Improved Assays for the Enzymes Relevant to the Mucopolysaccharidoses

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NIH Grant DK67859 (Salvatore Sechi, Program Official)

Today's Slides:
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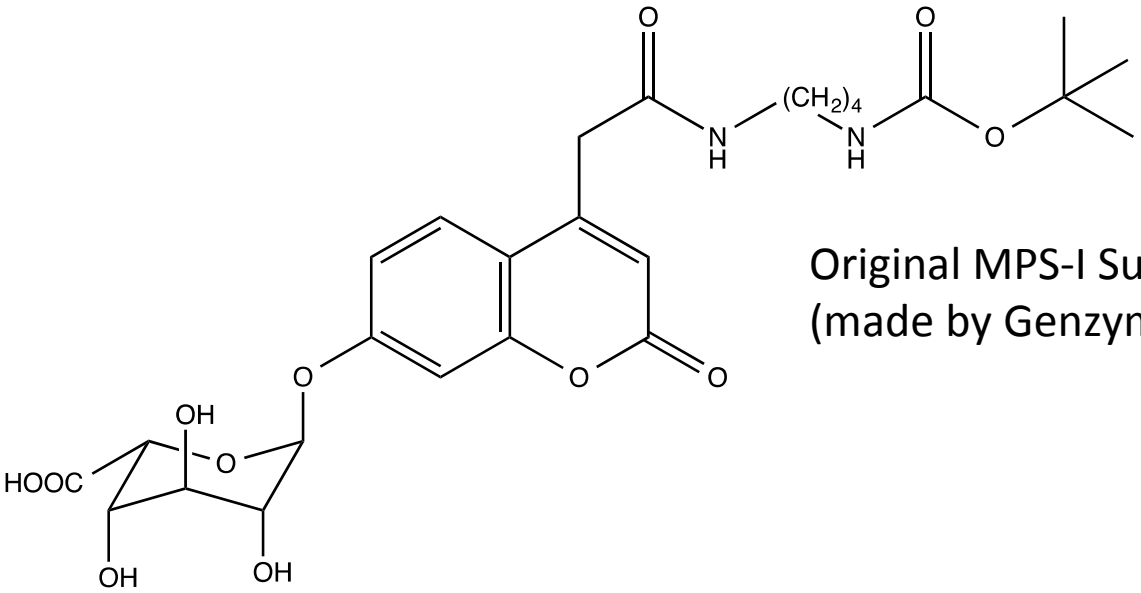
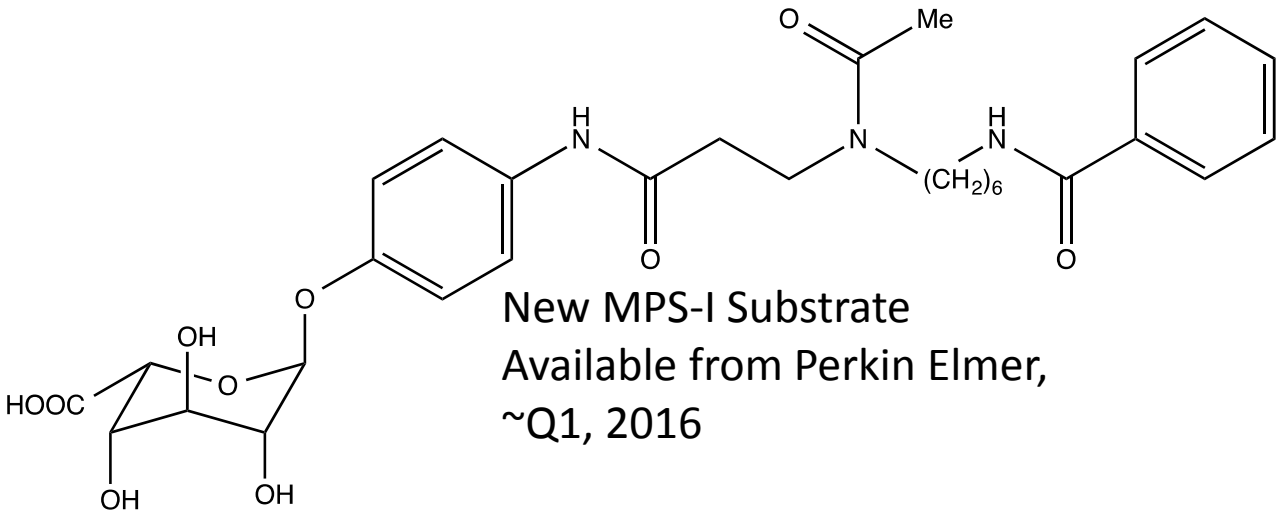
Alpha-Iduronidase for Assay of MPS-I (Hurler, Schei Syndromes)



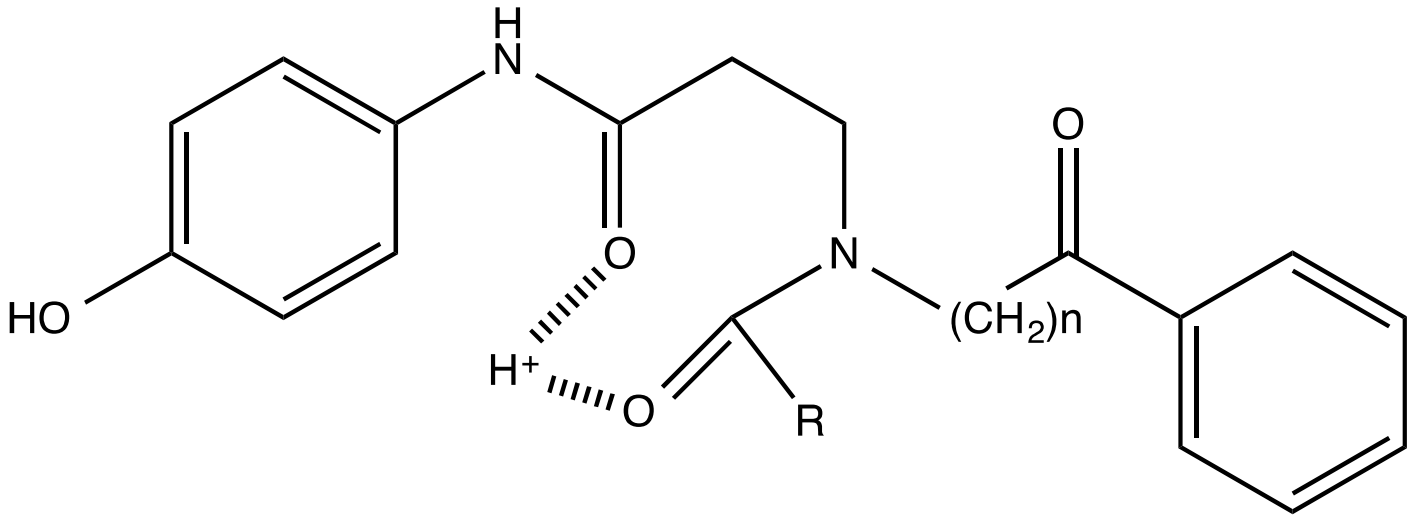
Original MPS-I Substrate
(made by Genzyme, distributed by CDC)



New MPS-I Substrate
Available from Perkin Elmer,
~Q1, 2016

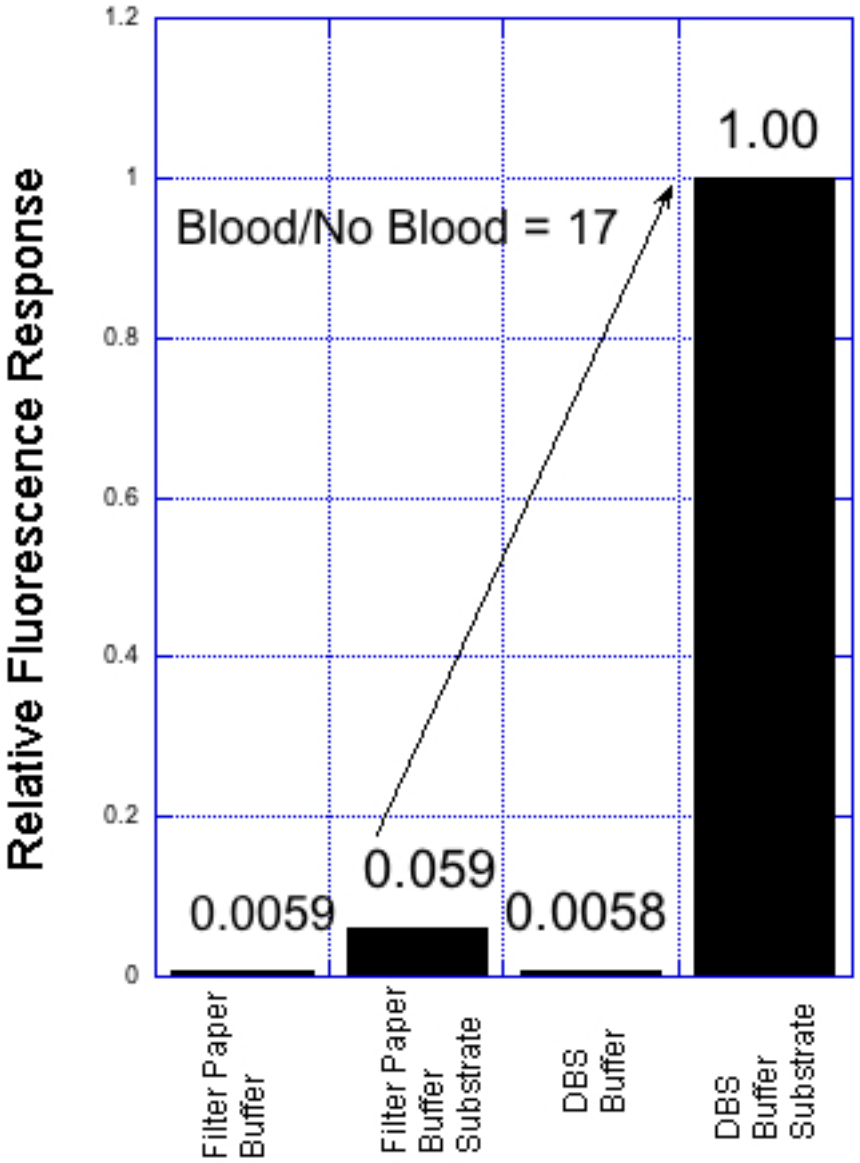
~10-fold more sensitive than original MPS-I substrate

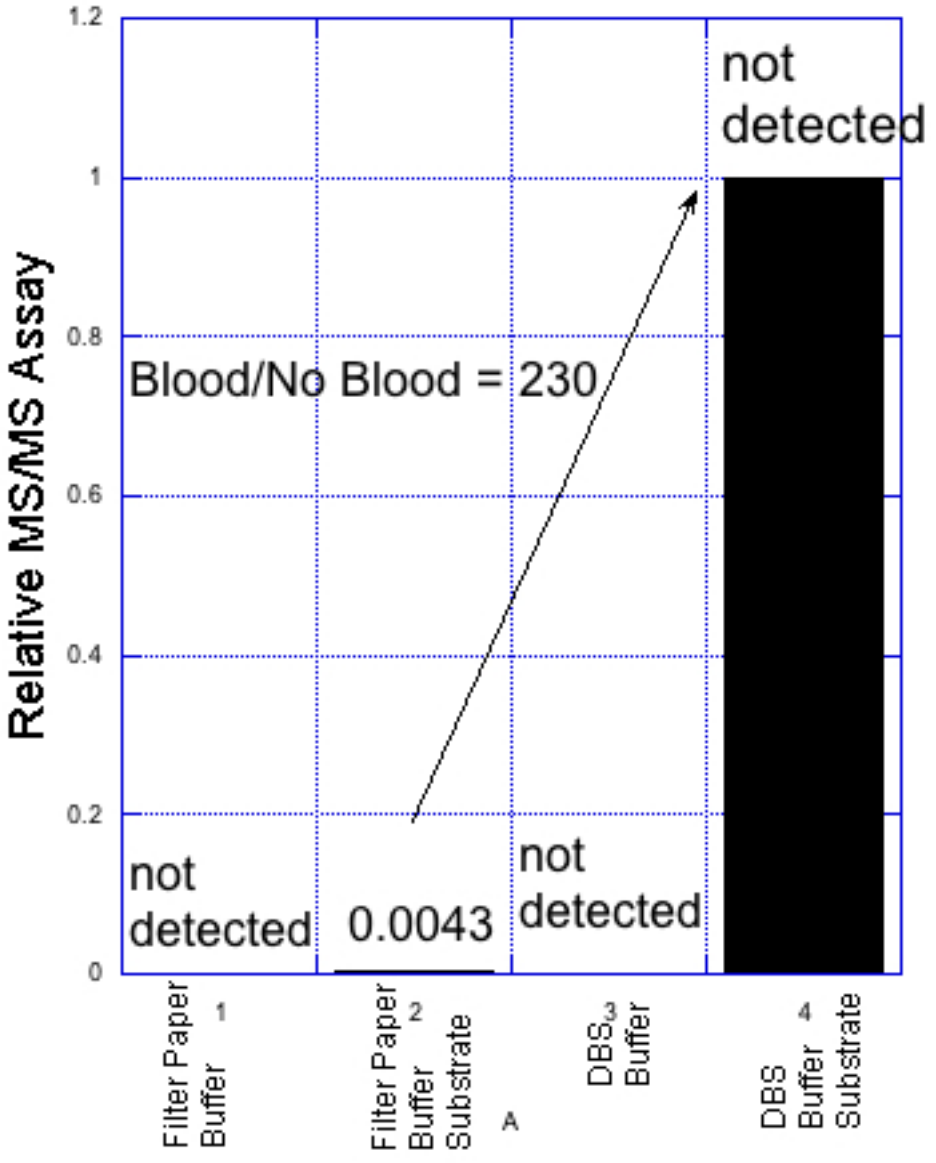
Design of the aglycone



1. Simple to synthesize
2. Readily extracts into ethyl acetate from buffer.
3. Readily protonates in the gas phase.
4. Fragments in the gas phase along a major pahtway.
5. Easily deuterated in the benzoyl portion for internal standard preparation.

Fluorescence vs MS/MS Assay of IDUA in DBS for MPS-I





Comparision of 2 large MPS-I Pilots

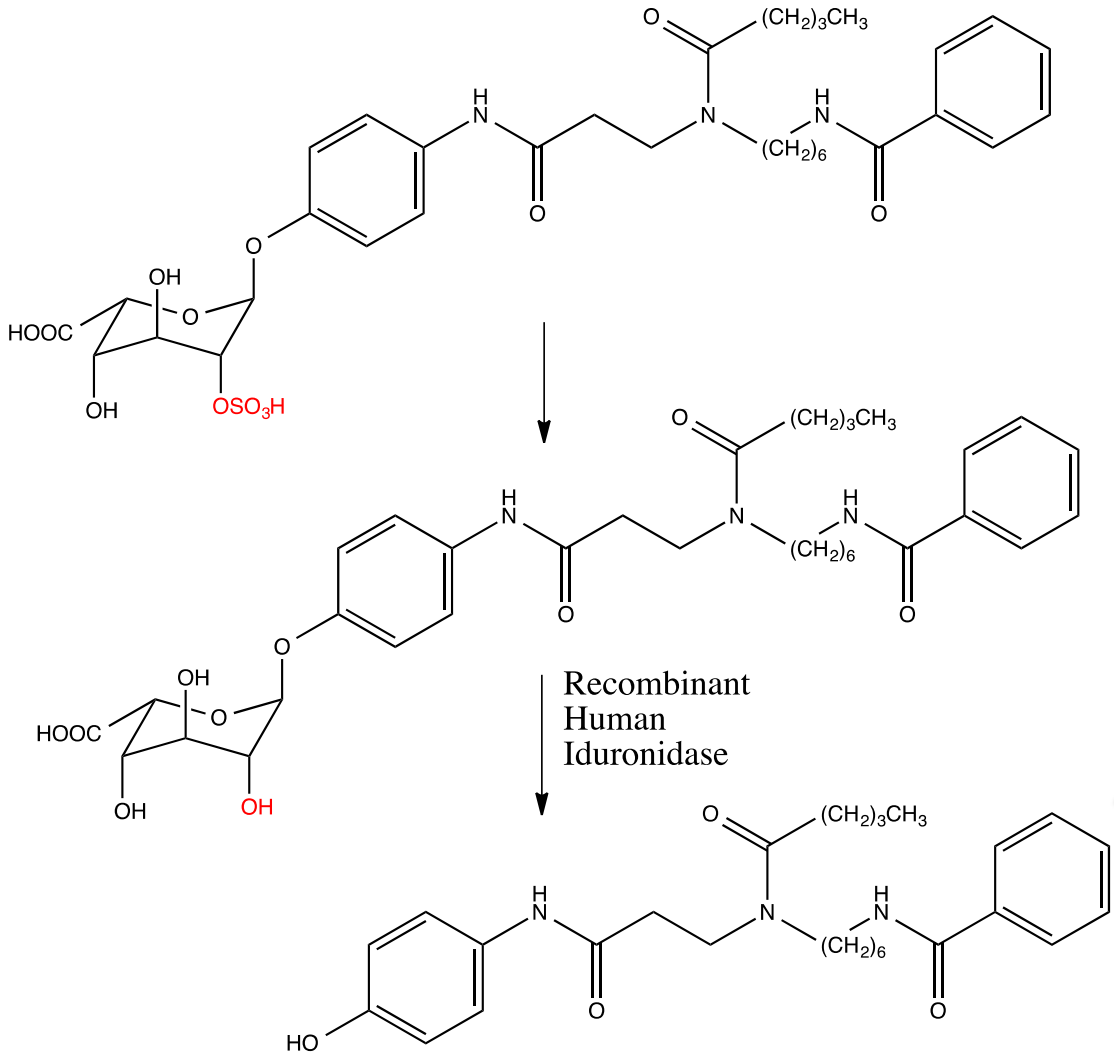
Study Site	Method	DBS tested	Screen cutoff MPS-I	Number of screen positives MPS-I	Number of positives after re-test MPS-I	Genotyping MPS-I
WA NBS Lab	n-1 version of UW/PE-FIA-MS/MS-2014 3-plex (MPS-I, Fabry, Pompe)	106,526	32% of mean activity	7	7	3 MPS-I 1 carrier 3 false pos 2 poor punch*
MO NBS Lab	Digital Microfl. Fluor. 4-plex (MPS-I, Fabry, Pompe, MPS-II)	117,000	20% of mean activity	57	20%	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).

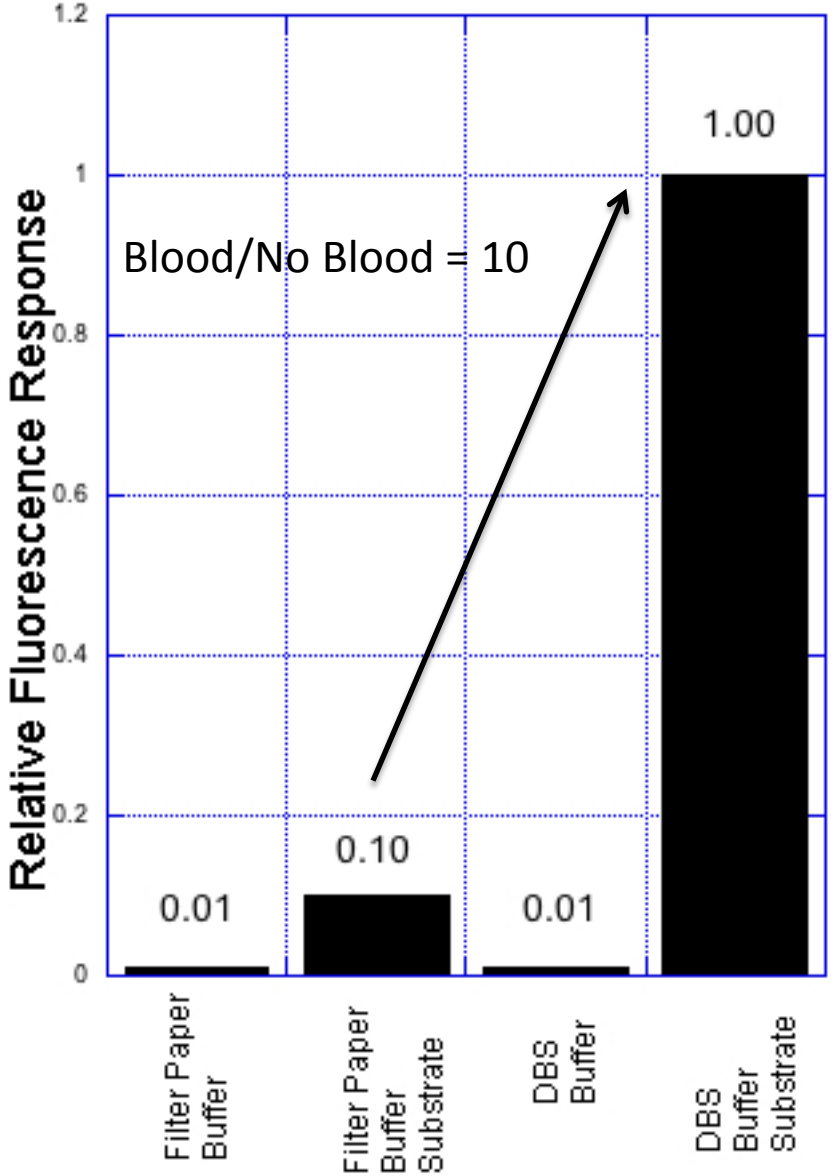
New MS/MS assay for ID2S for MPS-II

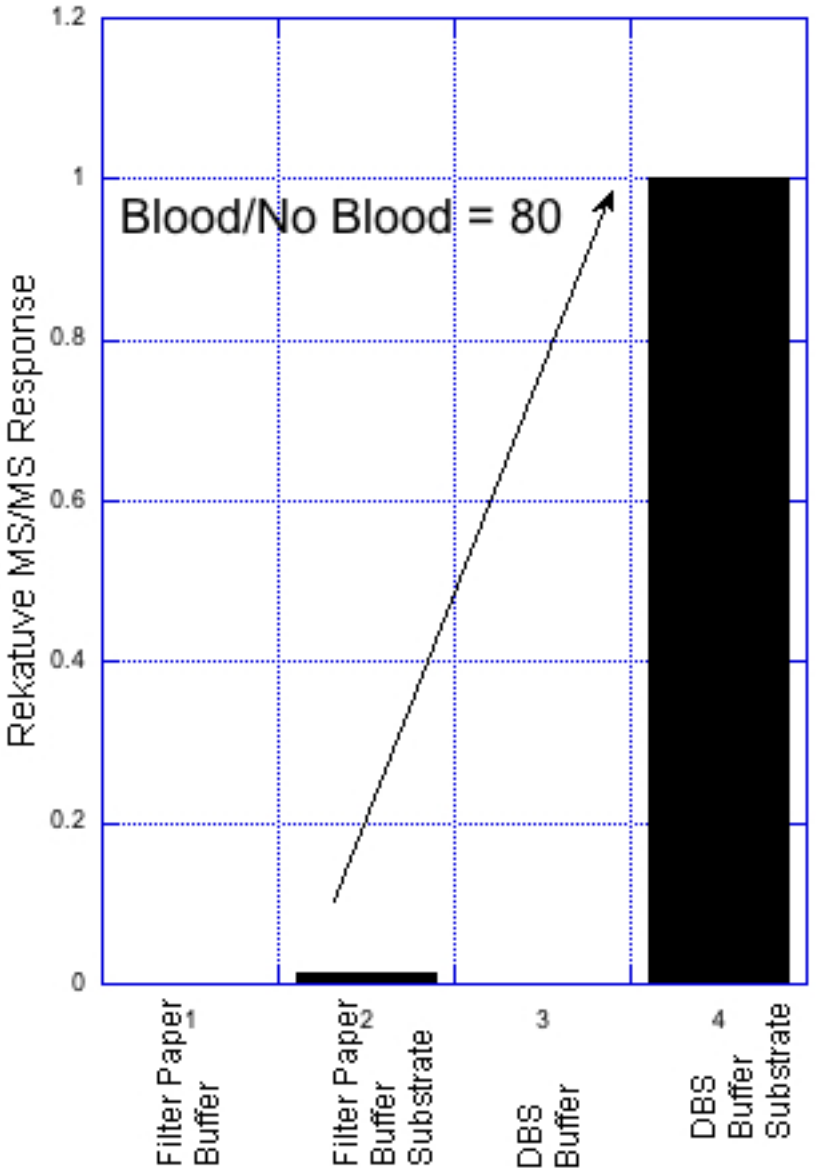


Detect by flow injection MS/MS

	Original Assay	New assay
Product ion counts (healthy)	~17,000	~1,200,000
Blood-to-no blood ratio	53	80

Fluorescence vs FIA-MS/MS assay of I2S (MPS-II) in DBS



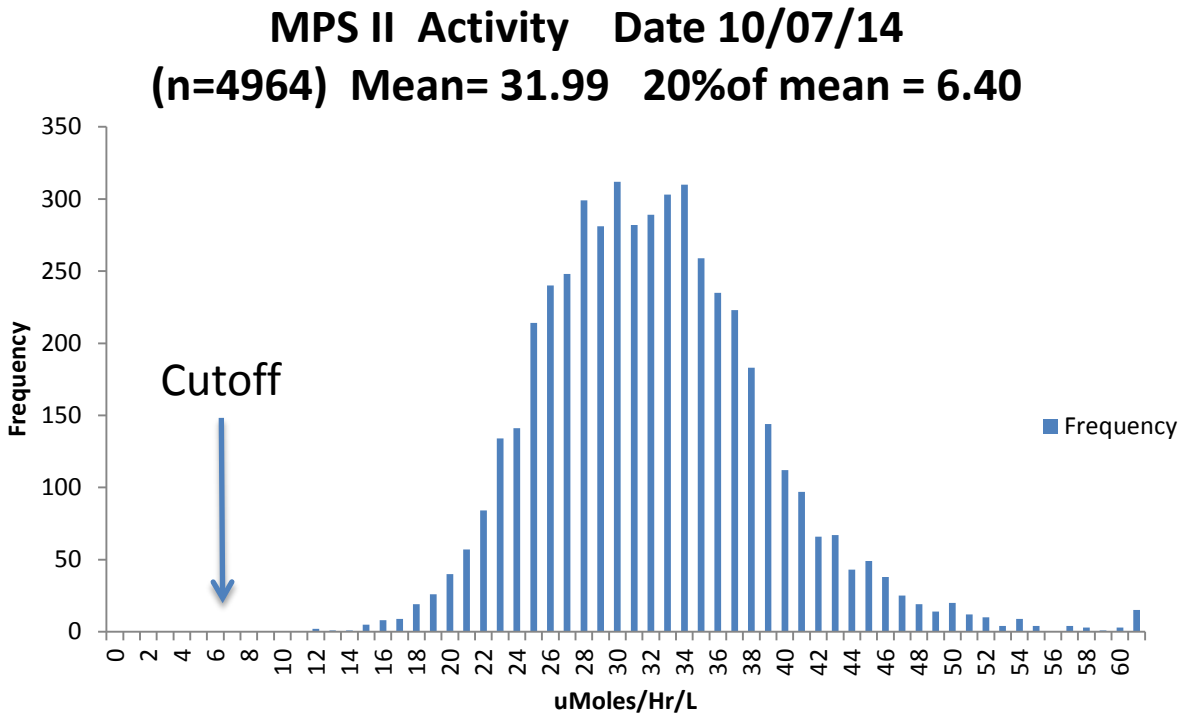


MPS-II FIA-MS/MS Pilot Study, WA state NBS Lab

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

Screen cutoff 20% of daily mean.

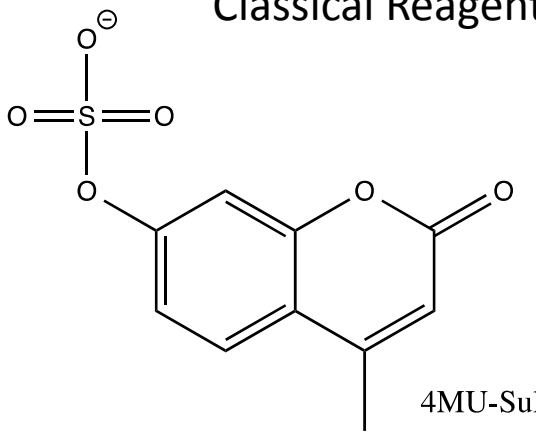
0 hits out of 4964



Pilot studies also started in Taiwan and Belgium

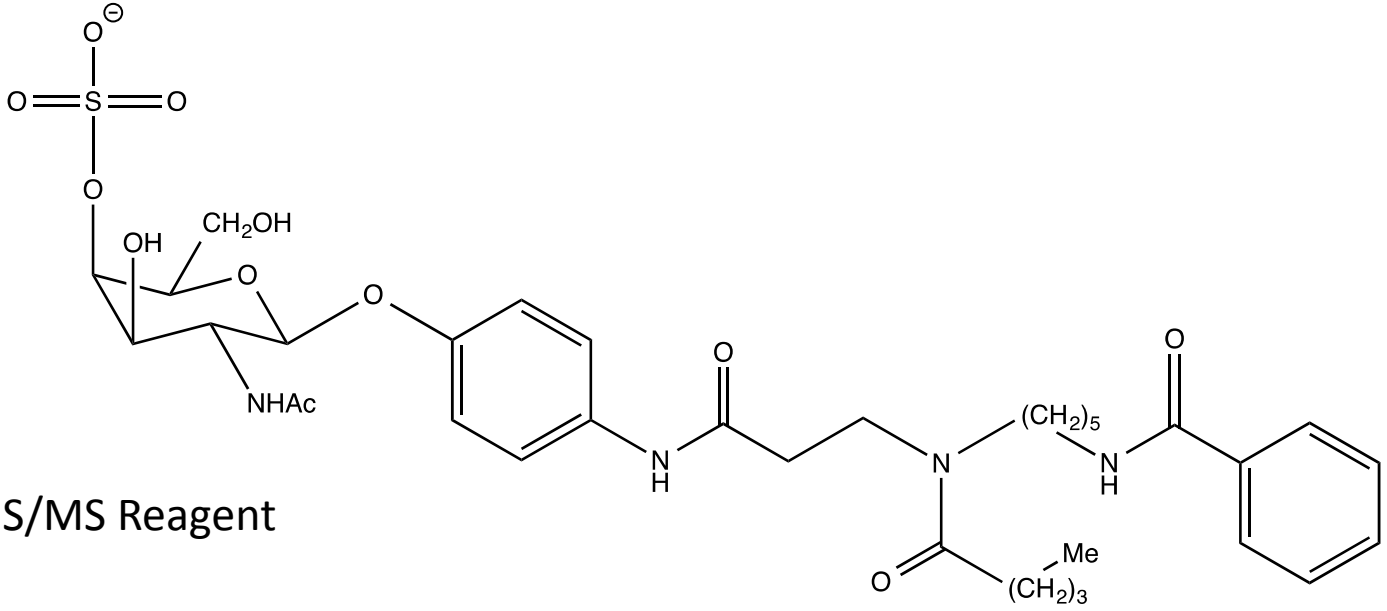
ASB (MPS-VI) Assay Reagents

Classical Reagent



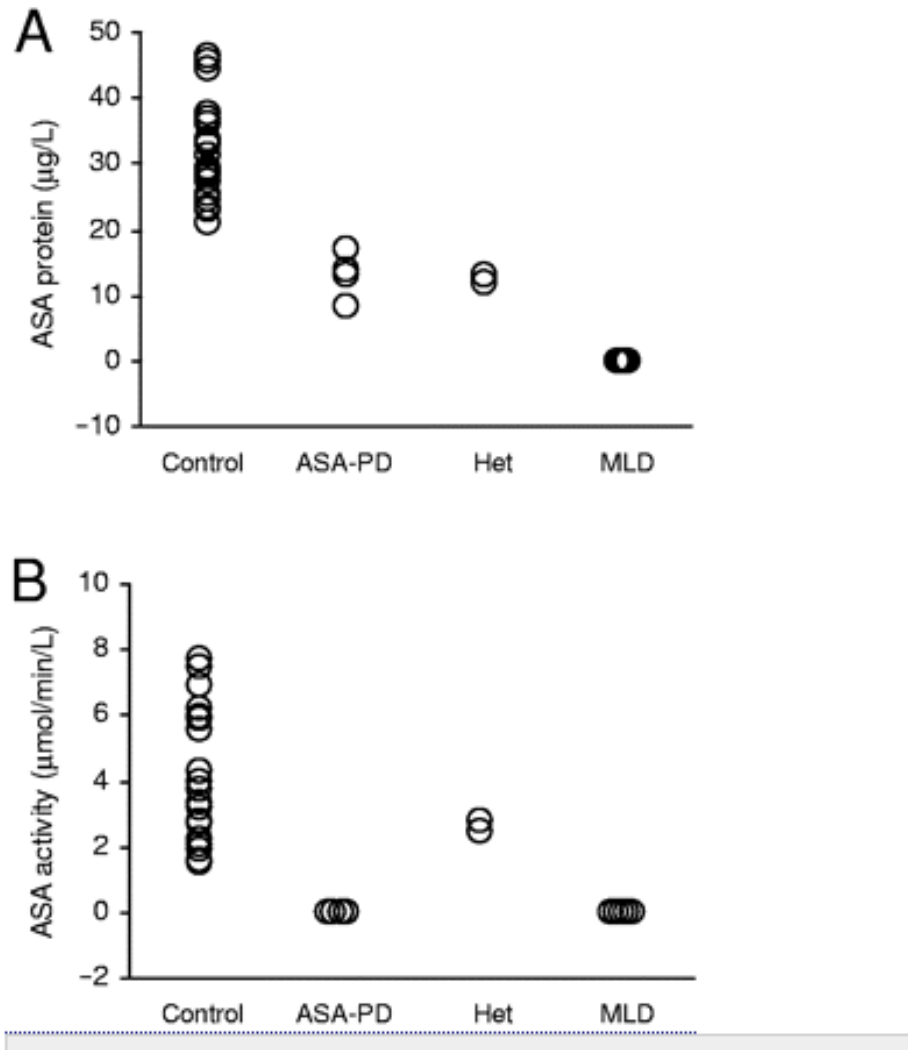
4MU-Sulfate

New MS/MS Reagent



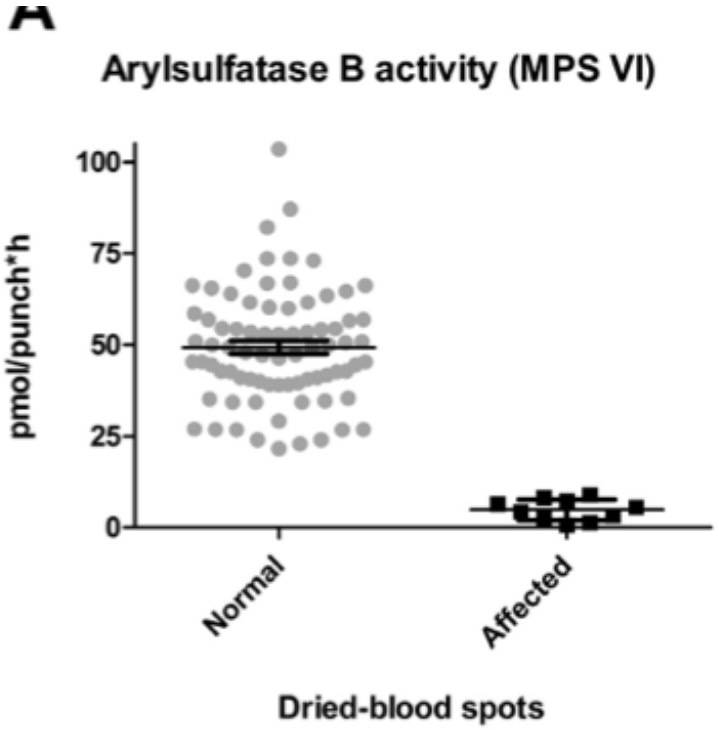
Assay with 4MU-Sulfate

ASA



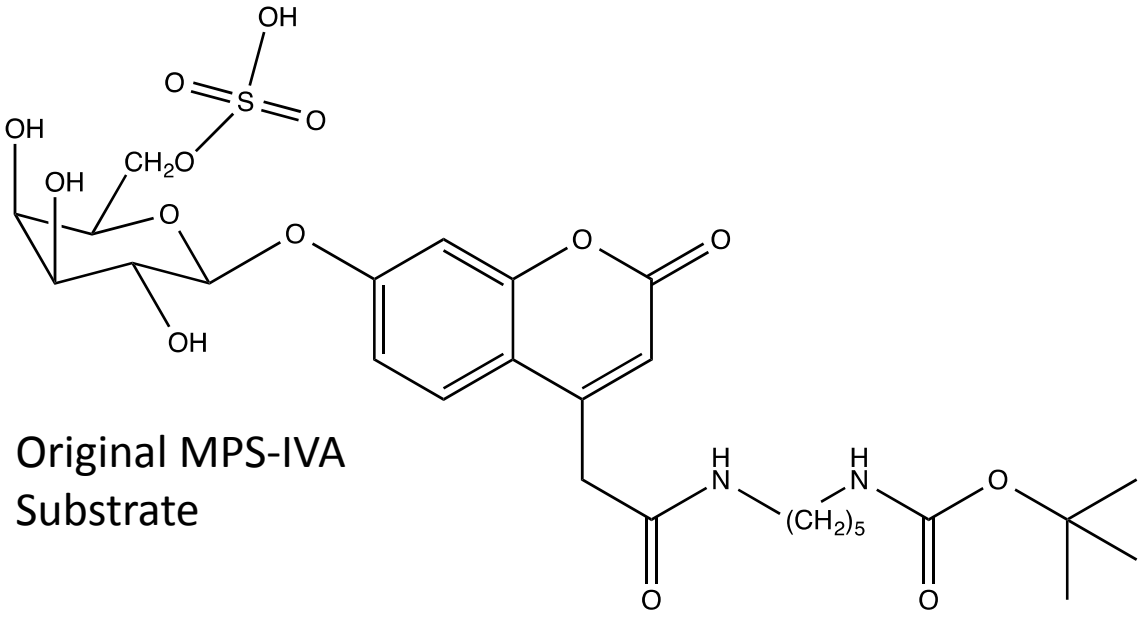
Clin. Chem. (2008) 54, 1925.

ASB

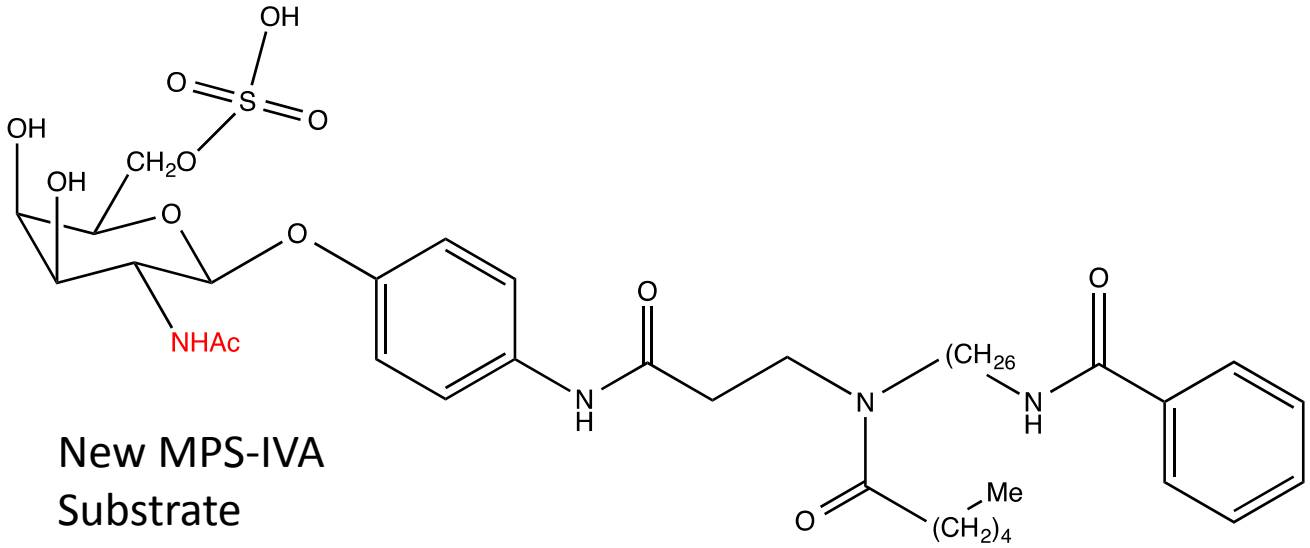


Molec. Genet. Metabol. Reports (2014) 1, 465

Improved Assay for GALNS for MPS-IVA (Morquio A)



Original MPS-IVA Substrate



New MPS-IVA Substrate

New MS/MS assays for MPS-IVA and VI

MPS-IVA

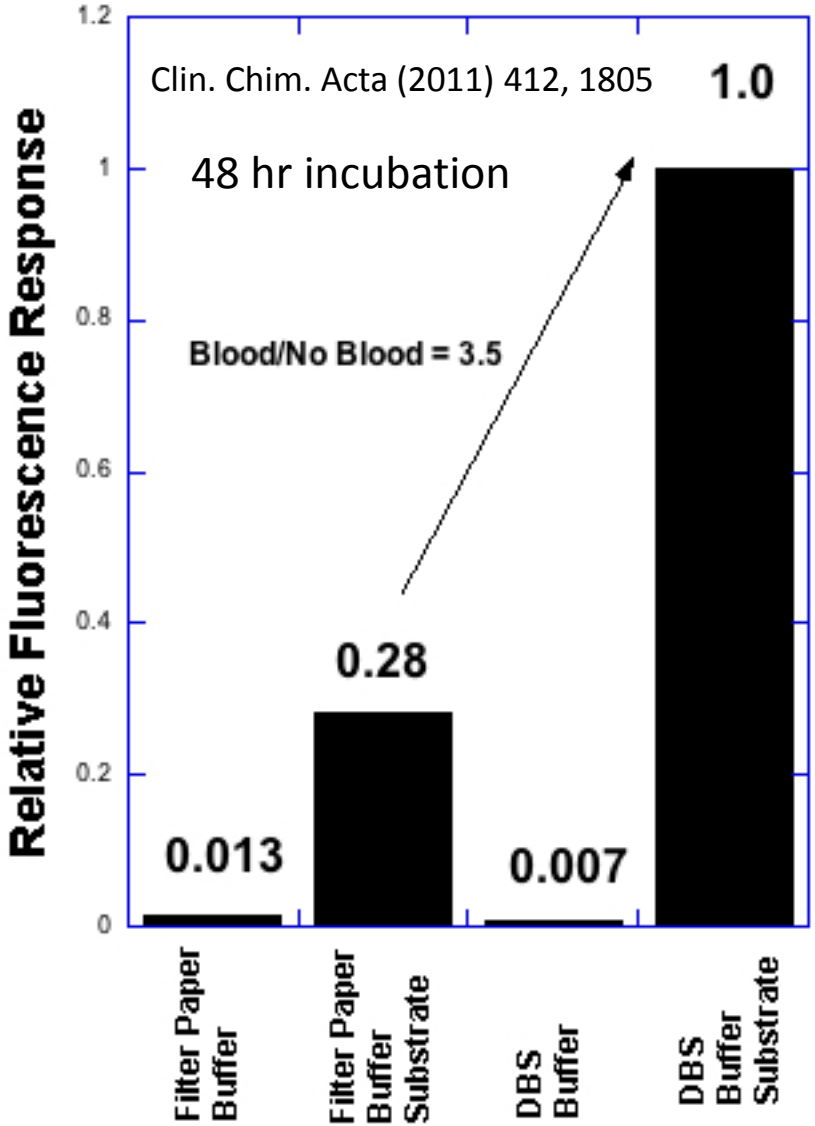
	Original Assay	New assay
Product ion counts (healthy)	~1,000-2,000	~400,000
Blood-to-no blood ratio	100	100

MPS-VI

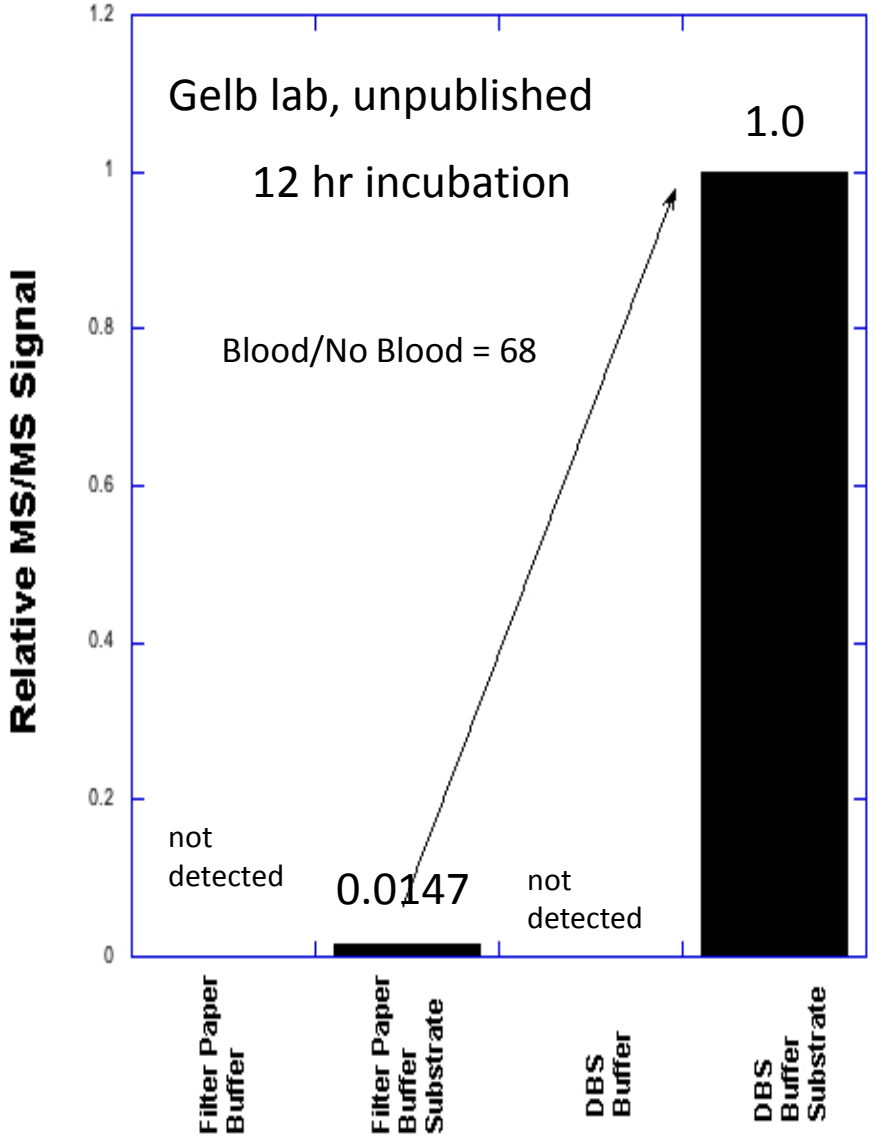
	Original Assay	New assay
Product ion counts (healthy)	~7,000	~600,000
Blood-to-no blood ratio	60	80

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

Fluorescence Asssay (4MU Substrate)



FIA-MS/MS Assay

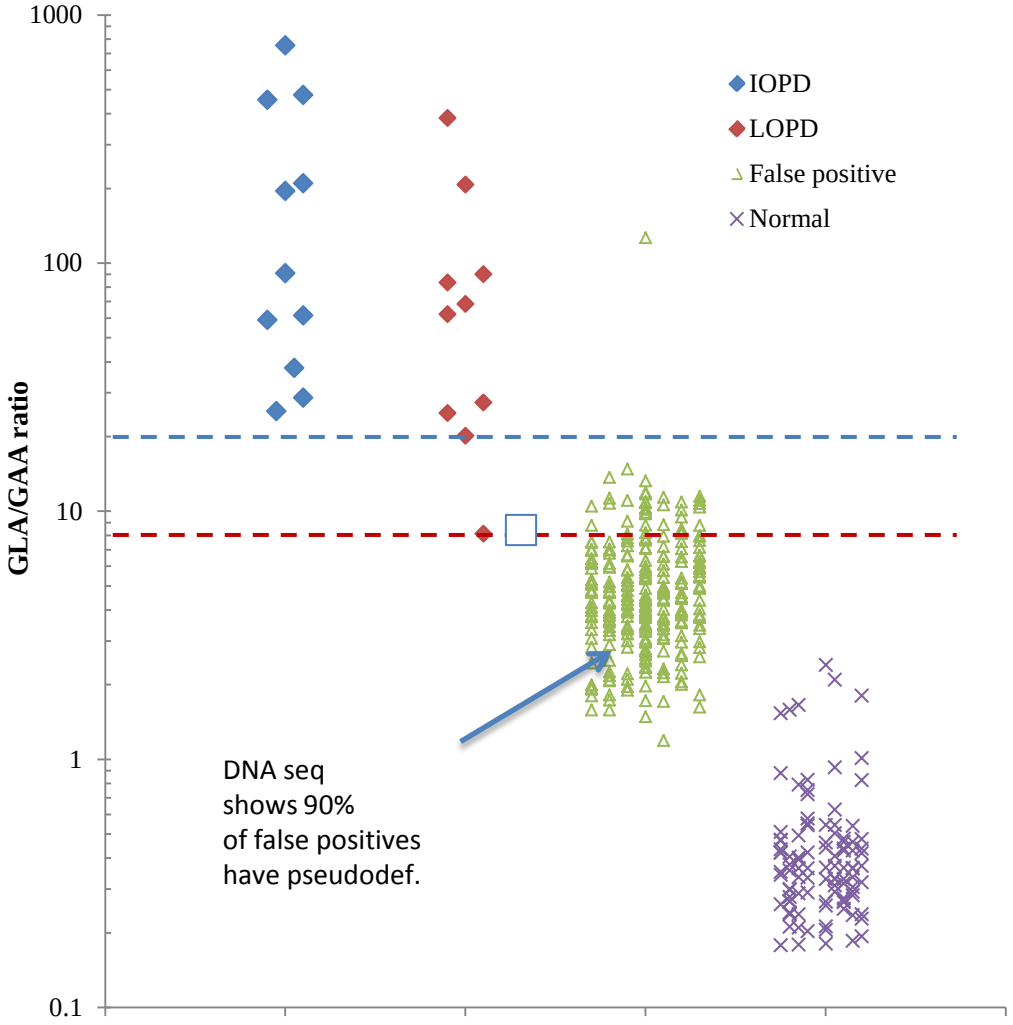


PE Genetics is gearing up to offer MPS-I, MPS-II, MPS-IVA, and MPS-VI MS/MS assays for outside labs that need an enzyme test.

Reagents will also be available to diagnostic labs that want to do their own assays.

Mack Schermer, Perkin Elmer
Marck Kuracina, Perkin Elmer

MS/MS for GLA/GAA activity distinguishes IOPD/LOPD from pseudodeficiencies



n = 250,000

Data from Joyce Liao, Chinese Foundation of Health, Taiwan (to be published)

Radiometric Assay for GALC Activity in Leukocytes for Evaluation of Screen Positive Krabbe Samples (NY)

- high risk < 3.6% of mean normal
- moderate risk 3.7-7% of mean normal
- low risk 7.1-12 % of mean normal
- no risk > 12% of mean normal

NY NBS program (Clin. Chim. Acta. 2013, 18, 73)

The Gold Standard?

4MU fluorescence enzyme assay with purified leukocytes

We think we can do better with MS/MS.

A collaborative study of 4MU-fluorescence vs MS/MS assays on several hundred leukocytes from LSD patients
(early onset, late onset, and pseudodeficiencies)
has been initiated with the Women's & Children's Hospital, Adelaide, Australia (Enzo Ranieri)

It seems clear that new diseases including additional LSDs will be added to the NBS panel in the coming years.

Reliable fluorimetric assays for Niemann-Pick-A/B, MPS-VI, Metachromatic Leukodystrophy and Wilson disease are not likely to be developed.

MS/MS not only provides the solution to assays for which a fluorimetric assay does not exist but also allows biomarker-based screening to be included in the same multiplex run as the enzyme assays.

IDUA activity for MPS-I
GAA activity for Pompe
Glu4 for Pompe
GLA activity for Fabry
Gal-Gal-Glu-Ceramide for Fabry
GALC activity for Krabbe
Psychosine for Krabbe
ABG activity for Gaucher
Glu-Sphingosine for Gaucher
C26-LPC for X-ALD
Biotinidase activity for biotinidase deficiency
Sulfatides for MLD
Enzyme activities for MPS-IIIA-D

GALNS activity for MPS-IVA
ASB activity for MPS-VI
GUS activity for MPS-VII
ASM activity for Niemann-Pick-A/B
Lysosphingomyelin for Niemann-Pick-A/B
Lysosomal acid lipase for Wilson disease

Summary

1. New MS/MS-based enzymatic assays for several MPS syndromes have been developed.

2. The new assays display a much larger dynamic range compared to first-generation MS/MS assays and compared to fluorimetric assays.

3. 4MU-fluorimetric assays have a low dynamic range because of intrinsic fluorescence of the 4MU-substrates.

4. Pilot studies with the new MPS-I and MPS-II MS/MS reagents show a dramatic reduction in the number of screen positives (less false positives and pseudodeficiencies).

5. Perkin Elmer Genetics will offer enzyme tests for multiple MPS diseases as well as reagents to labs that want to do their own tests.

6. MPS-I NBS is starting in IL, MO, NJ, PA, and Taiwan.

7. MPS-II will be added to the IL and NJ NBS program in the next several months.

8. Additional MS/MS assays are being developed: MPS-IIIA-D, MPS-VII, MLD, LAL

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