SHOULD WE SCREEN FOR (TREATABLE) LSDs: MPS I, II, IVA, VI, Pompe, Fabry, Gaucher

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EVA PCMA vzw

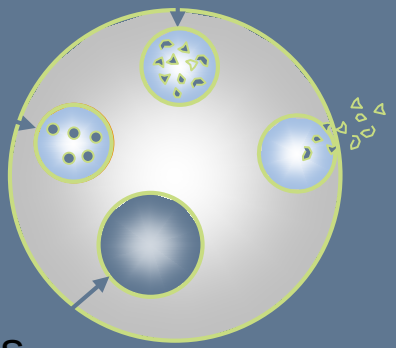
Cellular functions of lysosome

- Cells continually need to digest foreign materials (eg, bacteria), and damaged or old cellular components

Lysosomes are cell organelles that contain specific enzymes for digestion and degradation of complex waste molecules

Complex material is broken down by lysosomal enzymes

Lysosome containing complex material



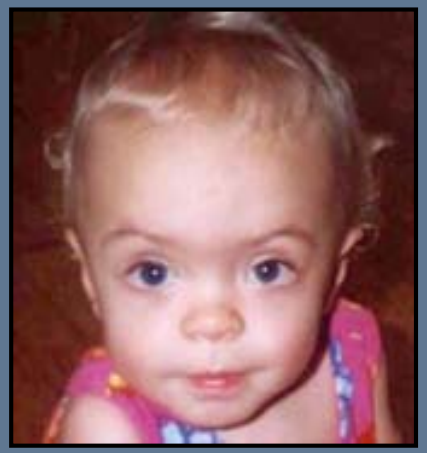
Degraded waste material is **excreted** or can be **reused**

Nucleus

Heese et al, *Semin Pediatr Neurol,* 2008

Lysosomal Storage disorders

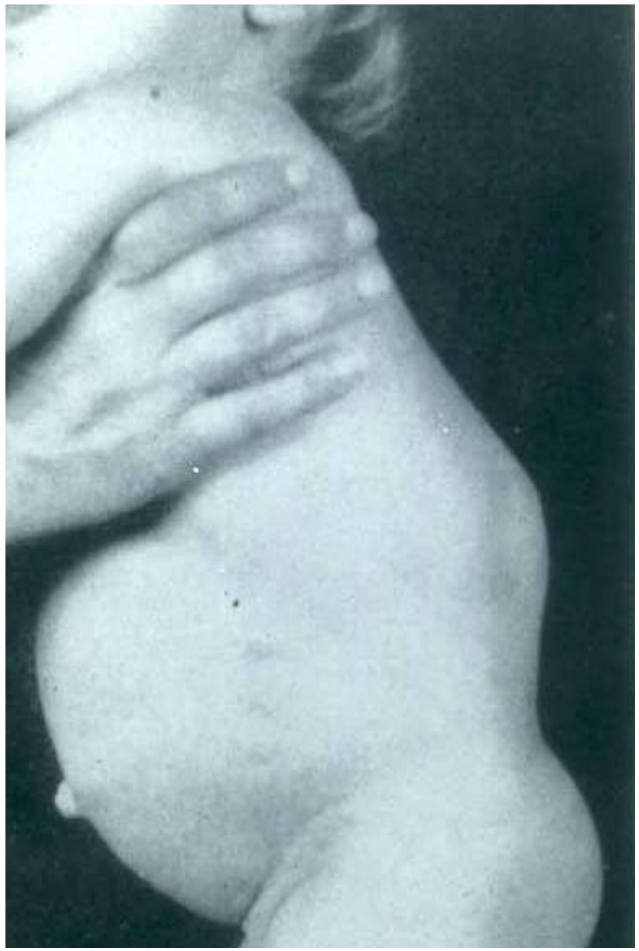
- Diagnosis on clinical grounds is very difficult:
  - Great clinical variability
  - Genetic heterogeneity
  - Age-dependent clinical symptoms & signs



MPS 1
Age 12 to 34 months

CASE:
Boy, age 5 years
Belgian ancestry
Clinical signs and symptoms:

- Lumbar gibbus
- Motor Dev. retardation
- Obstruction of upper airways
- Sleep apnea
- Growth retardation
- Slight facial dysmorphism
- Hepatosplenomegaly
- Rx hands: dysostosis multiplex



Project: creating awareness to look for clinical signs of MPS in young children

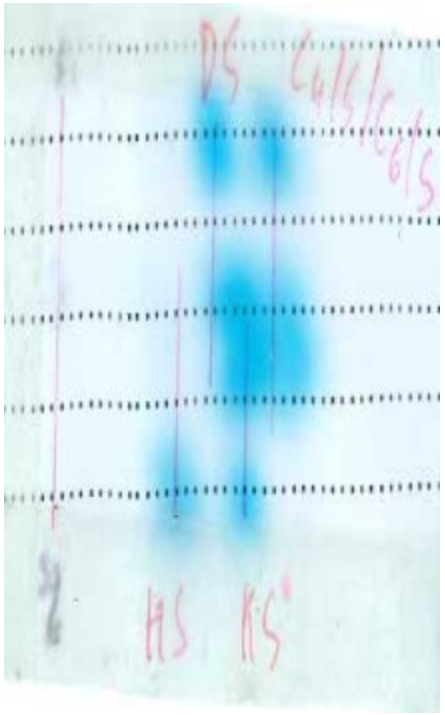
•Urinary GAG: 56 mg/mmol creat
448 µg/mg creat

1-dimensional electrophoretic separation of GAG species: dermatansulfate, chondroitine sulfate

-screening method

-laborious, time-consuming

-difficult to interpret



Lymphocyte Arylsulphatase B activity:

0.254/0.54 nmol/mg/min (RV: 2.2-18.6)

GalactoseNac-6-sulfatase: normal

2009:
LSD enzymatic analysis in DBS
Technically feasible?

Available Techniques

- Chamoles method (2001):
  - Fluorescence/enzymatic assay
  - Single assay-Single disease model
- Meikle et al (Hopwood)(2004-2006):
  - Multiplexed immune quantification
  - Specific antibodies-two-tier approach
  - Low sensitivity for detection of Pompe & Gaucher
- Gelb/Li et al (2004); Genzyme (Zhang et al)(2008)
  - ESI-MS/MS
  - Analytically multiplex screening
  - MPSI,II,VI, Pompe, Fabry, Gaucher, Niemann-Pick, Krabbe: specific substrates and Internal Standards
  - QC-CDC
- Millington
  - Digital microfluidics platform
  - =multiplex platform of Chamoles method
  - MPSI, VII, Pompe, Fabry

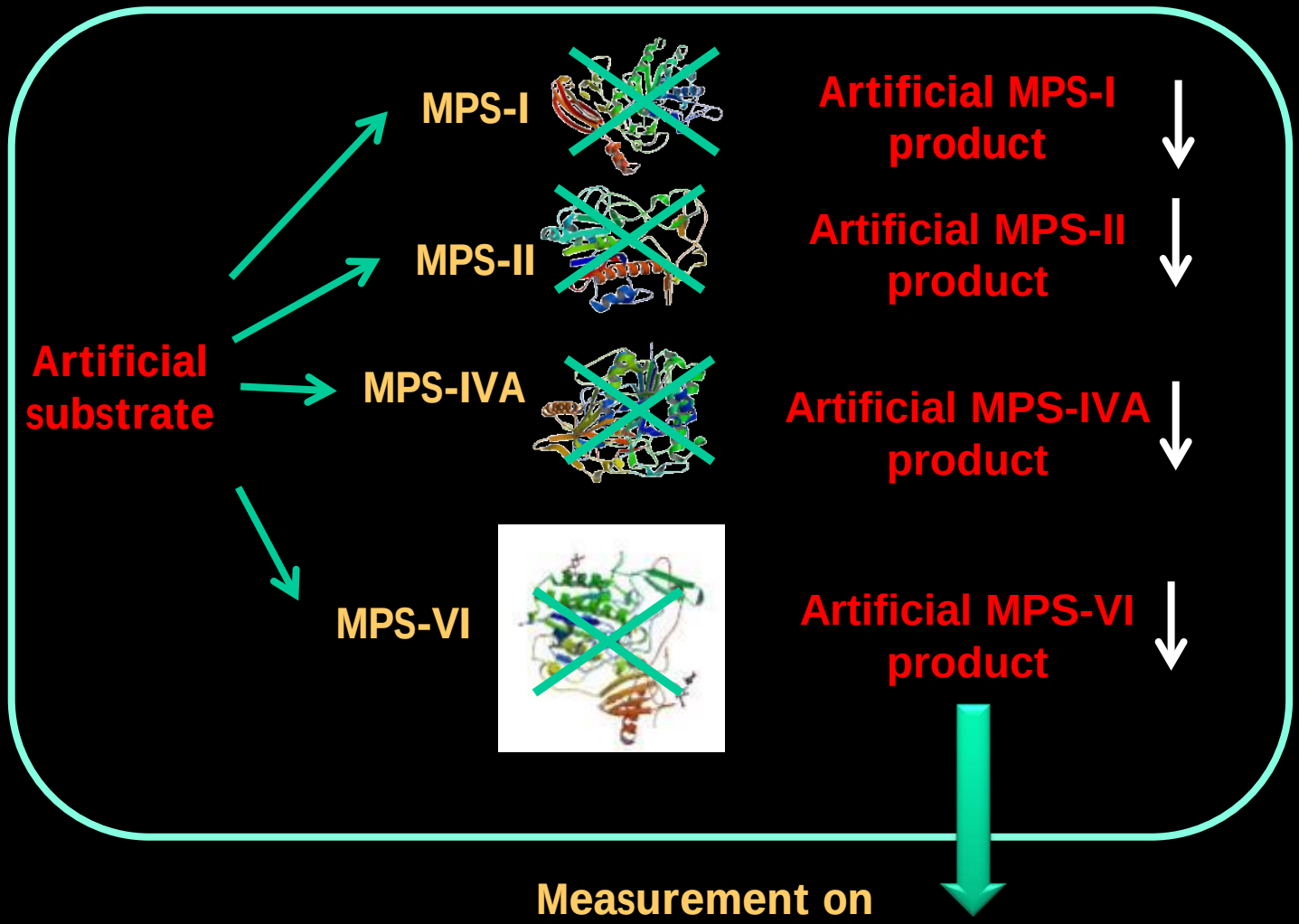
Collaboration:

- CDC:
  - QC DBS and calibration reagents
  - Reagents for Gaucher, Fabry, Pompe and MPS-I
- Dr. Gelb (University of Washington):
  - Buffer for 4+3 plex and 7 plex assay
  - Reagents for MPS-II (MPS IIIB; MPS VII)
- Dr. Gelb via BioMarin:
  - Reagents for MPS-IVA and MPS-VI
- LC-MS/MS:
  - LC = Acquity UPLC System (Waters)
  - MS/MS = Xevo TQ (Waters)

Method (Spacil et al. Clin Chem, 2013)

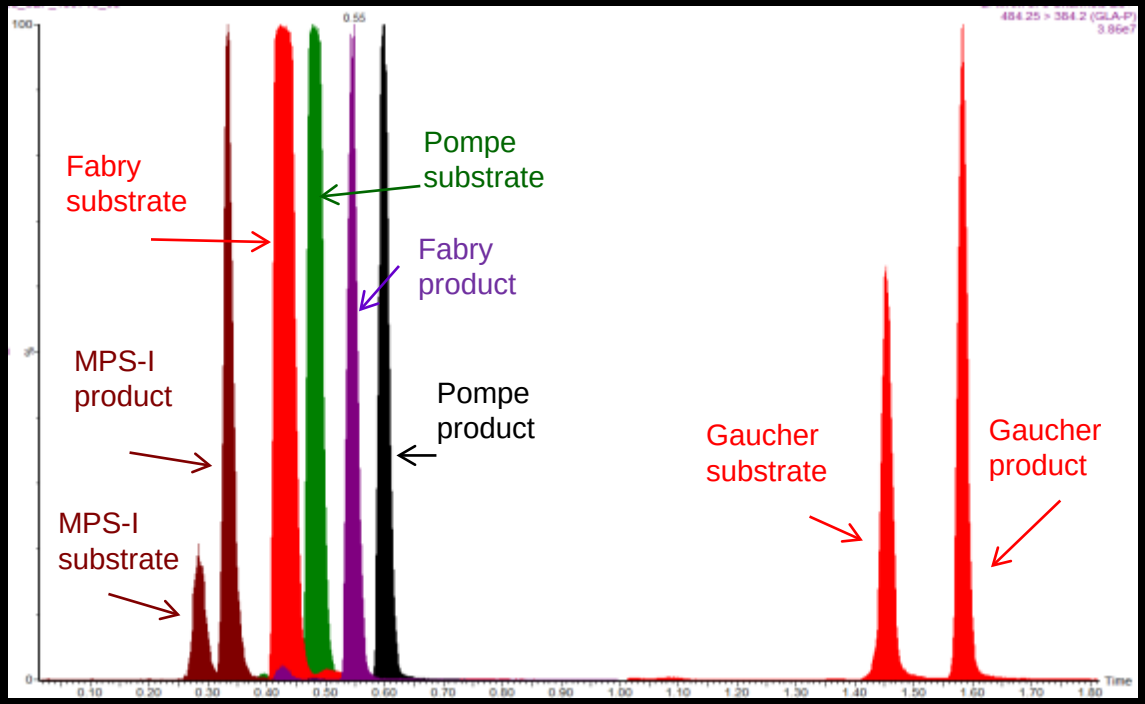
- Enzyme assay: blood spot diameter 3 mm
  - 7-plex assay: 3 DBS/ 3 buffers
    - Fabry, Pompe disease and MPS-I
    - Gaucher's disease (hydrophobic reagents)
    - MPS-II, IVA and VI
  - Assay/incubation duration 18h (overnight)
- UPLC separation:
  - Analyzing all 7 compounds (product and IS) in 1 run
  - Guard column (Xselect CSH; 10 mm x 2.1 mm, 3.5 µm) and analytical column (Xselect CSH; 50 mm x 2.1 mm, 3.5 µm)
  - Linear gradient - constant flow (0.8 ml/min) - total run time 3.2 min/sample
- ESI-MS/MS Selected Reaction Monitoring (SRM):
  - Measured in 3 time blocks
  - Parameters for ion source and mass analyzer optimized

Method for MPS screening (Spacil et al. Clin Chem, 2013)



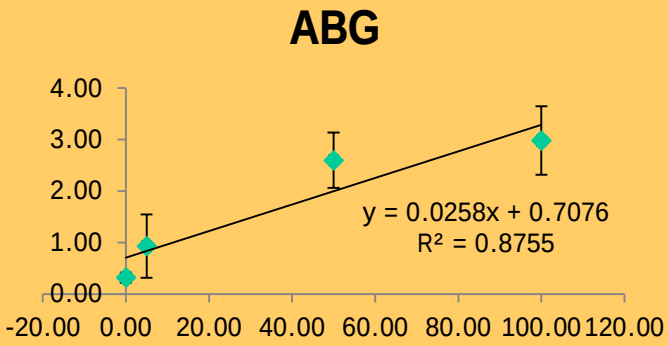
Validation:

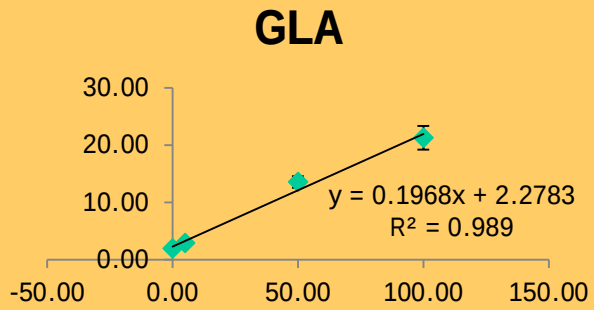
- Fabry, Pompe, Gaucher's disease and MPS-I
- Linear calibration curves
- Column carry-over is almost negligible
- All substrates are well separated from enzymatic product

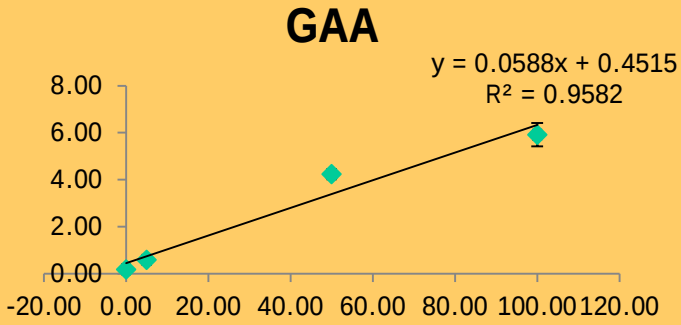


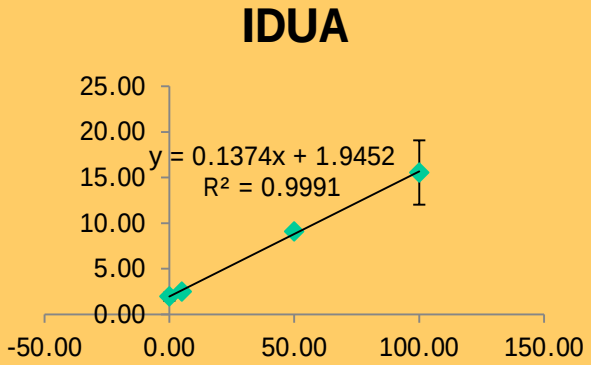
Validation:

- The method is linear: QC base – low – medium – high









Validation:

- CV% are < 20 CV%, except for Gaucher (glucocerebrosidase)
- Means and interday CV% in unprocessed cord blood (CDC QC material - high)
- High analytical range (comparison QC high with no blood control (dummy))

	Mean (n= 30, in µmol/lh)	Interday CV% (n=30)	Analytical range
Gaucher	10.8	28.7	42.7
Pompe	3.4	8.1	1373.1
Fabry	11.1	8.5	738.4
MPS-I	33	12.2	37.5
MPS-II	8.7	18	49.5

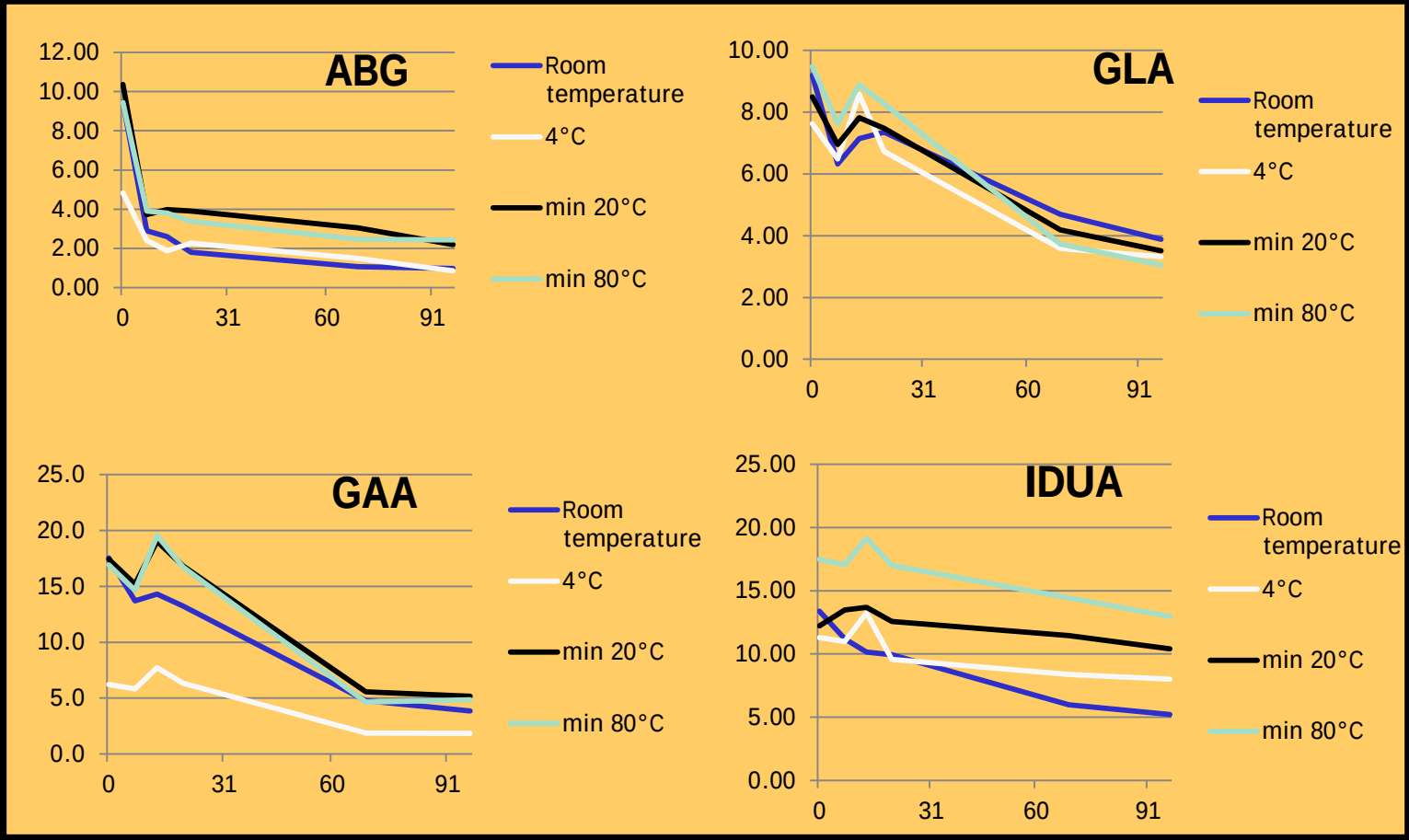
Validation:

- CV% are ≤ 20 CV%, except for GBA (Gaucher)
- Means and interday CV% in unprocessed cord blood (CDC QC material - high)
- High analytical range (comparison QC high with no blood control (dummy))

	Mean (n= 30, in µmol/lh)	Interday CV% (n=30)	Analytical range
Gaucher	10.8	28.7	42.7
Pompe	3.4	8.1	1373.1
Fabry	11.1	8.5	738.4
MPS-I	33	12.2	37.5
MPS-II	8.7	18	49.5
MPS-IVA	0.55	14.2	52
MPS-VI	0.99	14.6	262

Stability tests

- Pre-analytic stability test: Enzyme activity on DBS of healthy adults stored at different temperatures (in days of storage)



- Post-analytic stability test - 10 days after enzyme reaction: maximum 10% difference in product/Internal standard ratio

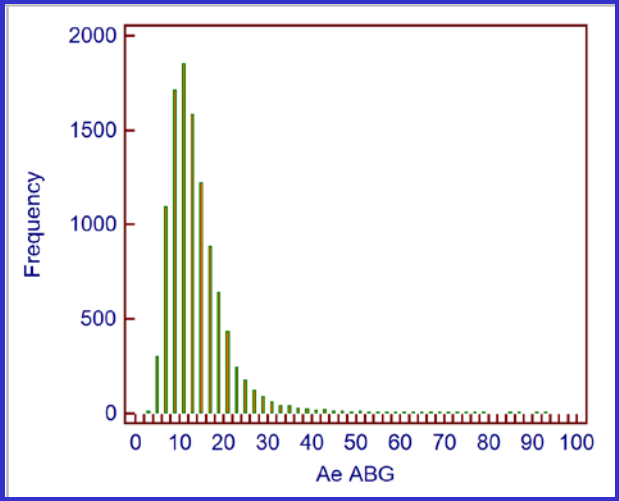
LSD study

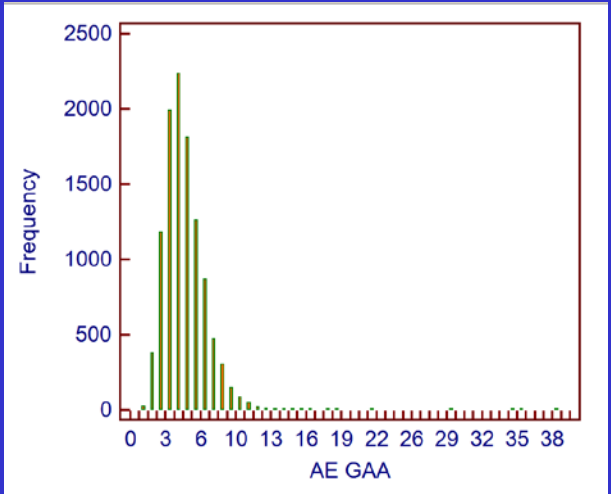
- Important to establish LSD reference values for each center (Müller et al. Diagnostic Pathology 2010)
- prospective study around 20 000 samples are screened for Fabry, Pompe, Gaucher's disease and MPS-I

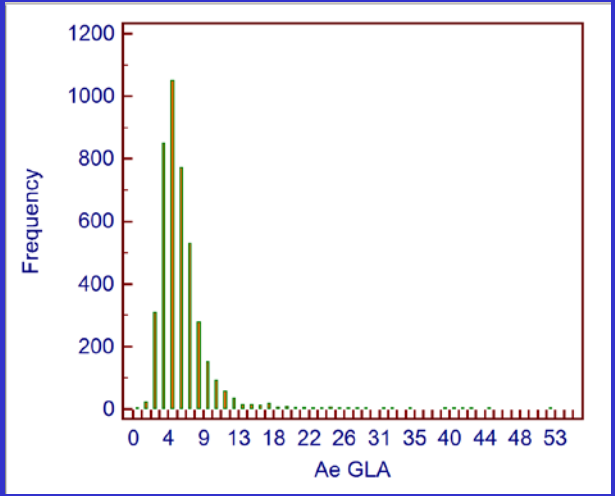
	Number of samples = n	Mean (Ae in µmol/lh)	Low cut-off (Ae in µmol/lh)	% recall
Gaucher	10716	14.5	4.0	0.103
Pompe	10892	4.9	1.41	0.101
Fabry	10553	6.1	1.63	0.094
MPS-I	10452	27.1	7.4	0.105

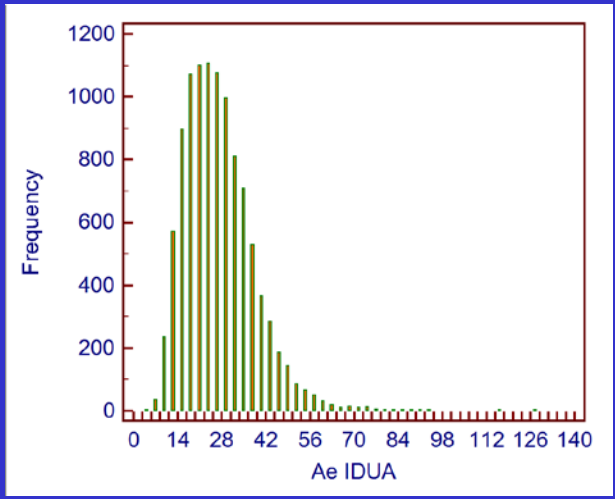
LSD study

- Enzyme activitity of ABG, GAA, GLA and IDUA are not normally distibuted



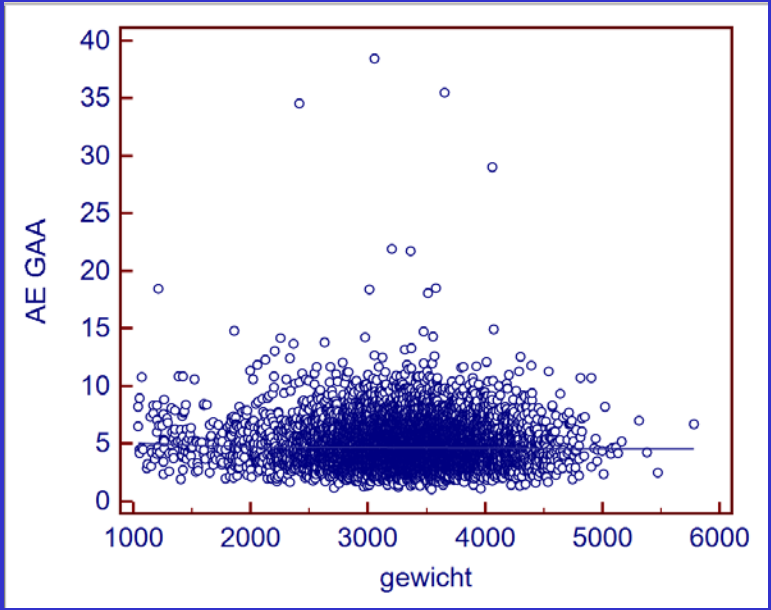


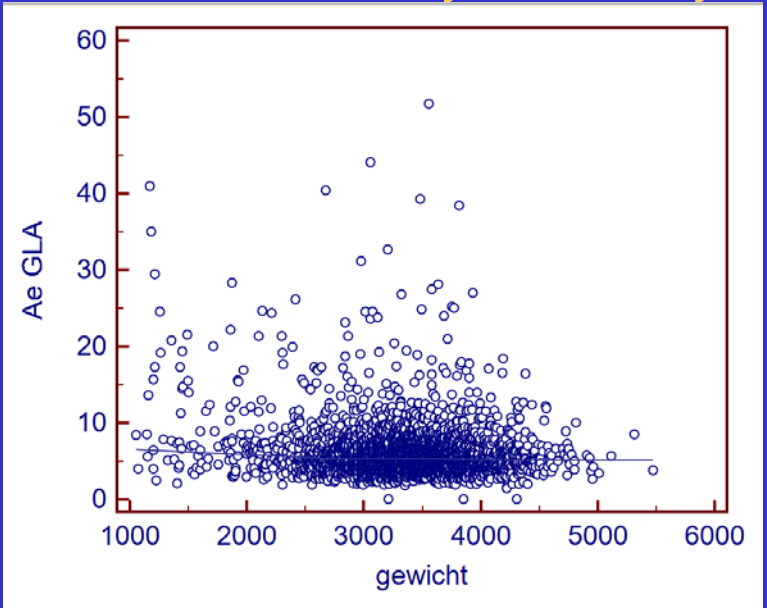




LSD study

- Enzyme activitity of GAA and GLA is statistically higher in female neonates, compared to male neonates. No statistically different ABG and IDUA activities are observed between the sexes.
- There is a statistically comfirmed negative correlation between both GAA and GLA enzyme activity and the neonates weight and gestation age. These correlations are not observed for ABG and IDUA.
- E.g. Weight correlation with GAA en GLA enzyme activity

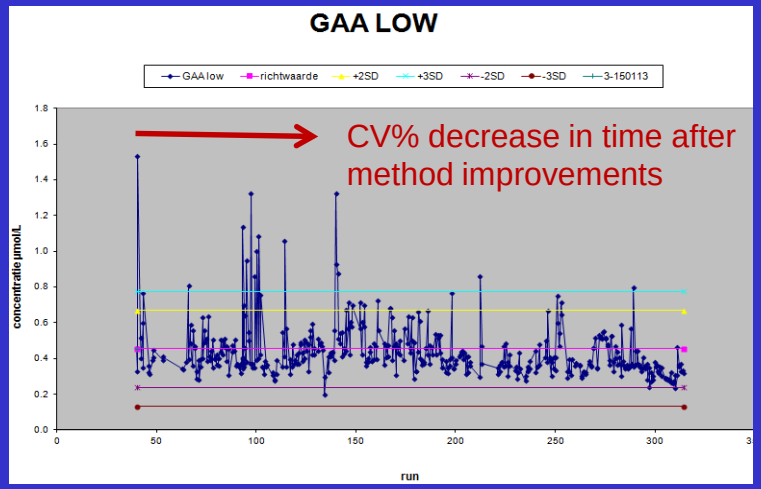


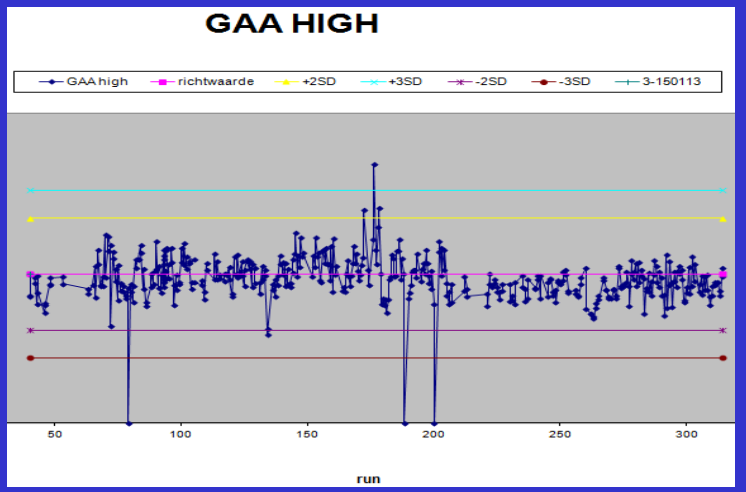


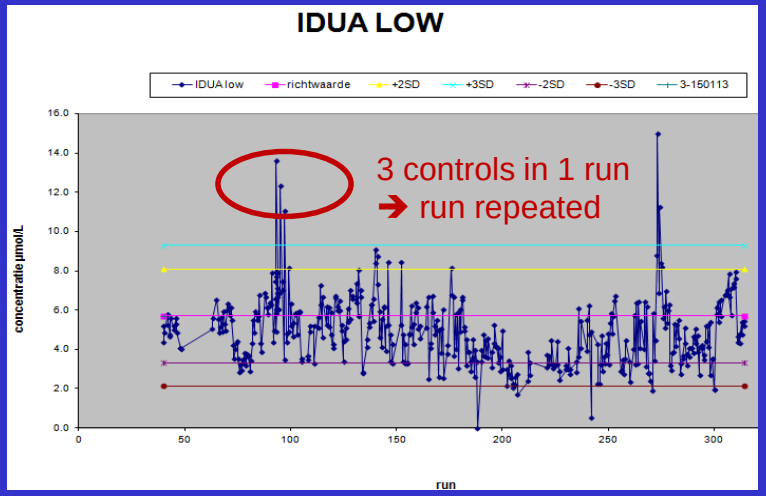
Method improvements (lower CV%)

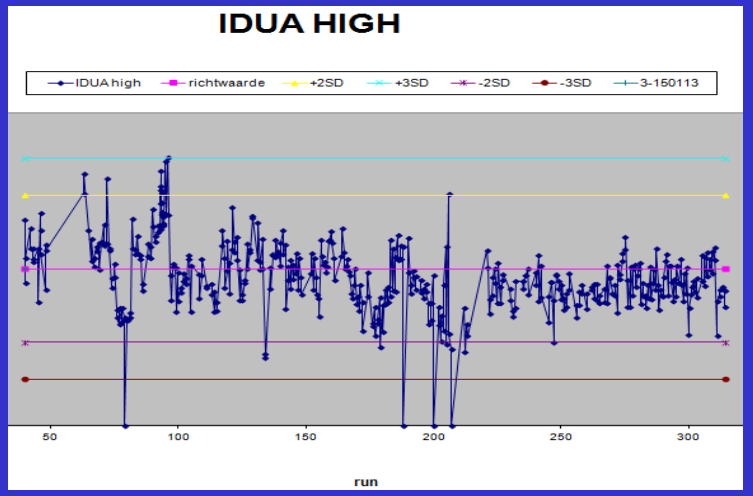
- Decreased incubation time: from 16h to 3h
- Increased concentration by lowering analyte volumes
- Omitting guard column
- Increased linear gradient for better peak separation
- Decreased capillary voltage

LSD daily QC low and high





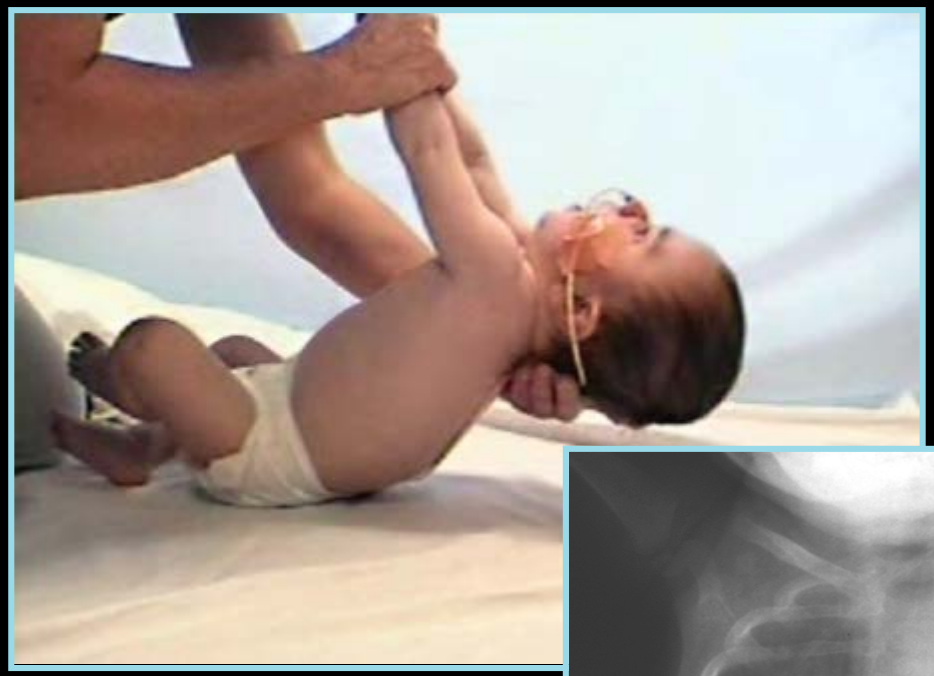


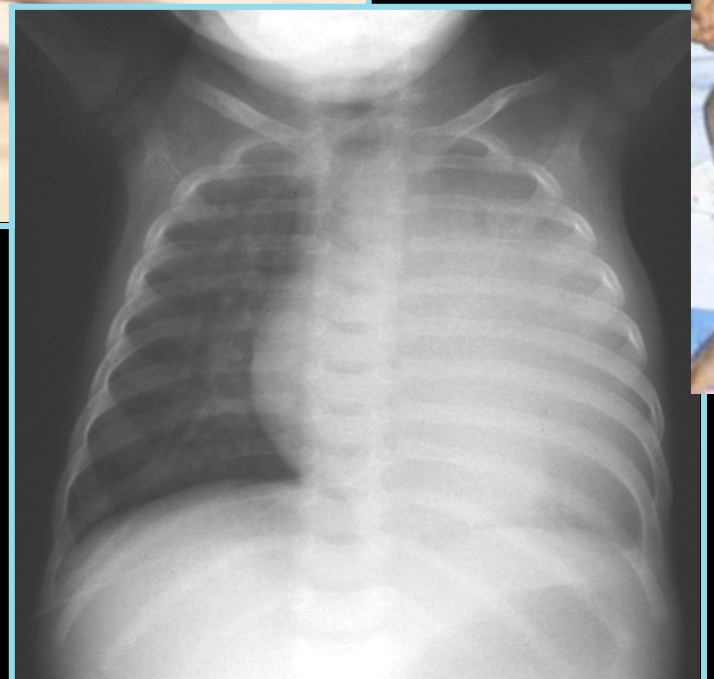


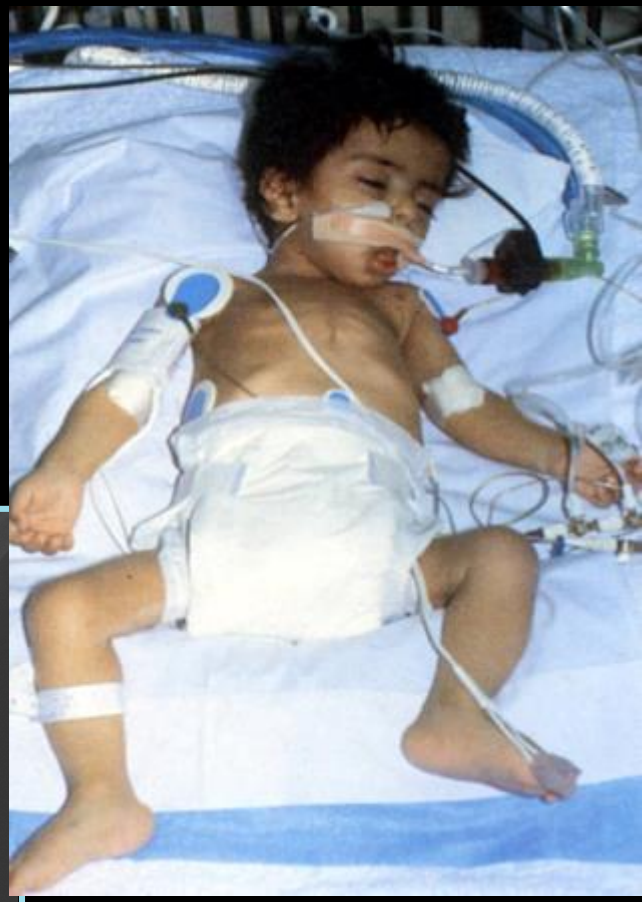
Screening method: Take-home messages

- LC-MSMS method provides an effective high-throughput multiplex screening method
  - Quality control of samples
  - Diagnostic yield (e.g. I cell disease)
- The method is robust (except for GBA), fast and cheap as it is performed on the same MS/MS used in the analysis of aminoacids and acylcarnitines
- Enzyme activities are not normally distributed
- Cut-off levels for GAA and GLA are different, depending of:
  - Gender
  - Gestational age and Birth Weight

Infantile-Onset Pompe Disease







False Positives: e.g. Pompe

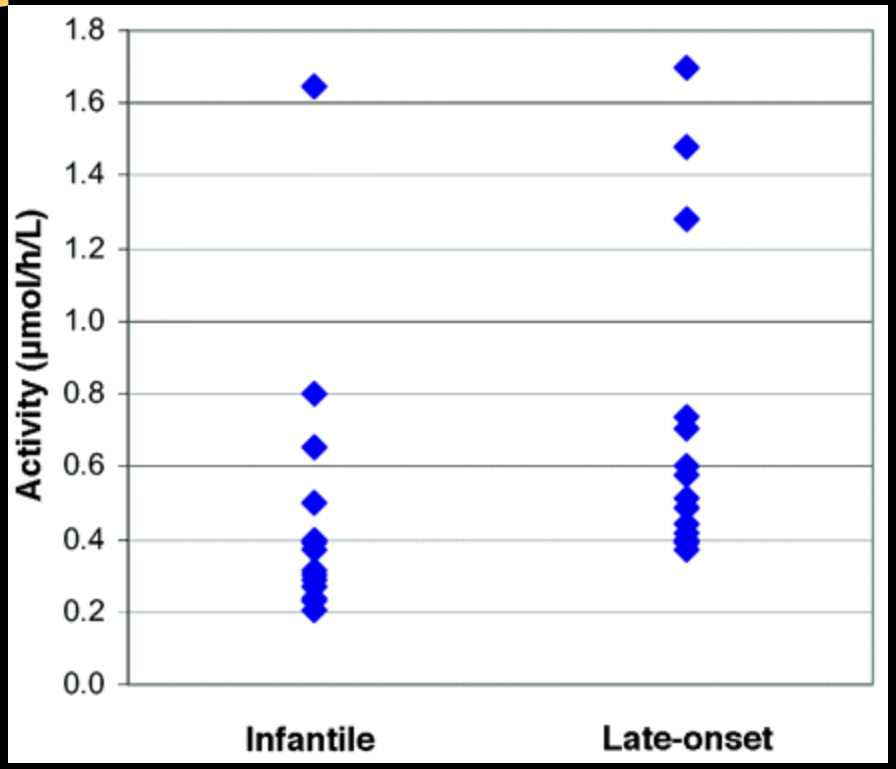
Method	Fluorescence Enzymatic assay	ESI-MS/MS Enzymatic assay
Population	Taiwan (2005-2008)	Austria (2008)
N	132,538	10,279
Recall rate %	0.82	0.039
False positives	117	4

Chien et al. Pediatrics 2009
MechtlerTP et al. Clin Chem 2011

False Positives: e.g. Pompe

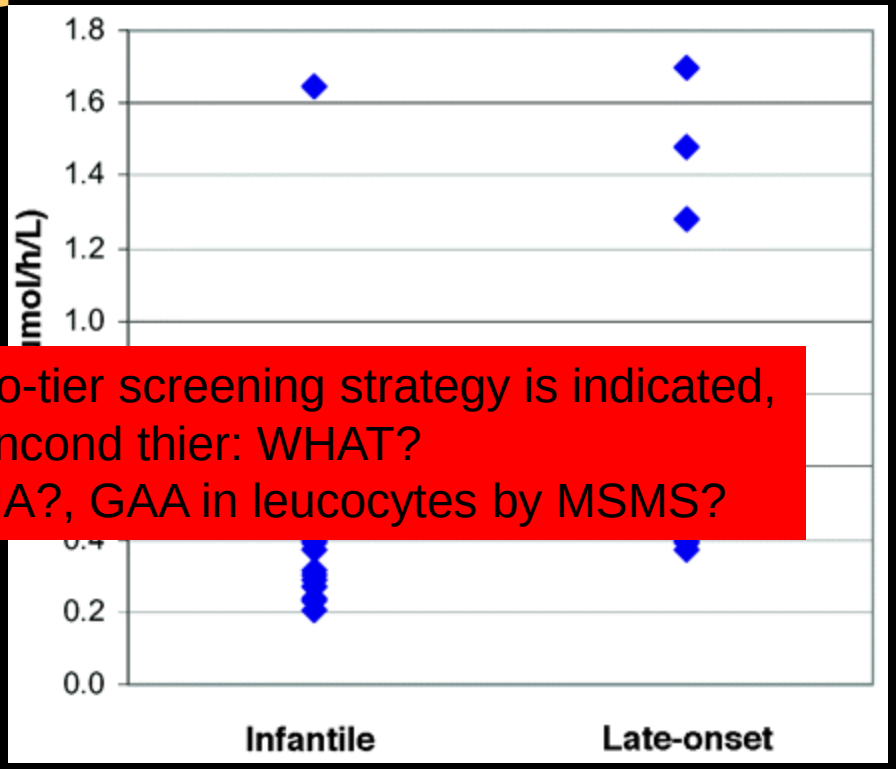
Method	Fluorescence Enzymatic assay	ESI-MS/MS Enzymatic assay
Population	Taiwan (2005-2008)	Austria (2008) Belgium (2014-2015)
N	132,538	10,279 20,000
Recall rate %	0.82	0.039 0.1
False positives	117	4 19
Specificity %	99.91	99.96 99.91

Pompe Screening by MS/MS Discrimination: infantile versus late-onset



(Dajnoki et al. Clin Chem 2008)

Pompe Screening by MS/MS Discrimination: infantile versus late-onset



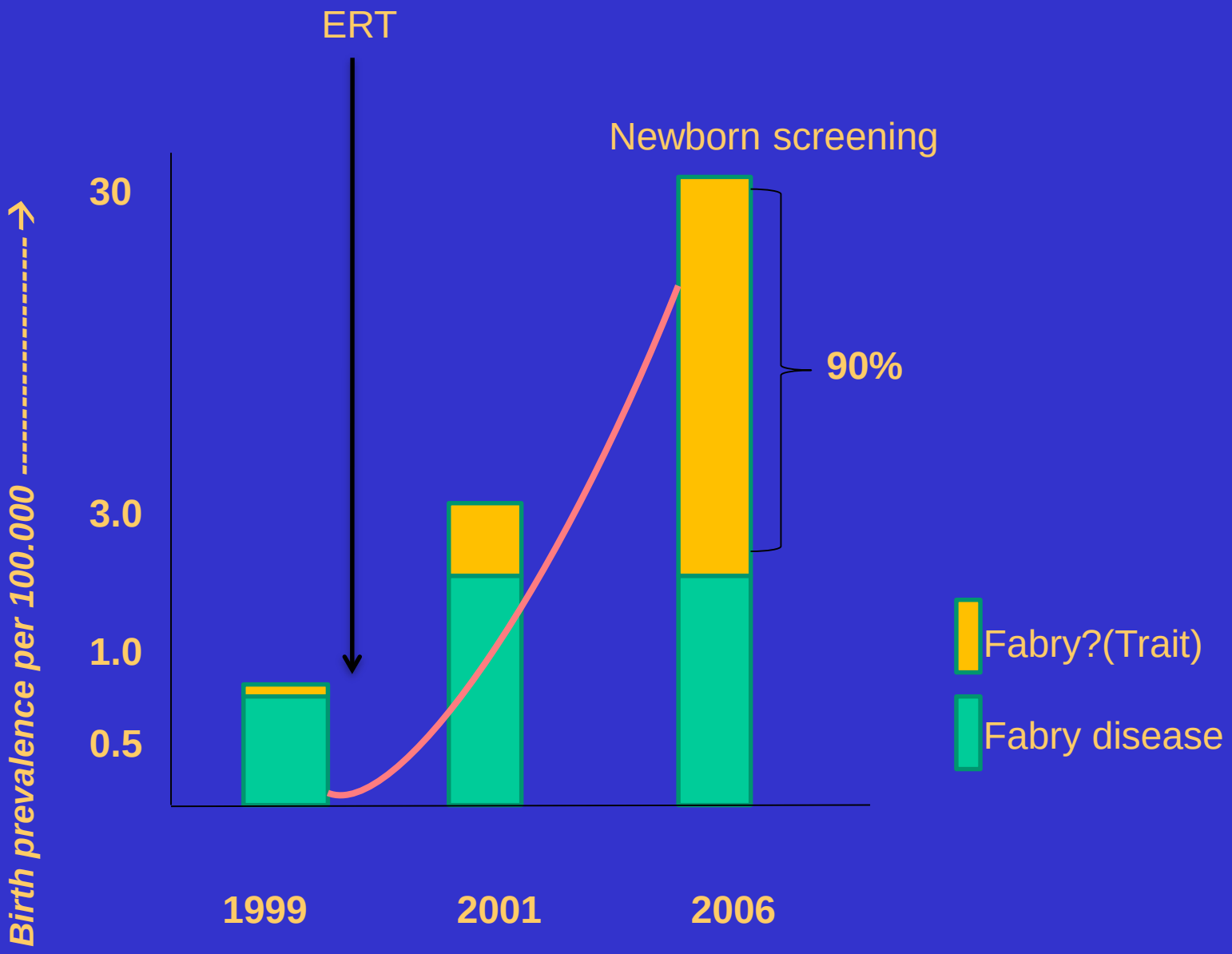
Two-tier screening strategy is indicated,
Sencond thier: WHAT?
DNA?, GAA in leucocytes by MSMS?

(Dajnoki et al. Clin Chem 2008)

LSD incidence:

Current view of LSD incidence underestimated:

- Incidence of Fabry in Italy: 1/3100 births (Spada et al, 2006, Am J Hum Genet)
- Incidence of Fabry in Taiwan: 1/6250 (Liao et al, 2014, Clin Chim Acta)
- Incidence of Pompe in Taiwan: 1/41000 (Chien et al, 2009, Pediatrics)
- Incidence of 1 per 2315 births (3 LSD) (Mechtler et al, 2012, Lancet)
  - *Gaucher: 1/17000*
  - *Pompe: 1/8700*
  - *Fabry: 1/3900*
- Incidence of Fabry, Pompe, and MPS-I is estimated at 1/7500 births (3 LSD) (Scott et al, 2013, J Pediatr)
  - *Fabry: 1/7800*
  - *Pompe: 1/27800*
  - *MPS-I: 1/35500*


ERT
Newborn screening
30
3.0
1.0
0.5
Birth prevalence per 100.000 ----------→
90%
1999
2001
2006
Fabry?(Trait)
Fabry disease

Ethics?

It is not about how we will screen, but What and Why we should screen?

Moving forward:

Proposal to our authorities:
start neonatal screening for
MPS I,VI, II, IVA
Evaluation performed in 2014

Towards a stand-still:

Implementation in….

Thank you!

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