Newborn Screening for Nine Lysosomal Storage Diseases

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2004: We reported a 5-plex assay for Niemann-Pick-A/B, Gaucher, Pompe, Fabry, and Krabbe diseases using dried blood spots and tandem mass spectrometry.

~2006: Giancarlo La Marca (Milan) reported a variation of our assay in which LC was used in-line with tandem MS to simplify the pre-MS sample preparation.

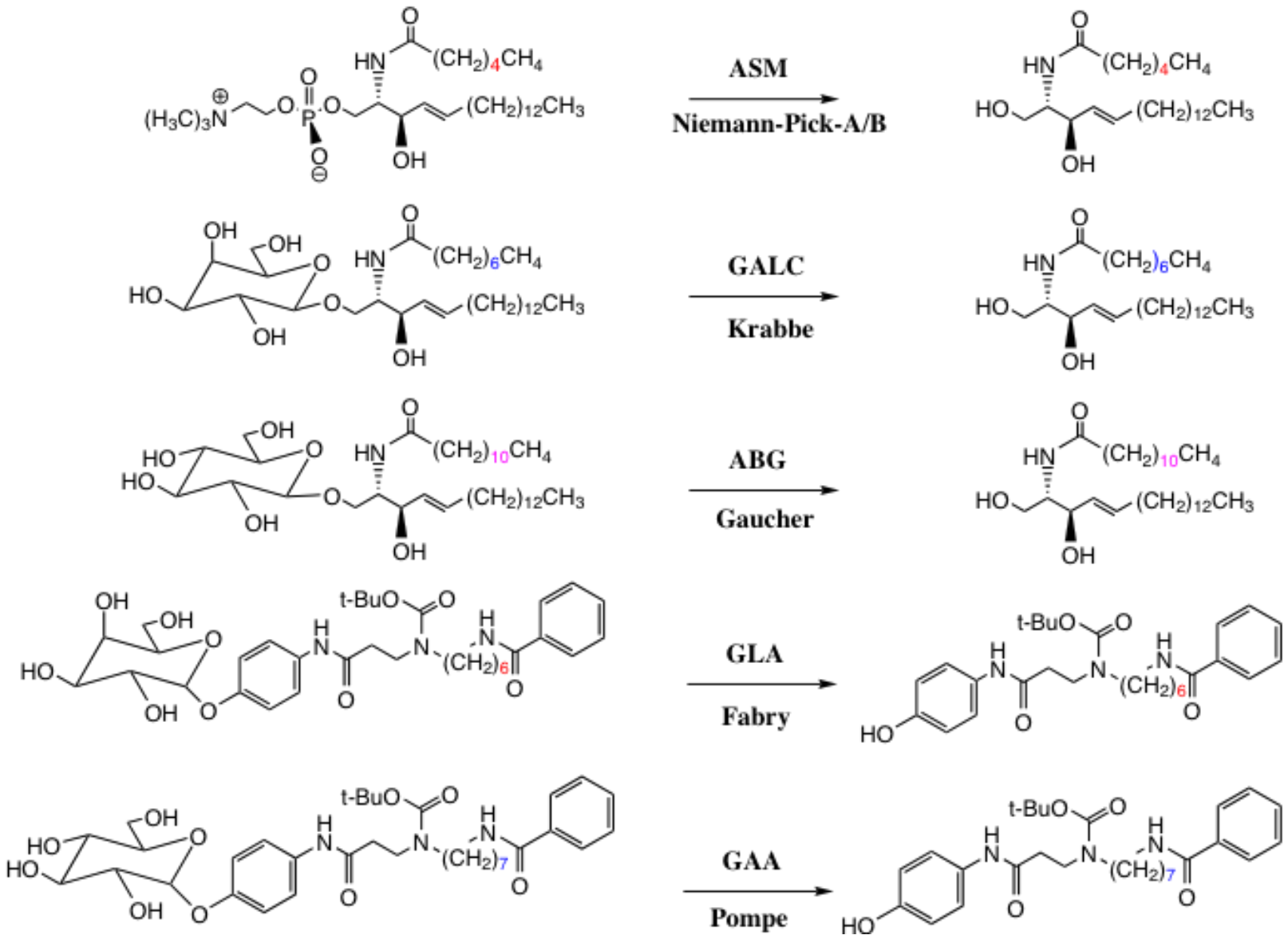
~2007: Our MSMS method was transferred to the NBS lab in NY for Krabbe disease NBS (*Joe Orsini et al.*).

2009/10: We expanded the tandem MS method to include MPS-I, MPS-II, MPS-IVA, and MPS-VI.

2010/11: We put these 9 assays together into a simplified 9-plex assay in which we reduced the number of enzyme buffers and simplified the pre-MS sample preparation.

2010/11: David Kasper (Vienna) also showed that LC in-line with MSMS can be used to simplify sample work-up. He also showed that the LC/MSMS method is very robust.

Synthetic Substrates for LSD Newborn Screening Using MSMS



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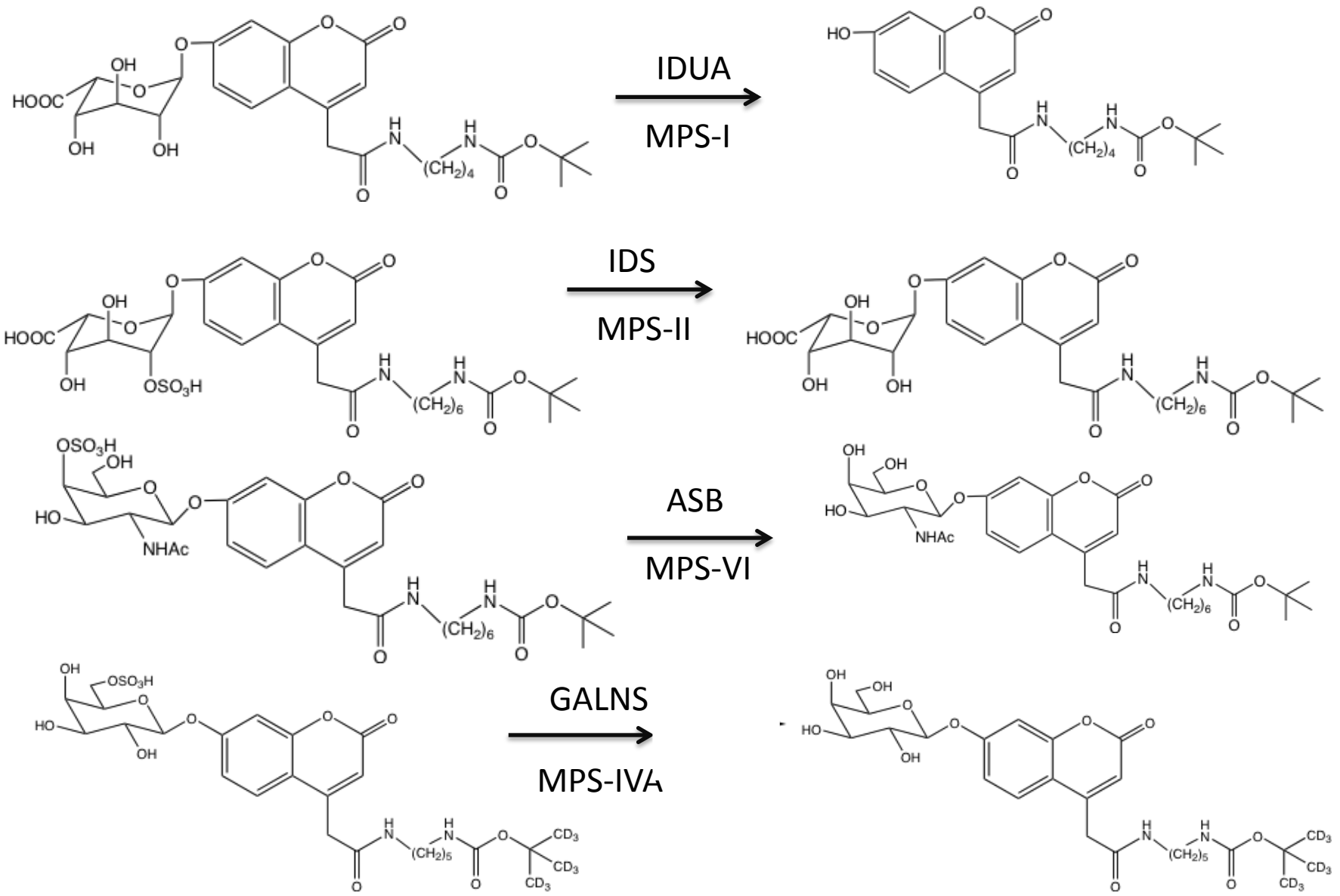


The New 9-Plex LSD Assay

1. Single 3 mm DBS punch incubated in a single well with assay cocktail 1:
   Gaucher, Fabry, Pompe, Niemann-Pick-A/B, Krabbe, MPS-I

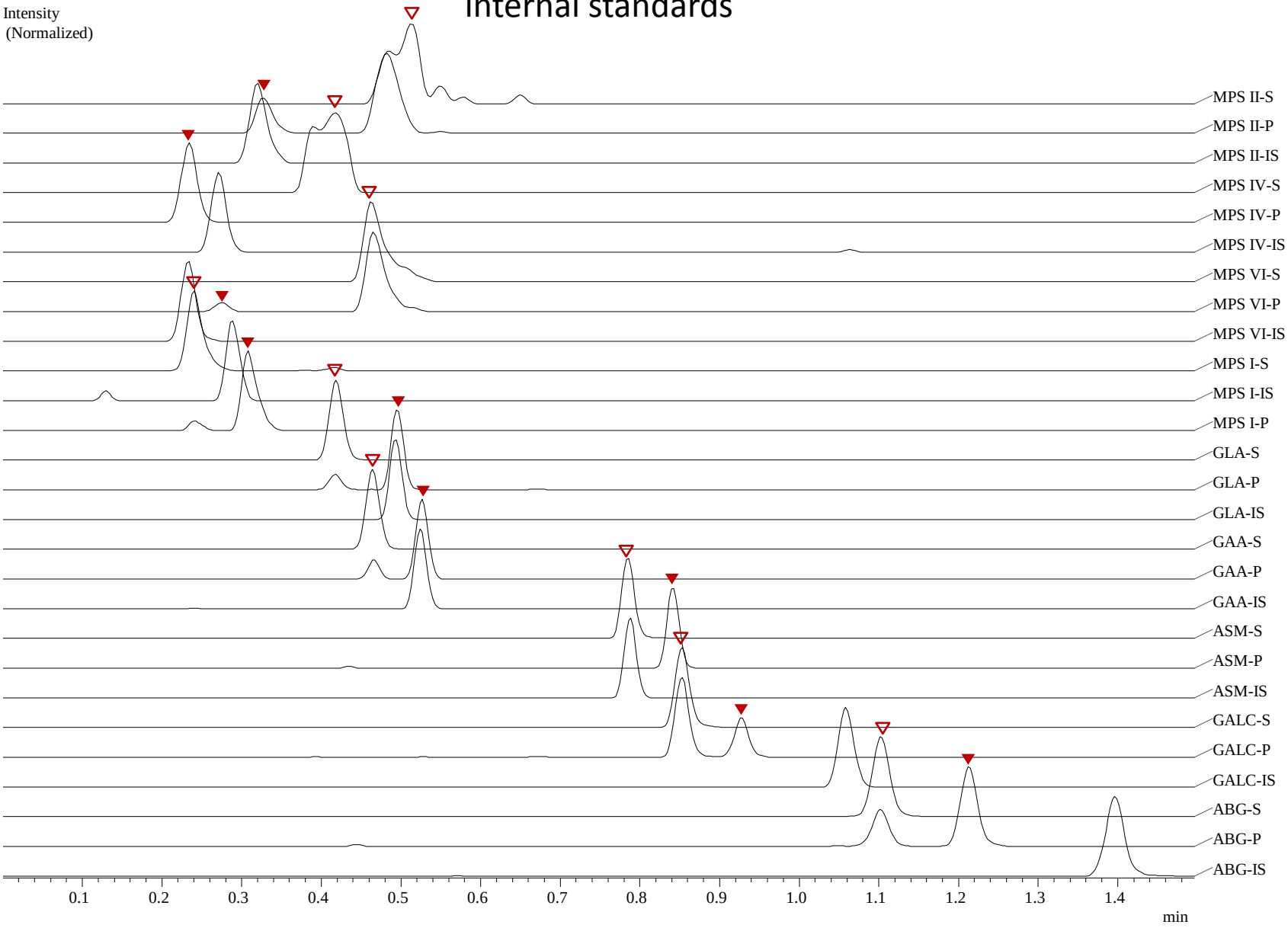
2. Single 3 mm DBS punch incubated in a single well with assay cocktail 2:
   MPS-II, MPS-IVA, MPS-VI

3. Quench reactions with acetonitrile and combine 2 wells into 1. Centrifuge plate for few min and transfer supernatant to a new plate. Add water and place on autosampler of UPLC/MSMS.

4. Analyze enzymatic products and internal standards by UPLC/MSMS with an inject-to-inject time of 1.5 min.

The New 9-Plex LSD Assay

Ion traces for 9 enzymatic products and 9 internal standards



The New 9-Plex LSD Assay

Summary of the features and advantages

1. All liquid transfers done with a manually operated 96-channel pipettor (i.e. Rainin Liquidator). No automatic liquid handling systems needed. No liquid-liquid or solid-phase extractions needed. Post incubation workup prior to MSMS < 1 hr.

2. Avoids pre-extraction of the DBS. Extraction of the DBS is problematic since different enzymes require different extraction conditions, extraction is not complete and some enzymes in extract are not fully dissolved and settle out.

3. Any subset of the 9 enzymes can be assayed with the identical protocol.

4. Entire process fits into 24 hr except for MPS-IVA which may require an extra day because it is inherently a slow enzyme (still under investigation).

5. UPLC is highly robust and does not add significantly to the cost of the instrumentation. It is used in Pharma for pharmacokinetic analysis on 1000s of samples per day. UPLC column cost is insignificant.

The New 9-Plex LSD Assay

Summary of the features and advantages

6. UPLC causes analytes to enter in the MSMS at a higher concentration compared to flow injection, thus the assay is more sensitive.

7. The major cause of down time in using MSMS in newborn screening is source loading due to direct infusion of a complex mixture (blood) into the instrument. With UPLC, most blood components elute in the void and can be easily diverted to waste rather than entering the MSMS source.

8. UPLC/MSMS allows biomarkers to be analyzed in the same run as the products of lysosomal enzymes. Biomarkers will be important for diseases such as MLD where a direct enzyme assay is probably not possible for newborn screening (pseudodeficiency problem). Biomarker analysis for diseases such X-ALD is the only reliable screening method. These types of biomarker analyses are not possible with fluorometric assays.

MSMS vs Fluorometric Assays of LSD

1. Not all LSDs can be analyzed by fluorometric methods.

2. A recent publication stated that fluorometric assays with digital microfluids is faster than the MSMS method. This is not the case.

3. The number of non-automated sample handling steps for digital microfluidic and UPLC/MSMS assays are similar.

3. Current pricing of fluorescence and MSMS LSD assays suggest that they are similar (including all reagents, instrumentation, servicing, personnel, etc.).

4. My sense is that MSMS data is of higher quality because the detection has essentially no background interferences (time will tell).

5. Many fluorometric assays of LSDs require a coupling enzyme to generate the fluorescent signal, and sources of coupling enzymes are not available for all LSD assays for which NBS is appropriate.

MSMS LSD Assays in place and pilot studies

1. Krabbe assay running for > 2 years in NY.

2. Perkin-Elmer Genetics now offers MSMS assays for 6 LSDs (Oct 2011) and will expand shortly to include the 9-plex.

3. A 7-plex LC/MSMS assay was transferred to the IL NBS lab from the Gelb lab and was found to be functional in < 2 weeks setup time.

4. The 9-plex UPLC/MSMS assay will be piloted shortly in NBS labs in WA, Taiwan, Austria, and NY (Mt. Sinai).

5. David Kasper (Austria NBS lab) has run a pilot UPLC/MSMS assay on 6 LSDs (~80,000 DBS analyzed) See his talk this thurs.