NBS
3. Development of the new UW/PE-FIA-MS/MS-2014 6-Plex Assay
4. Detailed comparision of the UW/PE-FIA-MS/MS-2014 and digital microfluidics fluorimetry methods.
5. Large Scale Pilot studies for LSDs

Historical Summary of MS/MS LSD NBS Developments

2004-2009: UW team develops FIA-MS/MS assay for Pompe, Fabry, Niemann Pick-A/B, Krabbe, MPS-I, Gaucher. Genzyme modifies the method a bit and manufacturers the reagents. CDC distributes them and developes QC DBS **UW/Genzyme/CDC-2008 FIA-MS/MS Method**

2006: NY State (J. Orsini et al.) goes live for Krabbe NBS using a slight modification of the UW/Genzyme/CDC-2008 FIA-MS/MS Method.

2009-2013: D. Kasper, G. La Marca, UW team simplify the pre-MS/MS sample prep by using LC coupled to MS/MS. **UW-LC-MS/MS-2013 Method.**

2012: D. Kasper reports a large scale (n=40,000) pilot study of Gaucher, Fabry, NP-A/B, Pompe using LC-MS/MS showing a very low false positive rate thus validating LSD NBS
Lancet (2012) 379, 335.

2012: Perkin Elmer and UW team up. Push back from some NBS labs to add LC so we return to FIA-MS/MS. Reagents and buffer optimized. **UW/PE-FIA-MS/MS-2014 Method.**

2012: IL NBS lab sets up UW-LC-MS/MS-2013 Method. Plans to go live for Pompe, Fabry, NP-A/B, MPS-I, Krabbe, Gaucher now (see their poster)

2013: WA NBS lab reports a large pilot study (n=110,000) of Pompe, Fabry, MPS-I using n-1 version of UW/PE-FIA-MS/MS-2014 assay. *J. Pediatr. (2013) 163, 498.*

2014: WA NBS lab starts large pilot (n=100,000) of UW/PE-FIA-MS/MS Method for 6 LSDs.

Worldwide Update on LSD NBS Labs (Live or near-Live)

MS/MS

NY	Krabbe	**UW/Genzyme/CDC-2008 method**
IL	Pompe, Fabry, MPS-I, Krabbe, NP-A/B, Gaucher	**UW-LC-MS/MS-2013 method**
NJ	Pompe, Fabry, MPS-I, Krabbe, NP-A/B, Gaucher	**UW/PE-FIA-MS/MS-2014 method**
WA	Pompe, Fabry, MPS-I, Krabbe, NP-A/B, Gaucher	**UW/PE-FIA-MS/MS-2014 method**
PA	Pompe, Fabry, MPS-I, Krabbe, NP-A/B, Gaucher	**UW/PE-FIA-MS/MS-2014 method**
PE-Genetics	Pompe, Fabry, MPS-I, Krabbe, NP-A/B, Gaucher	**UW/PE-FIA-MS/MS-2014 method**
Greenwood Ctr	up to 9 LSDS	**UW/PE-FIA-MS/MS-2014 method**
CFH-Taiwan	Pompe, Fabry, Gaucher, MPS-I, MPS-II	**UW/PE-FIA-MS/MS-2014 method**
Taipei Inst Path.	Pompe, Fabry, Gaucher, MPS-I	**UW/PE-FIA-MS/MS-2014 method**

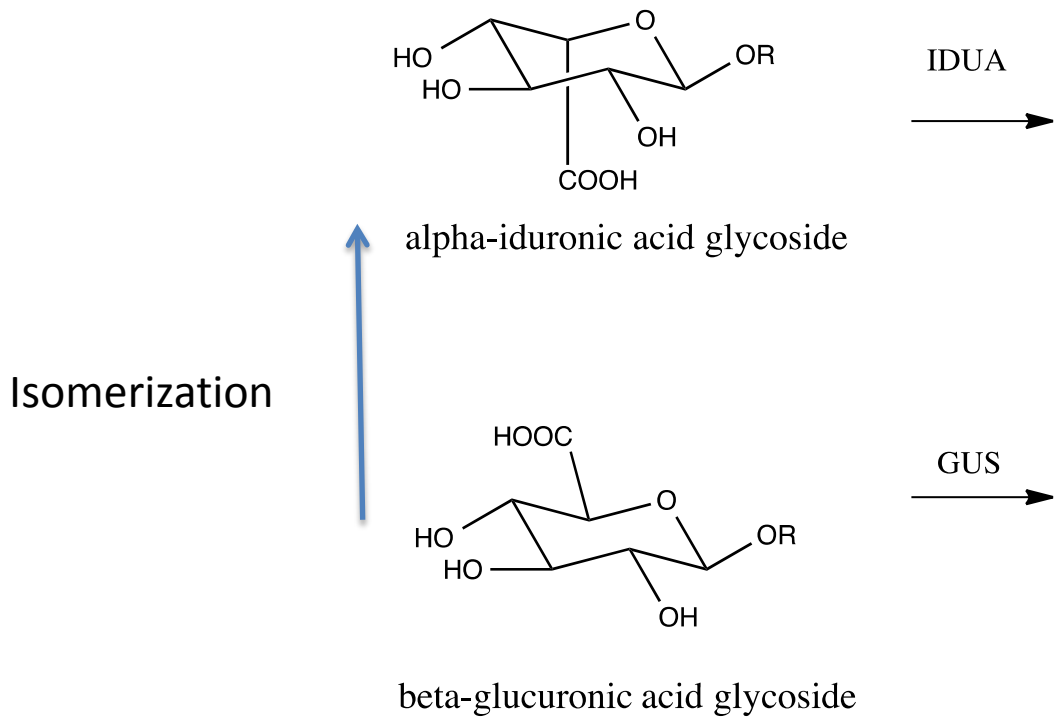
Digital Microfluidics Fluorimetry

MO	Pompe, Fabry, MPS-I, Gaucher

96-well Plate Fluorimetry

Nat. Taiwan U. Hosp.	Pompe, Fabry

Home Brew Reagents for LSD NBS? Maybe for a few but not for most.



Past problems with iduronic acid-4MU:

1. Availability has been off and on (synthesis is 10-12 steps).
2. Contains variable amounts of isomeric glucuronic acid-4MU (no good for MPS-I assays since beta-glucuronidase will produce 4MU and thus give variation in % activity at the low end.

UW/PE-FIA-MS/MS-2014 Reagents

for Gaucher, Krabbe, Fabry, Niemann-Pick-A/B, Pompe, and MPS-I

1. Commercialized by Perkin Elmer Corp.

2. Anticipated launch date for kit and reagents Q1, 2016, reagents are being supplied now to IL and NJ.

Mark Kuracina, Mack Schermer, Perkin Elmer Life Sciences

PE/UW 6-Plex Reagents

1. Similar or identical to the reagents original developed by the Gelb lab and manufactured by Genzyme but improved to increase rate of dissolution in assay buffer, to increase the dynamic range of the MS/MS assay, and to include internal standards that are chemically identical but isotopically distinguished by MS/MS.

2. GMP manufactured by a company in the USA that has 45 years of experience in production of related molecules. Syntheses optimized for production scale by collaboration between Mike Gelb and the manufacturer.

3. Checked by LC/MSMS to be devoid of enzymatic products (< 0.005% product) and to be devoid of interfering isomers (i.e. < 0.005% glucuronide in the iduronidate-based MPS-I substrate). This is critical for enzymatic activity assay at the low end.

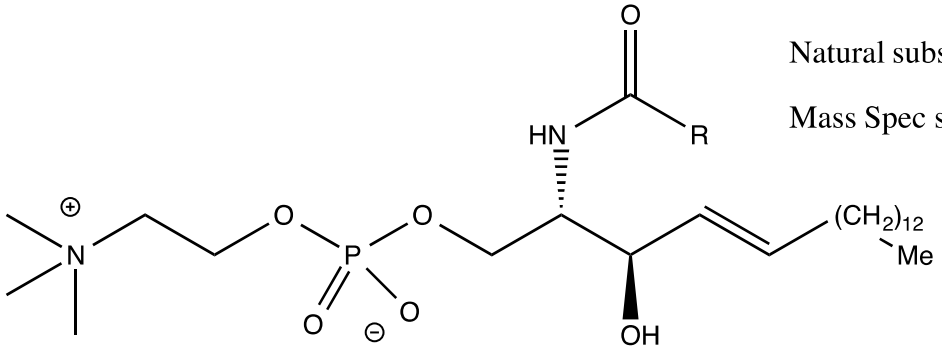
4. Checked in-house with the MS/MS NBS assay using the same SOP as provided with the kit.

Arun Babu Kumar, Sophia Blanchard, Naveen Chennamaneni (Gelb lab, UW)
Joe Trometer (Perkin Elmer Life Sciences)

UW/PE-FIA-MS/MS-2014

Single buffer for 6 LSDs optimized over a 2 year period by systematic variation of additives that have been previously reported to modulate lysosomal enzyme activities

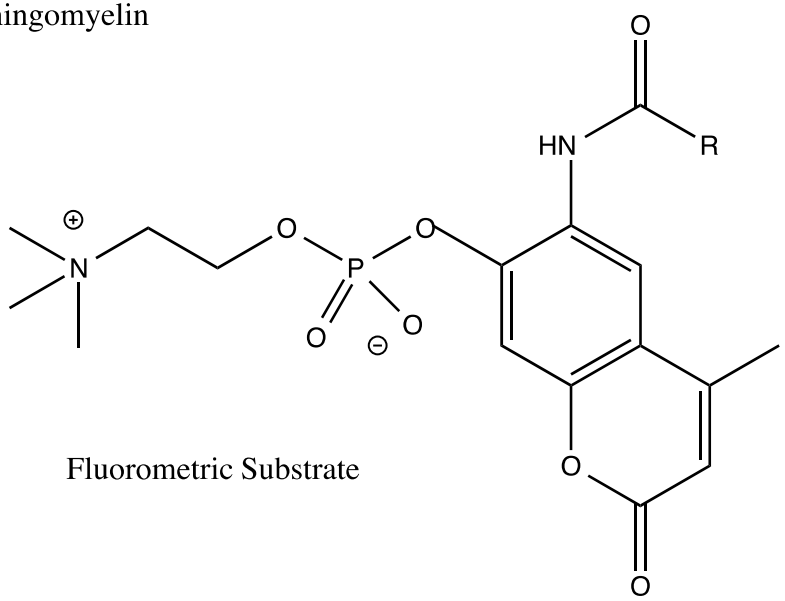
Fluorescence Assay for Niemann-Pick has a serious false negative problem and should not be used for NBS



Natural substrate, R = long chain

Mass Spec substrate, R = shorter chain

Sphingomyelin



Fluorometric Substrate

15-20% cases of NP-AB are missed with the fluorometric assay due to a mutation that has no activity with the natural substrate but has normal activity with the fluorometric substrate. This is a deal breaker !

Neuropediatr. (2003) 34, 301
J. Inherit. Metab. Dis. (2005) 28, 733

Use of MS/MS allows enzymes assays to be carried out with the natural substrate or a close analog. Recent data in the Gelb lab shows that use of the MS/MS substate solves the false negative problem (to be published). Fluor. substrate for Gaucher, and Krabbe are also not like the natural substrates. Reasons for concern? Let's see.

Detailed comparison of FIA-MS/MS Method to the Digital Microfluidics Fluorimetric Method

LSDs covered as of Oct. 2014:

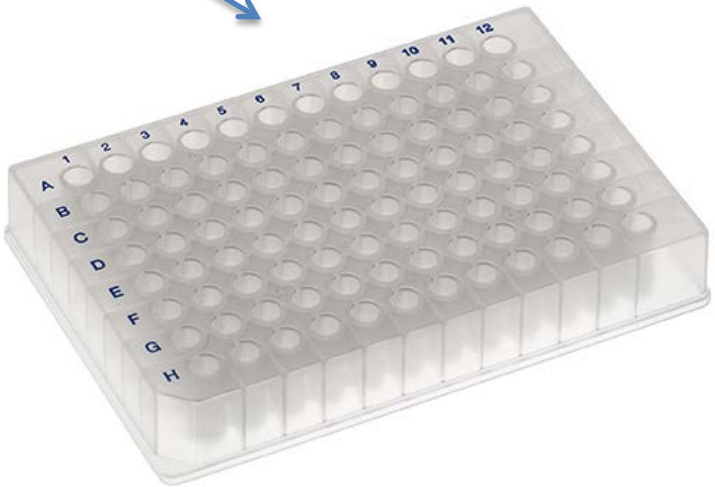
FIA-MS/MS: MPS-I, Fabry, Gaucher, Pompe, MPS-II, Krabbe, Niemann-Pick-A/B, MPS-IVA, MPS-VI

DMF: MPS-I, Fabry, Gaucher, Pompe, MPS-II

UW/PE-FIA-MS/MS-2014 Method

Note IL is using the UW-LC-MS/MS-2013 method and will likely switch to the UW/PE-FIA-MS/MS-2014 method in 2016, easy for them to do, just remove the LC column.

Step 1: Punch DBS into 96-well plate



UW/PE to be published

UW/PE-FIA-MS/MS-2014 Method

Step 2: Add 30 μL 6-plex assay cocktail containing 6 substrates and 6 int. stds. to each well (96 newborns per plate)

Rainin Liquidator 96-channel pipettor

NO ROBOT needed

1 liquid transfer per 96 newborns

UW/PE to be published

UW/PE-FIA-MS/MS-2014 Method

Step 3: Place 96-well plate in shaker/incubator for overnight incubation.

Already present in NBS labs.

UW/PE to be published

UW/PE-FIA-MS/MS-2014 Method

Step 4: Add 200 μL water then 400 μL ethyl acetate to each well, mix up and down with pipettor 4-5 times.

2 liquid transfer per 96 newborns

Rainin Liquidator 96-channel pipettor

UW/PE to be published

UW/PE-FIA-MS/MS-2014 Method

Step 5: Centrifuge plates 5 min to separate organic and aqueous layers

8 inch diameter plate centrifuge: $4500

UW/PE to be published

UW/PE-FIA-MS/MS-2014 Method

Step 6: Transfer 200 μL of upper ethyl acetate layer to new 96-well plate.
Step 7: Evaporate ethyl acetate .
Step 8: Add flow injection solvent
Step 9: Place on autosampler for flow injection MS/MS

2 liquid transfer per 96 newborns

Already present in most NBS labs

UW/PE to be published

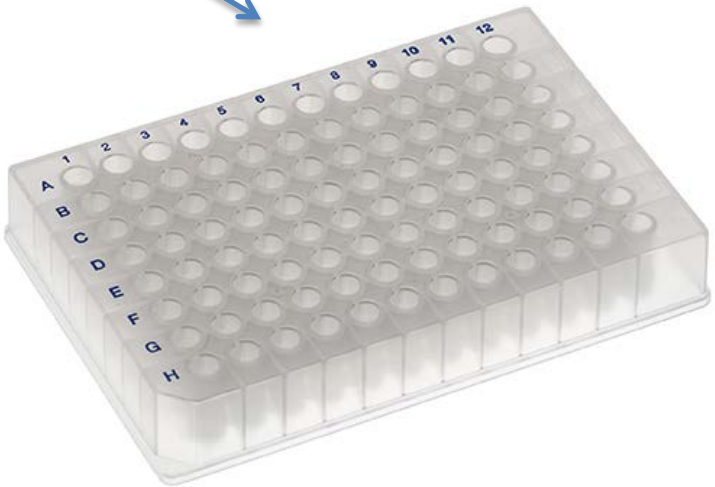
UW/PE-FIA-MS/MS-2014 Method

Over past 3 years, a SINGLE FTE in the WA NBS lab has run 113,000 DBS per year on a SINGLE FIA-MS/MS instrument.

No backup MS/MS purchased since the lab already has 1 for the amino acids/acyl carnitines/organic acid NBS (same machine as used for LSD NBS).

Digital microfluidics fluorimetric 5-plex

Step 1: Punch DBS into 96-well plate, identical to FIA-MS/MS method



R.S. Sista et al. / Clinica Chimica Acta 424 (2013) 12–18

Digital microfluidics fluorimetric 5-plex

Step 2: Add DBS extraction solvent to each well.

Not sure how this is done but a 96-channel pipettor seems appropriate.

Rainin Liquidator 96-channel pipettor

1 liquid transfer per 96 newborns

R.S. Sista et al. / Clinica Chimica Acta 424 (2013) 12–18

Digital microfluidics fluorimetric 5-plex

Step 3: Place 96-well plate in shaker/incubator for 30 min to extract enzymes.

Already present in NBS labs.

R.S. Sista et al. / Clinica Chimica Acta 424 (2013) 12–18

Digital microfluidics fluorimetric 5-plex

Step 4: Centrifuge plates 5 min.

8 inch diameter plate centrifuge: $4500

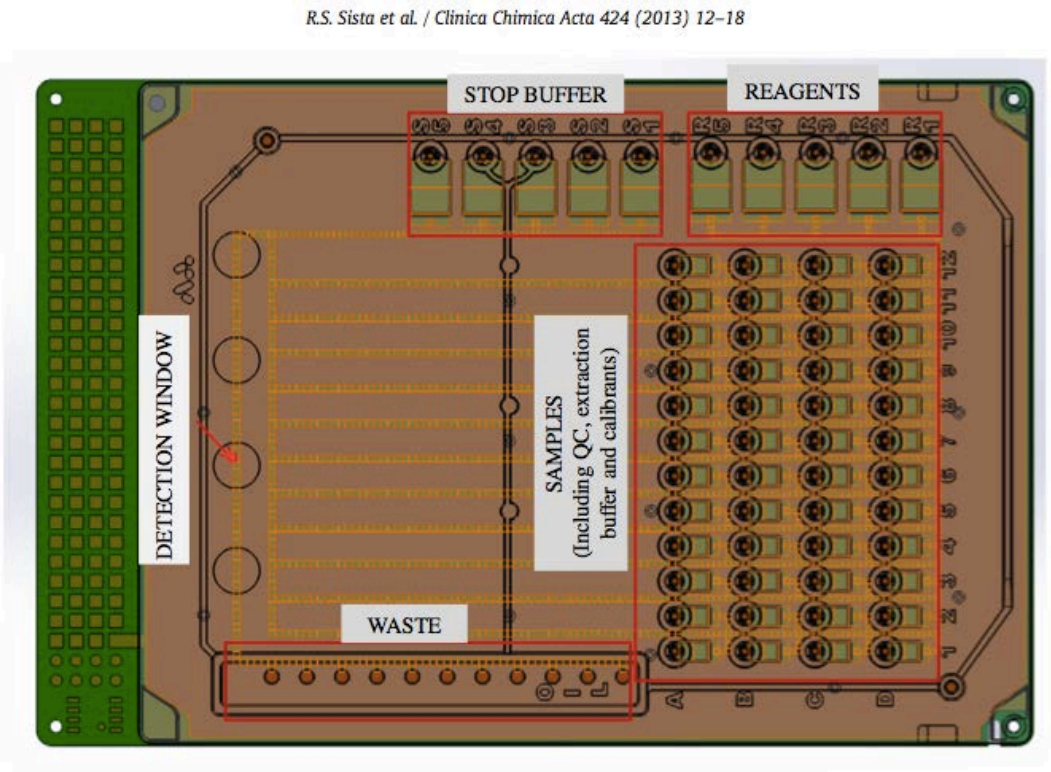
R.S. Sista et al. / Clinica Chimica Acta 424 (2013) 12–18

Digital microfluidics fluorimetric 5-plex

Step 5: Transfer 1.6 μL of DBS extract to sample well on microfluidics chip.

Not sure how the small volume is transferred. Each chip accepts 44 enzyme extracts and 4 calibrators.

2 liquid transfer per
96 newborns if done with
a 48-channel pipettor



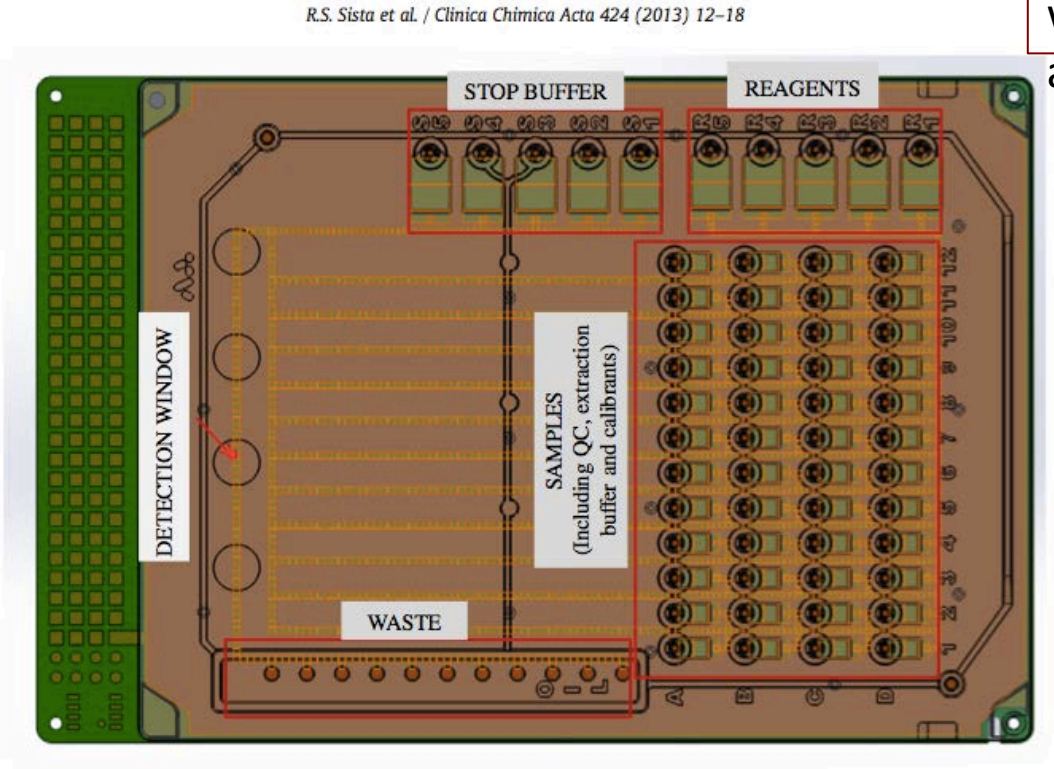
R.S. Sista et al. / Clinica Chimica Acta 424 (2013) 12–18

Digital microfluidics fluorimetric 5-plex

Step 6: Transfer 5 different assay reagents to 5 different reservoirs on chip

2 liquid transfer per 96 newborns if done with

a 5-channel pipettor

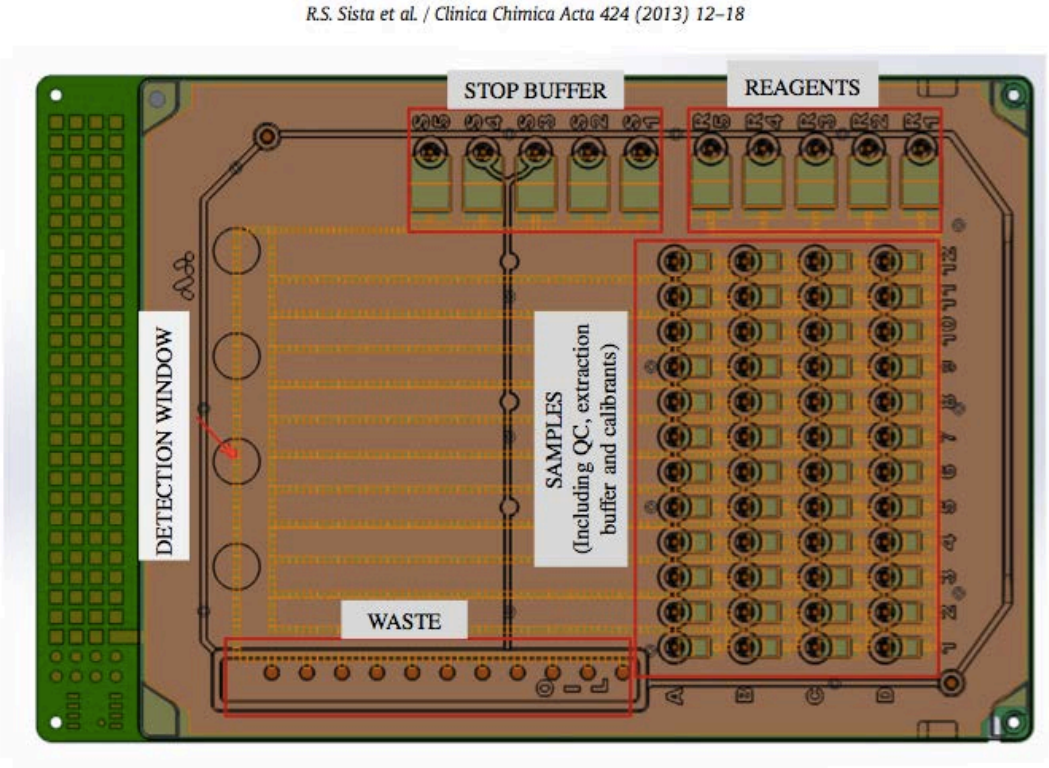


R.S. Sista et al. / Clinica Chimica Acta 424 (2013) 12–18

Digital microfluidics fluorimetric 5-plex

Step 7: Transfer 5 different stop solutions to 5 different reservoirs on chip.

2 liquid transfer per
96 newborns if done with
a 5-channel pipettor



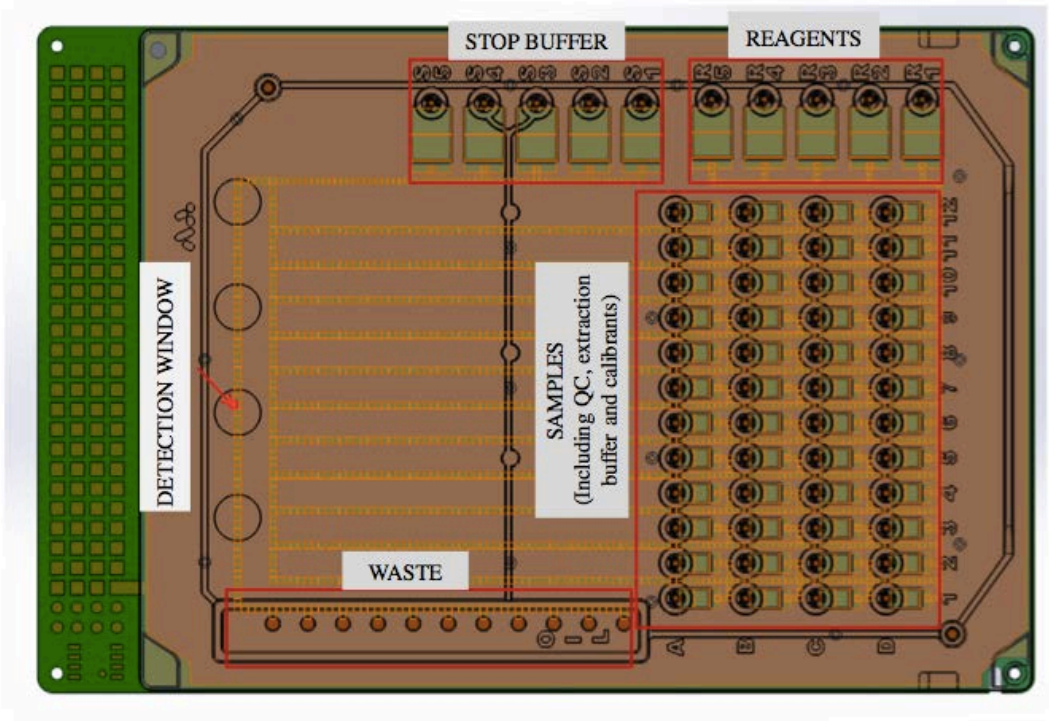
R.S. Sista et al. / Clinica Chimica Acta 424 (2013) 12–18

Digital microfluidics fluorimetric 5-plex

Step 8: Transfer silicone oil to cover chip.

2 liquid transfer per 96 newborns

R.S. Sista et al. / Clinica Chimica Acta 424 (2013) 12–18



R.S. Sista et al. / Clinica Chimica Acta 424 (2013) 12–18

Digital microfluidics fluorimetric 5-plex

Step 9: Place chip in chip reader for reagent mixing, incubation, and fluorimetry.
One workstation is a set of 4 readers

R.S. Sista et al. / Clinica Chimica Acta 424 (2013) 12–18

Summary

Method	Number of liquid transfers assuming a multi-channel pipettor is used	Volume of smallest volume transferred	Equipment required
UW/PE MS/MS 6-plex	5	30 μL	DBS puncher plate incubator plate centrifuge multichannel pipettor solvent evaporator one MS/MS with flow injection
Digital microfluidics fluorimemtric 5-plex	9	1.6 μL	DBS puncher plate incubator plate centrifuge multichannel pipettor plate readers

WA state NBS lab, 1 FTE (B.S. level) has been running 6-7 x 96 well plates per day doing all steps except punching the blood spot (170,000 newborns per year) using a SINGLE Waters TQD MS/MS with a flow-injection autosampler. A backup machine may be required, but you could use the backup for amino acid/ acyl carnitine/organic acid analysis since it is the SAME instrument. Throughput of 1 MS/MS = throughput of 7.8 digital microfluidics machines.

Space Requirements are Similar

MS/MS

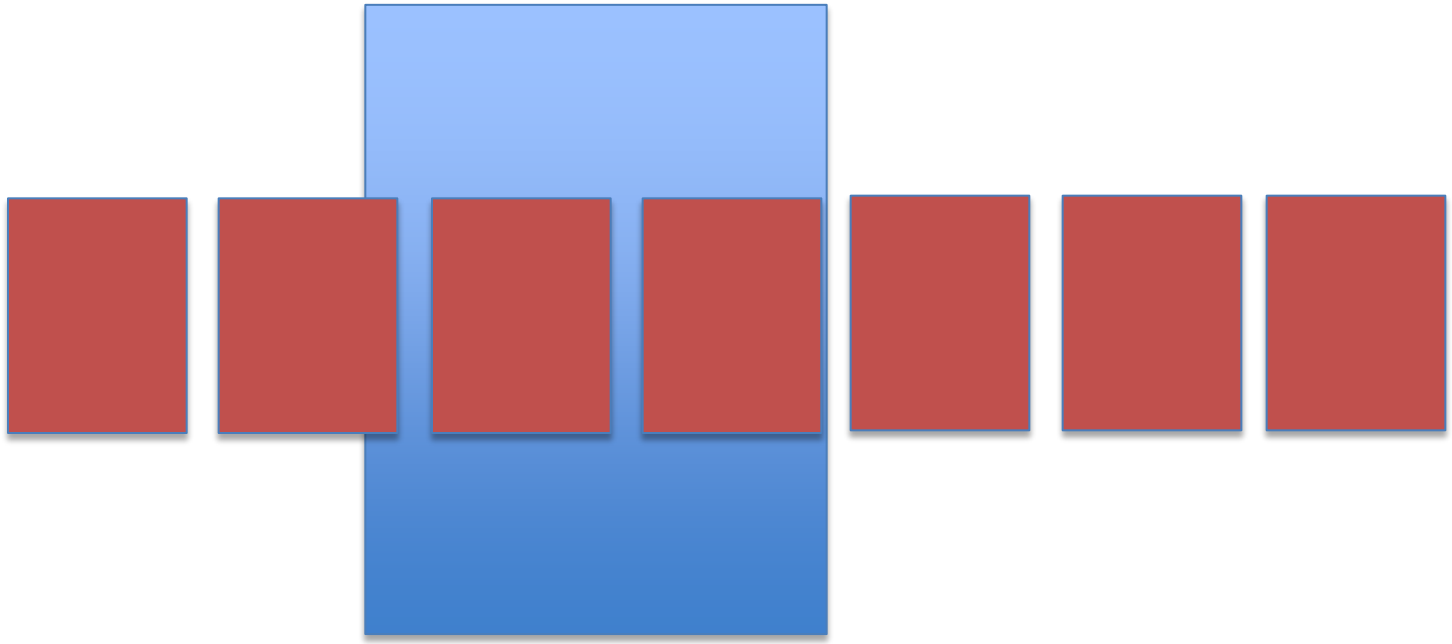
one MS/MS instrument

Volume: 14 cu ft

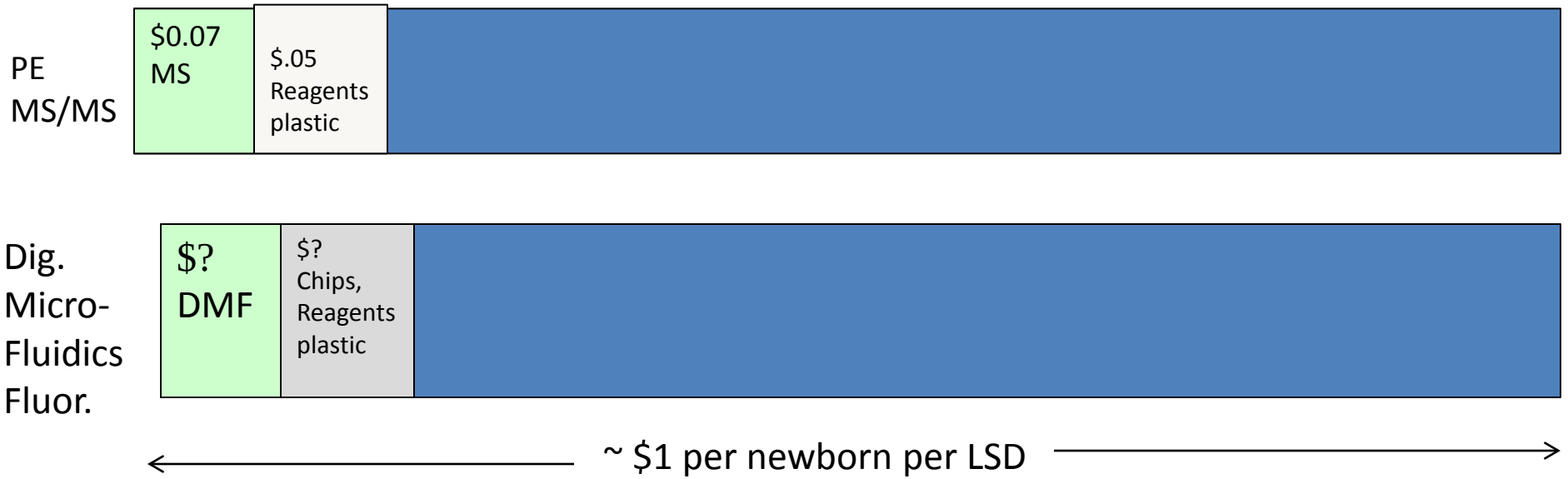
Digital microfluidics fluorimetry

7 Fluorimeter plate readers

Volume: 12.6 cu ft



Estimated costs per newborn per LSD



MS/MS instrumentation cost is the cost of instrumentation purchasing, installation, maintenance, nitrogen, and electricity divided by the number of LSD enzymes analyzed before the instrument needs to be replaced (8-10 yrs). Does not include a backup, presumably most labs already have one for their ongoing MS/MS NBS. Does not include capital other than installation, but space requirements for MS/MS is similar to that for digital microfluidics.

Comparison of new UW/PE-MS/MS assay to digital microflluidics fluorimetric assay.

The critical parameter is the assay signal with blood divided by the signal without blood, the so-called blood/no blood ratio, aka dynamic range.

Disease	Ratio of mean activity for random newborns to mean activity of LSD-affected		Ratio of mean activity for random newborns to mean activity in filter paper blank (no blood)
	MS/MS	Digital microfluidics	MS/MS
Fabry	28	6.1	109
Gaucher	67	3.7	216
Pompe	63	5.0	367
MPS-I	168	7.4	230
MPS-II	60	3.9	80
Krabbe	27	no data	85
Niemann-Pick-A/B	26	no data	104

MS/MS: to be published *Jason Cournoyer, PE Life Sciences*

Digital microfluidics: Clin. Chim. Acta (2013) 424, 12-18

Comparision of 2 large MPS-I Pilots

Study Site	Method	DBS tested	Screen cutoff MPS-I	Number of screen positives MPS-I	Number of positives after re-test MPS-I	Genotyping MPS-I
WA NBS Lab	n-1 version of UW/PE-FIA-MS/MS-2014 3-plex (MPS-I, Fabry, Pompe)	106,526	32% of mean activity	7	7	3 MPS-I 1 carrier 3 false pos 2 poor punch*
MO NBS Lab	Digital Microfl. Fluor. 4-plex (MPS-I, Fabry, Pompe, MPS-II)	117,000	not stated, cutoff was decreased as study progressed	57	not stated	1 MPS-I 24 pseudodef 3 carriers 24 false pos 4 pending 1 lost to followup

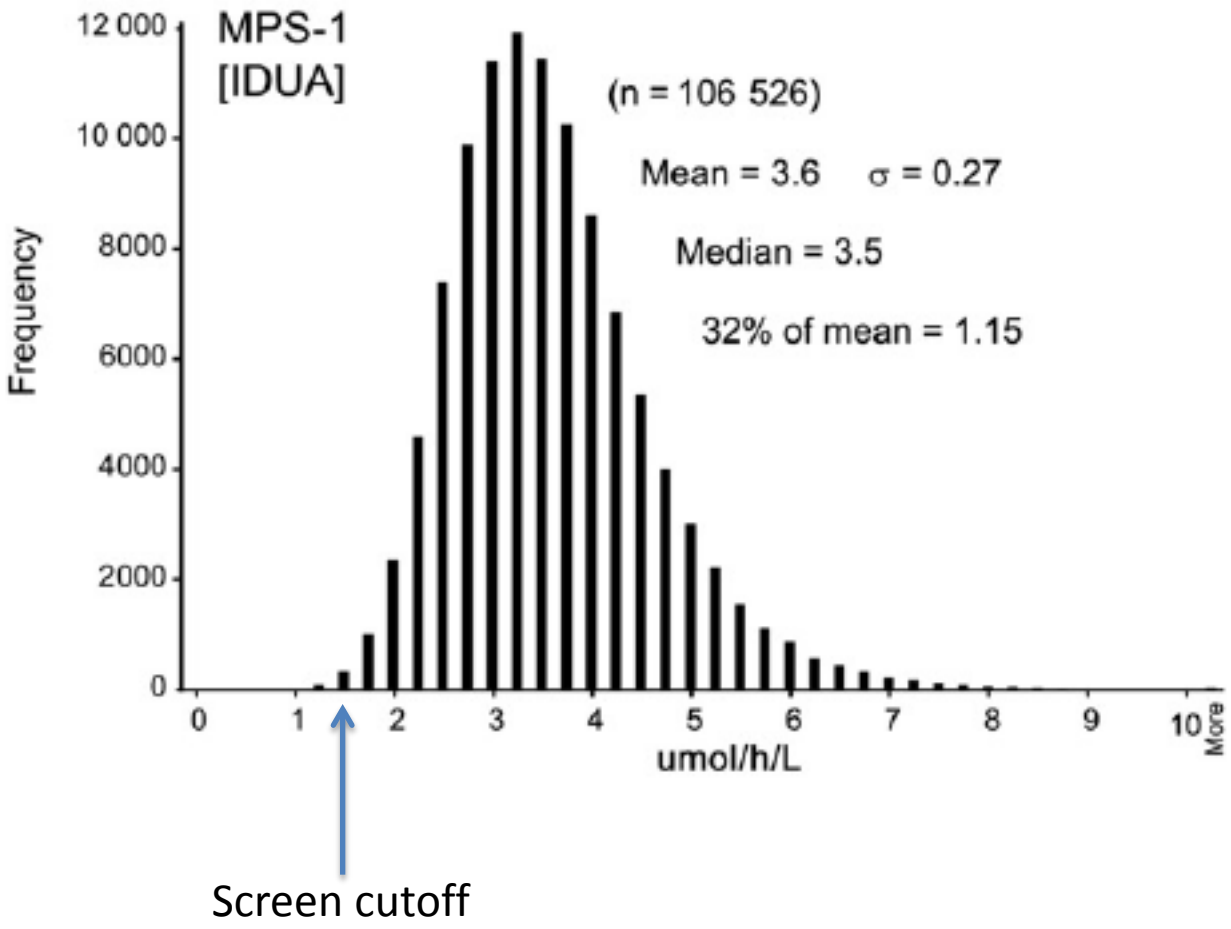
WA study: J. Pediatr. (2013) 163, 498.

MO study: Newborn screening for MPS-I: Interim report from the Condition Review Group, Alex R. Kemper, Sept. 11, 2014

*Poor punch: All 3 enzymes read low, and punch was found to be deficient in blood (white paper showing).

WA state NBS lab 3-plex pilot study

Distribution of IUDA enzymatic activity measured with n-1 version of UW/PE-FIA-MS/MS-2014 Method



Putting all the large-scale NBS pilot studies together

WA vs MO large scale NBS lab comparison: The UW/PE-FIA-MS/MS-2014 method outperforms the digital microfluidics fluorimetry method in terms of dynamic range and estimated false positive rates.

Mayo Clinic Pilot: ~100,000 DBS submitted to 4 assays:

1) Standard microtiter plate fluorimetric assay
2) UW/Genzyme/CDC-2008-FIA-MS/MS assay
3) Digital microfluidics fluorimetry assay
4) Luminex immunoquantification of protein abundance assay

Matern D, Oglesbee D, Tortorelli S.Dev Disabil Res Rev. 2013 Jun;17(3):247-53.

NBS assay performance of assays 2-4 are similar and better than 1. False positive rates of all four are unacceptably high.

Putting it all together: UW/PE-FIA-MS/MS-2014 assay is the top performing LSD NBS assay. Furthermore the UW/Genzyme/CDC-2008 assay is becoming obsolete and is being replaced by the UW/PE-FIA-MS/MS-2014 assay.

Comparison of UW/PE-FIA-MS/MS-2014 6-plex to UW-LC-MS/MS-2013 6-plex

Number of Screen Positives per 10,000 DBS

Lab/Method	GLA	GAA	IDUA	ASM	GALC	GBA	**TOTAL**
WA NBS lab UW/PE-FIA-MS/MS-2014 Method	0	3	3	0	3	3	**12**
IL NBS lab UW/LC-MS/MS-2013 Method	4	8	14	1	7	4	**38**

Data in WA is based on the ongoing pilot, n=7,000 DBS completed.

Data in IL is based on the validation study with n=12,000 DBS.

LC and FIA MS/MS are likely to be equivalent, better performance in WA likely due to improved reagents and buffer in assay cocktail

Summary

1. There are multiple fluorimetric and MS/MS methods for LSD NBS.

2. MS/MS methods are converging to the UW/PE-FIA-2014 method for 3 reasons:
   1) It is simpler to carry out the pre-MS/MS steps.
   2) It outperforms the earlier developed MS/MS methods in terms of false positives.
   3) The PE kit will likely be the only long-term source of MS/MS reagents, and home-brew is probably not feasible.

3. The comparison of 2 large pilot studies (~110,000 DBS), the WA state NBS lab MS/MS 3-plex study (n-1 version of UW/PE-FIA-MS/MS-2104 method) and the MO state NBS lab 4-plex study (digital microfluidics fluorimetry) shows that the MS/MS method greatly outperforms the fluor. method in terms the number of screen positives and the false positive rates. Large scale pilot studies using conventional fluorimetry shows enormously high false positive rates.

4. Pre-instrumentation sample preparation is easier for the UW/PE-FIA-MS/MS-2014 method compared to digital microfluidics fluorimetry.

5. Manpower and space requirements of the two methods are similar

6. Cost of the two methods are likely to be similar.

UW

Mariana Barcenas
Sophie Blanchard
Naveen Chennamaneni
Trisha Duffey
Scott Gerber
Frances Hocutt
Tanvir Khaliq
Arun Babu Kumar
Yijun Li
Sophia Masi
Martin Sadilek
Zdenek Spacil
Haribabu Tatipaka
Ding Wang
Brian Wolfe

Perkin Elmer

Jason Cournoyer
Anna Butterfield
Carole Elbin
Joe Trometer
Alex Cherkasskiy
Mack Schermer
Mark Kuracina

Genzyme Corp.

Joan Keutzer
Helmut Kallwass
Kate Zhang
Petra Olivova
Daniel Scheidegger

Shire Corp.

David Whiteman

BioMarin Corp.

Nicole Miller
Tom Lester

Frank Turecek
Dept. of Chemistry
Univ. of Washington

C. Ronald Scott
Dept. of Medicine
Univ. of Washington

Funding: NIH, Genzyme Corp., BioMarin Corp., Perkin Elmer, Shire

The Perkin Elmer Development Team

MO NBS lab announced 187 screen positives out of 117,000 for MPS-I, Pompe, Gaucher, Fabry *APHL NBS Mtg, Oct, 2014 online abstracts*

WA NBS lab announced 42 screen positives out of 111,000 for MPS-I, Fabry, Pompe *J. Pediatr. (2013) 163, 498.*

Gelb commentary:

Births in both states is ~80,000/yr.
MO still has to add NBS for Krabbe, Niemann-Pick-A/B, MPS-II so they are likely to have > 500 screen positives per year (10 per week). Can second-tier analyses keep up with that demand? Note, in NY > 100 DBS are screen positive for Krabbe per year.

Mayo Clinic Pilot Study

Started ~ Sept. 2011, to reach 100,000 anonymous DBS over 2 yrs.

UW/Genzyme/CDC-2008 FIA-MS/MS method

Luminex Immunoquantificaton Metohd

Digital Microfluidics Fluorimetric Method

Chamoles Fluorimetric Method

all 4 run in parallel.

D. Matern et al. Dev. Disabil. Res. Rev. (2013) 17, 247

Gelb commentary: Matern has presented the outcome of this pilot (~40,000 DBS) at conferences in which also presented. All methods show similar false positive rates, and these rates may be too high such that additional methods are needed, i.e. biomarkers. The MS/MS that he used is now obsolete and is being replaced by the UW/PE-FIA-MS/MS-2014 method that greatly outperforms the older method. Matern's data would tend to suggest that the new MS/MS method will also outperforms the digital microfluidics, Chamoles fluorimetric and Luminex immunoquantification methods. There is general concern in the field that immunoquantification may lead to too many false negatives in cases where stable, but enzymatically poorly active, protein is made (i.e. ~10-15% of MPS-II patients).

A Useful Advantage of Multiplexing

If all the enzymes in the multiplex set read low it suggests a miss-punched DBS.

This is how we found the 2 miss-punched DBS in the 110,000 pilot study. After examination of the enzyme activities, we inspected the DBS and found it to be deficient in blood (punched near the DBS perimeter).

A second utility of multiplexing is that a low reading for all enzyme activities in the set with a properly punched DBS would signify a low white cell count. Over time we should try to use a normalization scheme to help filter out some of the noise in the screening due to differential white cell counts.

Hypotheses of why UW/PE-FIA-MS/MS-2014 outperforms digital microfl. fluorimetry

1. Variability in amt of enzyme added to the assay:

FIA-MS/MS:	amt of blood per DBS punch
DMF:	amt of blood per DBS punch % of enz extracted from punch amt of extract added to the chip (1.6 μL)

2. Dynamic range of FIA-MS/MS is more than an order of magnitude larger than for fluor due to the relatively large background of fluor. (fluor. of blood and substrate)

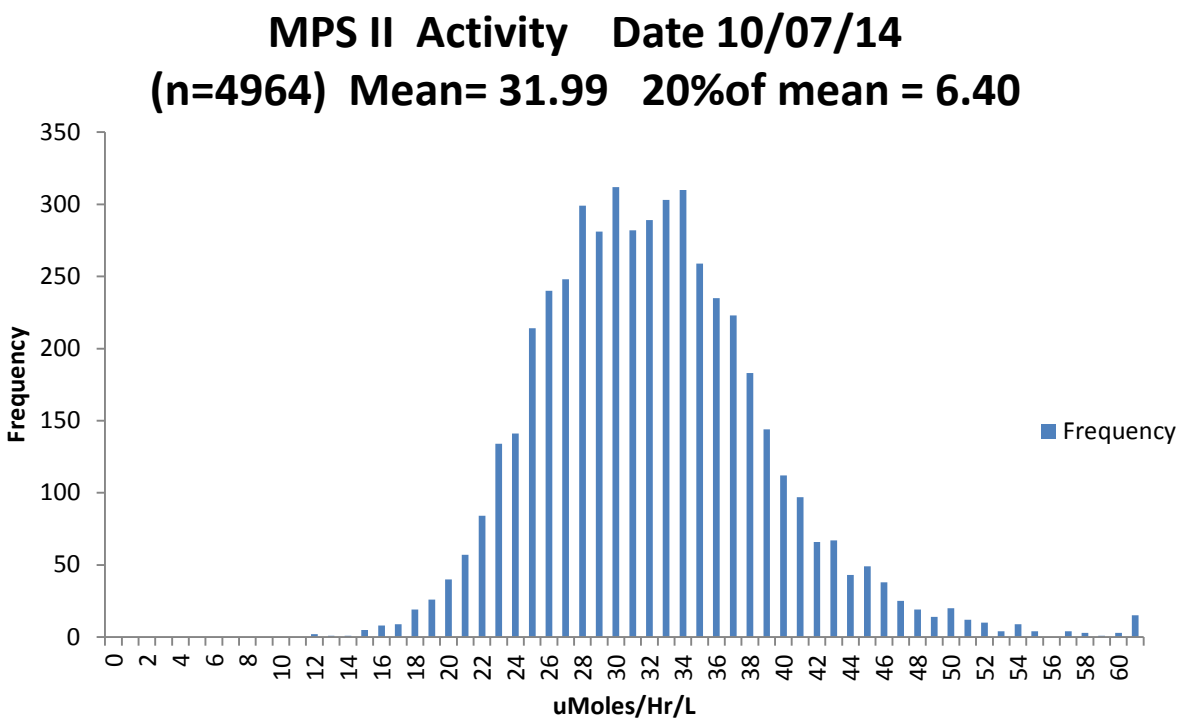
3. FIA-MS/MS uses a chemically identical internal standard that contains heavy isotopic substitution and is thus quantified independent of the product. Not possible with fluor. assays.

MPS-II FIA-MS/MS Pilot Study, WA state NBS Lab

Started Aug, 2014. To reach n = 100,000.

Screen cutoff 20% of daily mean.

Number of hits: 0 out of 4964



Large Scale Pilot Studies (1/3 of Taiwan, done by 96-well plate fluorimetry)

National Taiwan Univ. Hospital

Fabry (Chamoles Fluor. method):

171,977 DBS gave 913 hits gave 94 after more enzyme tests gave after leukocyte enzyme test: 5 refusals, 19 (>30% act.), 66 (<30% act.), 11 (<5% act.)
Human Mutat. (2009) 30, 1397.

Pompe (Chamoles Fluor. method):

473,738 DBS gave 2,241 hits, gave 219 after more enzyme tests gave after DNA seq: 10 refusals, 212 normals, 19 LOPD, 9 IOPD.
Molec. Genet. Metabol. (2012) 106, 281.

Mackay Memorial Hospital, Taiwan

MPS-I (Chamoles Fluor. method):

35,285 DBS gave 58 hits, gave 19 after assay again with first-tier method, gave 1 MPS-I after urinary GAGs/leukocyte enzyme activity/DNA seq.
Orphanet. J. Rare Disease (2013) 8, 147.

False positive rates are very high, see the publications

Pilot Studies

1. A pilot study of the new PE 6-plex kit (MPS-I, Krabbe, Pompe, Fabry, Niemann-Pick-A/B, Gaucher) started in the WA state NBS lab in July, 2014. Will reach 100,000 DBS.
   Funded by NIH and Perkin Elmer Life Sciences. So far number of hits are tracking with what we found the published 3-plex study (Scott et al., (2013) J. Pediatr.)

2. A pilot study of MPS-II has started in the WA state NBS lab to reach 100,000 DBS, MPS-IIIA, -IIIB, and -VI will be added shortly. Method: FIA-MS/MS
   So far 0 screen positives out of 4,964 DBS with a cutoff of 25% of mean activity.
   Funded by Shire, David Whiteman and BioMarin, Nicole Miller

3. A pilot study of MPS-II/MPS-VI in the Chinese Foundation of Health (Taiwan) will start in fall, 2015. Method: FIA-MS/MS
   Drs. C. C. Chiang and H. C. Liao, Chinese Foundation of Health

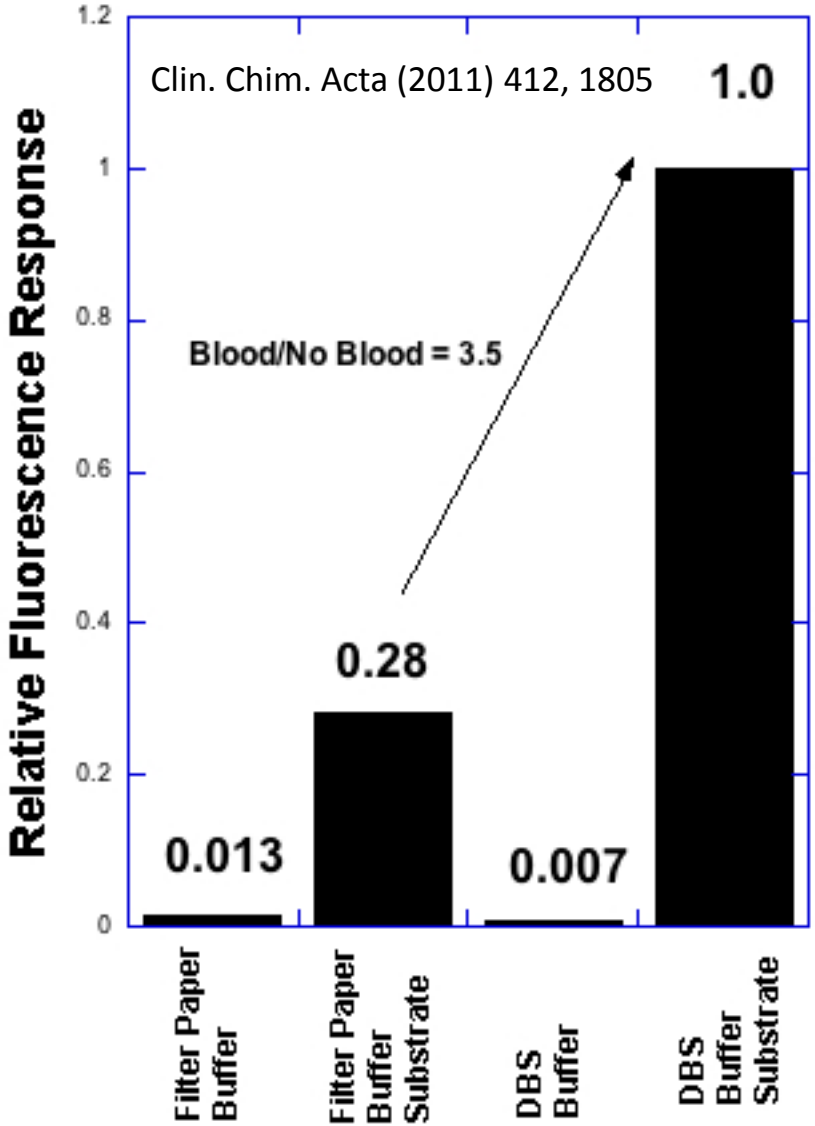
4. A pilot study for 9 LSDs has started in Belgium 2013 using UW-LC-MS/MS-2013 method
   S. Devos, PCMB, Belgium

- **Additional commercial developments.**

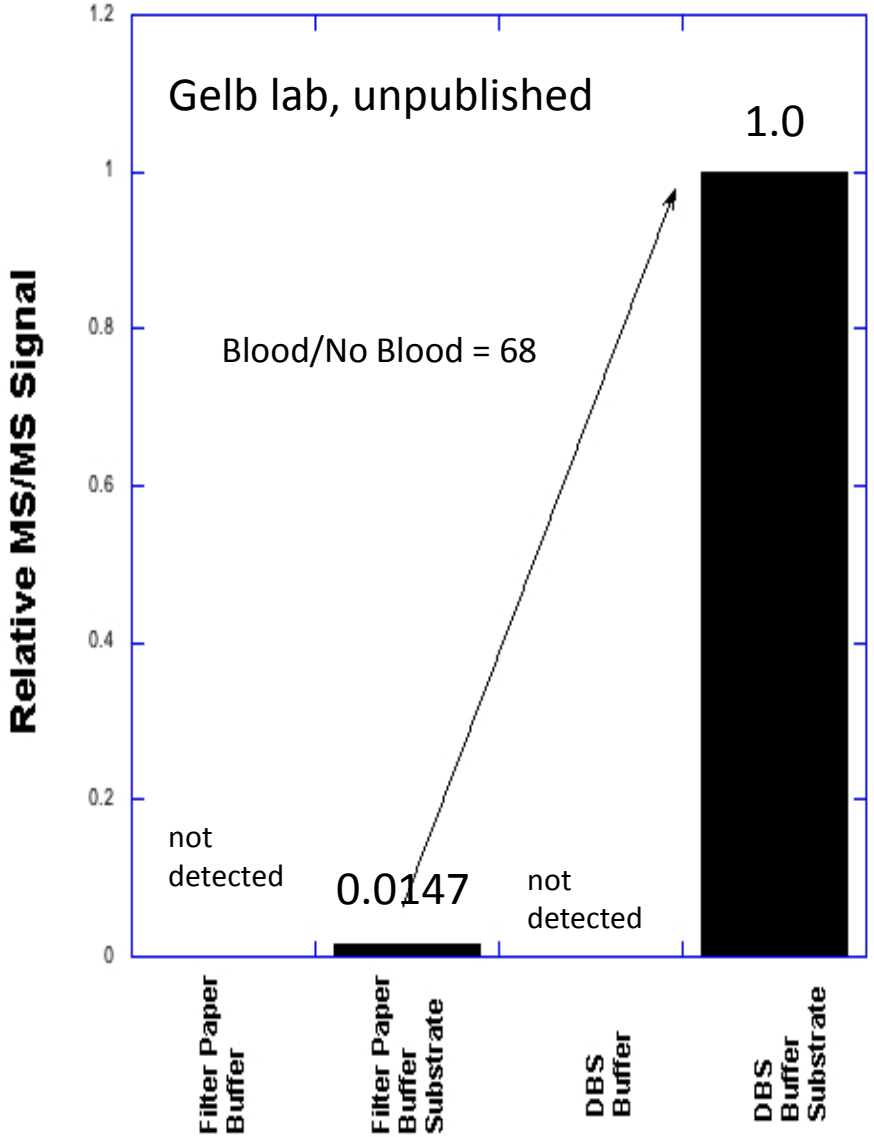
- Perkin Elmer Genetics will shortly offer worldwide testing for MPS-IVA and MPS-VI,
- and addition of MPS-II, -IIIA, and –IIIB is expected to follow.
- *Mack Schermer, PE Life Sciences*

Fluorescence vs FIA-MS/MS assay of GALNS (MPS-IVA) in DBS

Fluorescence Asssay (4MU Substrate)

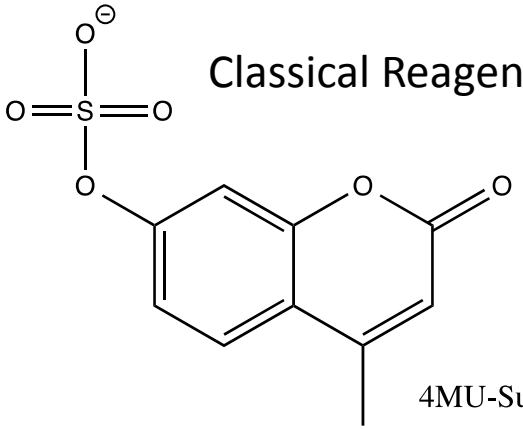


FIA-MS/MS Assay

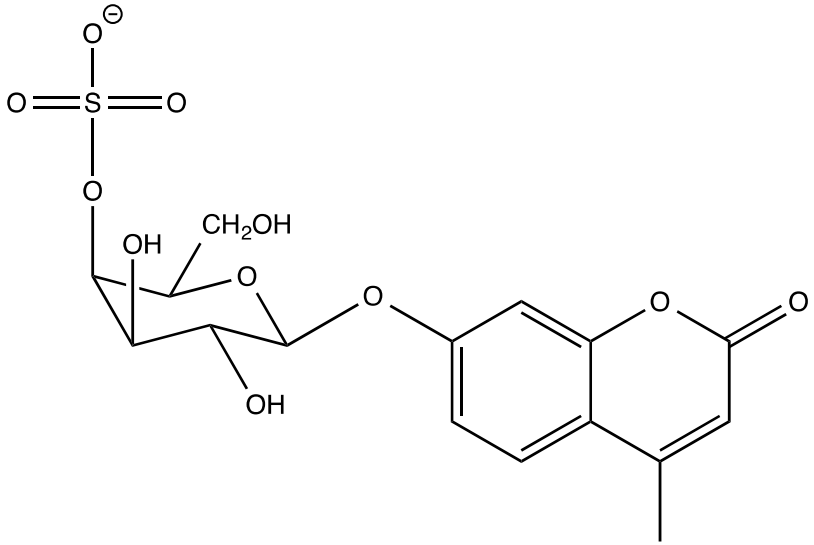


ASB (MPS-VI) Assay Reagents

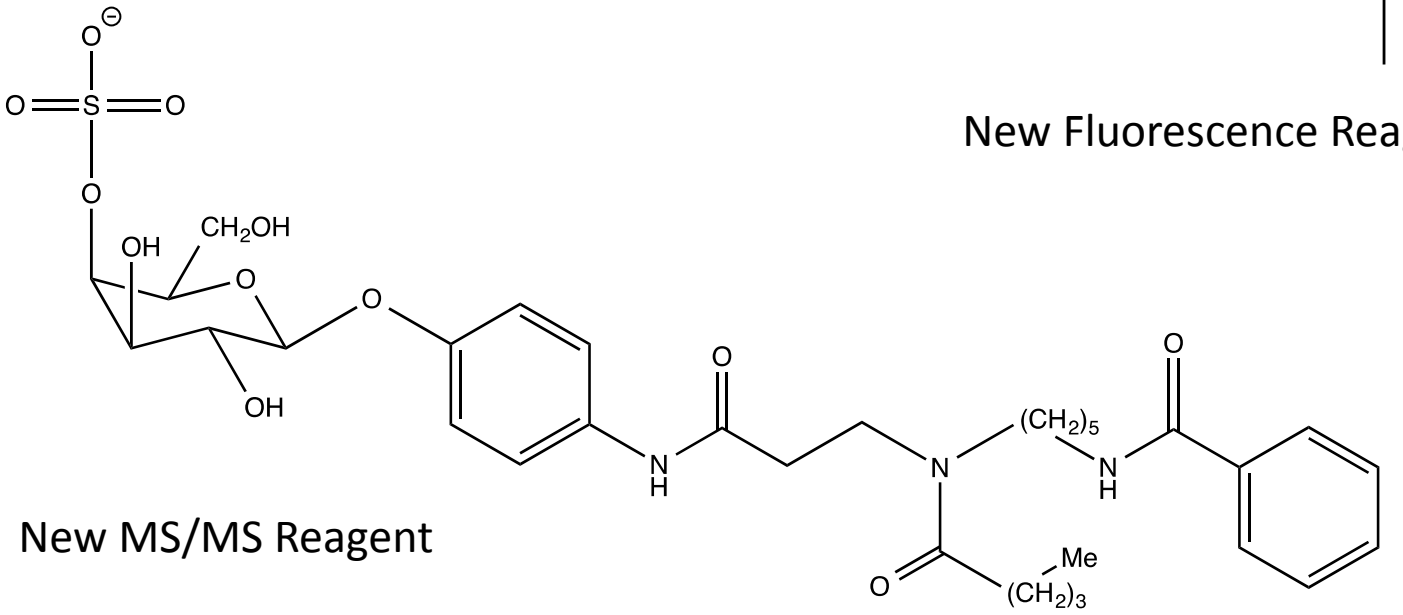
Classical Reagent



4MU-Sulfate



New Fluorescence Reagent



New MS/MS Reagent

Why don't we detect more pseudodeficiencies with a cutoff of 32% of mean activity?

A "true" pseudodeficiency is when the actual enzyme activity is low but the patient does not have significant disease. This occurs when deficient but finite enzyme is sufficient to prevent pathogenicity.

Another possibility is that the assay is not very accurate at the low end and exaggerates the degree of deficiency. This is a "pseudo-pseudodeficieny"

Hypothesis: Because the dynamic range of the MS/MS assay is so large, the accuracy of the % activity at the low end is higher than assays that have a lower dynamic range. The data comparing MS/MS to fluor. assays of MPS-I suggest that the "pseudodeficiency" problem is less severe than we thought.