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Comparison of use of cutoffs to CLIR in screening for Pompe disease and Krabbe disease

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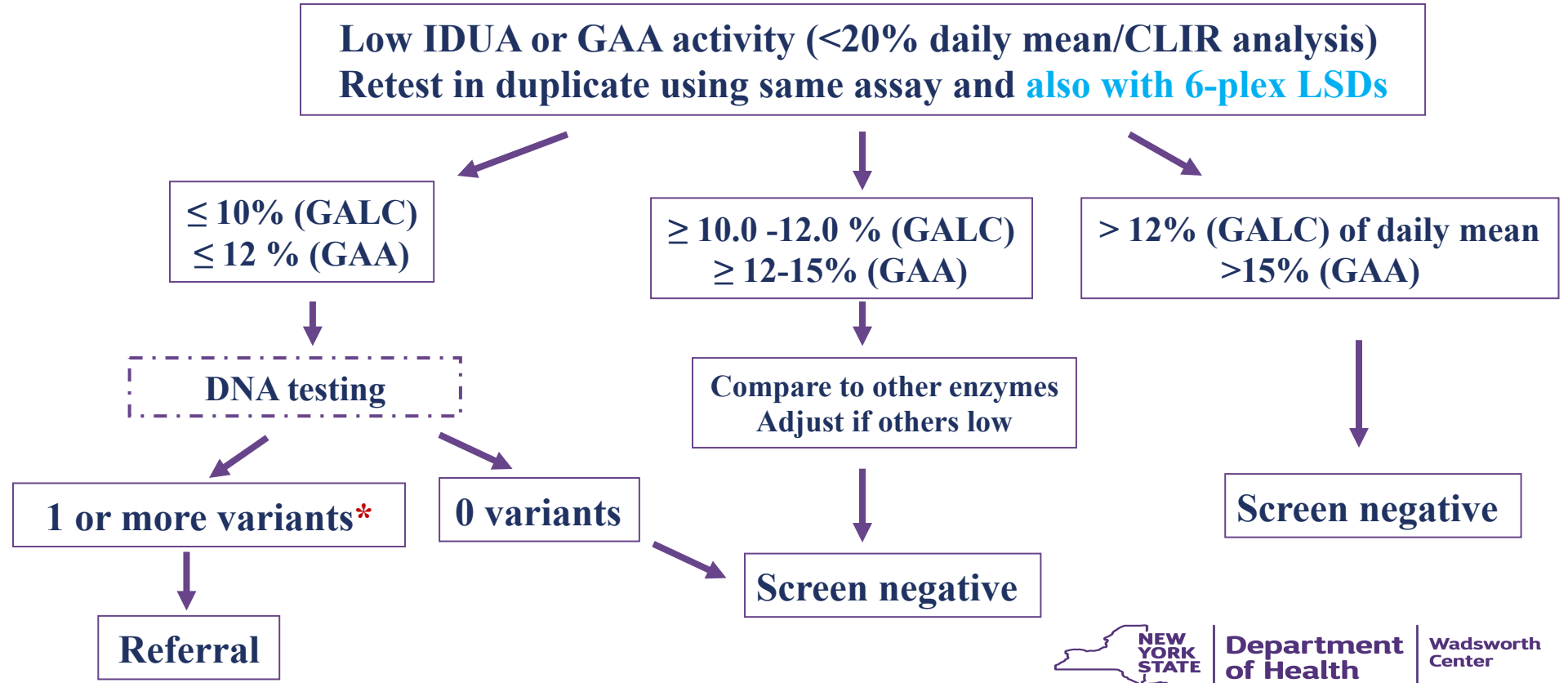
Krabbe/Pompe Screening Algorithm



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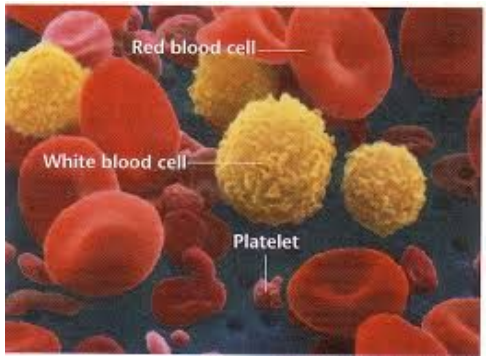
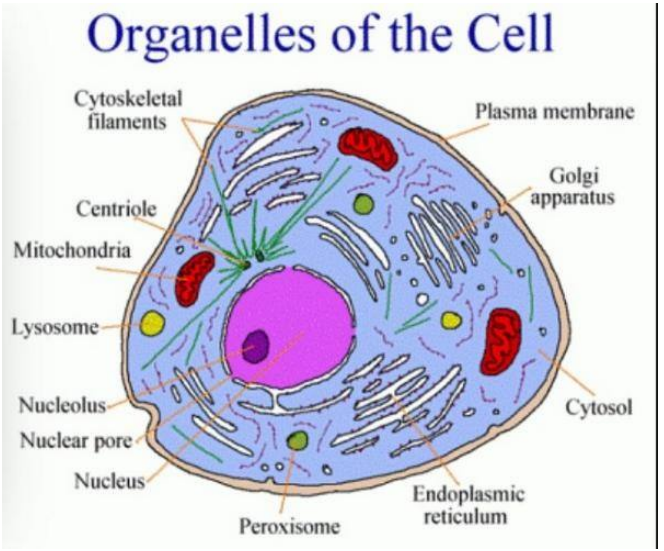
Enzyme data: GALC example

Samples with:	% GALC	% GAA	% IDUA	% GLA	% GBA	% ASM
GALC <12%	8.3	60.9	73.2	48.8	64.3	95.2
GALC >300%	464	130	116	309	136	86

** Observation: when GALC very low (<12%) or very high (e.g.>300%), the other enzymes follow

Dried blood spot screening





Markers:
Can be present in serum, red cells, white cells or some combination
Diagnostic tests: target a specific component of the blood

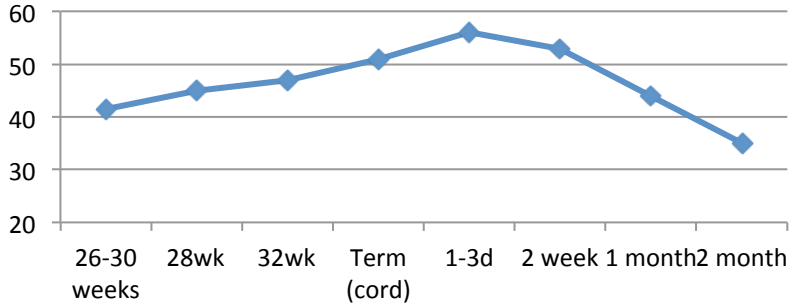
Dried blood spot variables

Dried Blood Spot variables: not accounted for in calculating marker concentrations

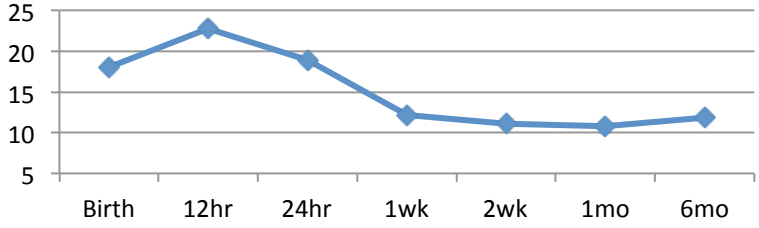
1. Red cells (hematocrit): affects **volume** of blood in punch: affecting all calculated marker concentrations
2. White cells (leukocytes): **contain lysosomes** – for LSDs, the measured enzyme activity dependent on number of white cells
3. Exposure to heat, humidity in transport - affect enzyme activities

Variables in dried blood screening

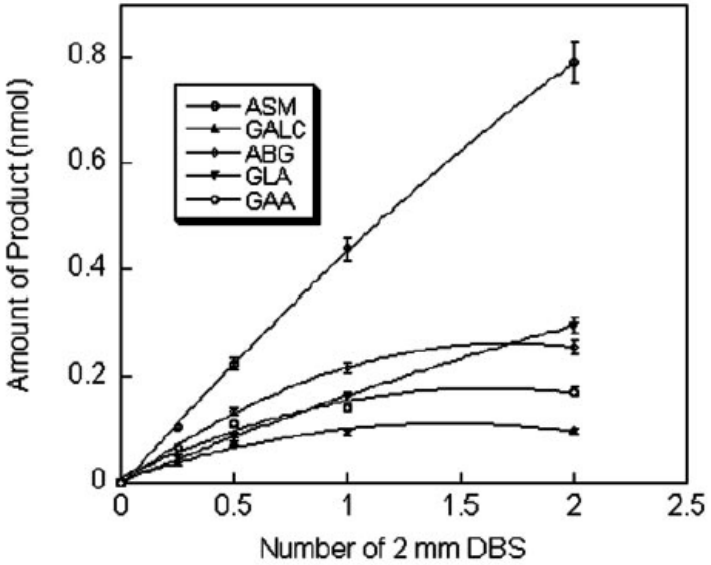
HCT(%)



Total Leukocytes (x1000/mm^3)

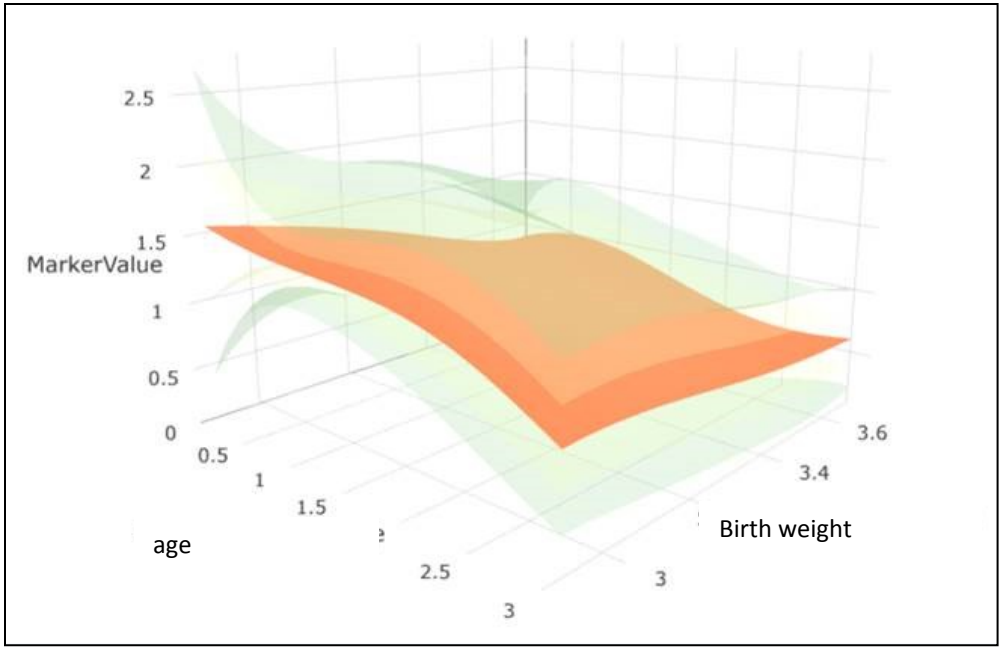


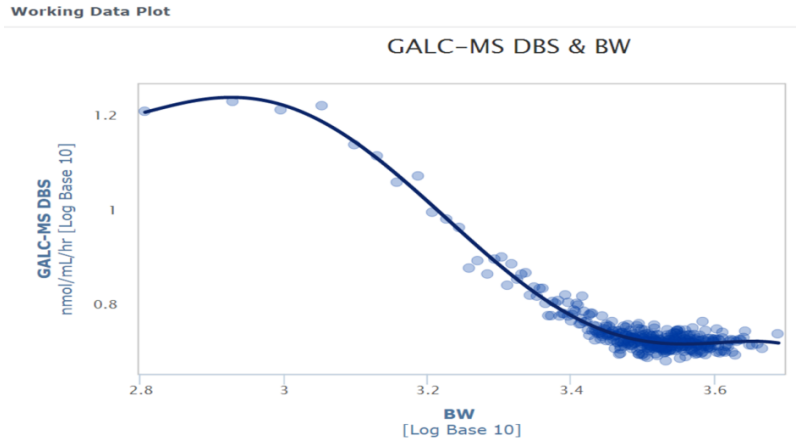
Data from The Harriet Lane Handbook



Li, Gelb et al, Clinical Chemistry, 2004

GALC versus birth weight and age: Marker Profile





Plots from CLIR/Mayo

Profile of GALC activity: vs. bwt and age

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Value of multi-marker approach

1. **Biochemical dependency** of markers with biochemical dependencies can be handled (phenylalanine and tyrosine)
2. **Physical effect** of hematocrit and blood filling circle:
    a. for many markers the concentrations will increase with increased hematocrit – simply more blood in 3 mm punch
    b. some marker concentrations will be lower, as less serum in high hematocrit sample punches.
3. **Biological variables:** Markers **primarily present** in white or red cells

CLIR: looks at markers and all possible ratios of markers that are evaluated in the screen. At simplest level, using ratios corrects for variables having a common affect on all markers (Enzymes).
May also detect other relationships between markers

Live Screening Summary

Krabbe

Started: 08-07-06

Samples Tested: ~2,650,000

Referrals: 485

Infantile cases: 5

Possible LOKD: 21

PPV* = 26/485 = 5.4%

Pompe

Started: 10-01-14

Total Tested: 760,393

Referrals: 109

Infantile Cases: 5

Possible LOPD: 50

PPV* = 55/109: 50.4%

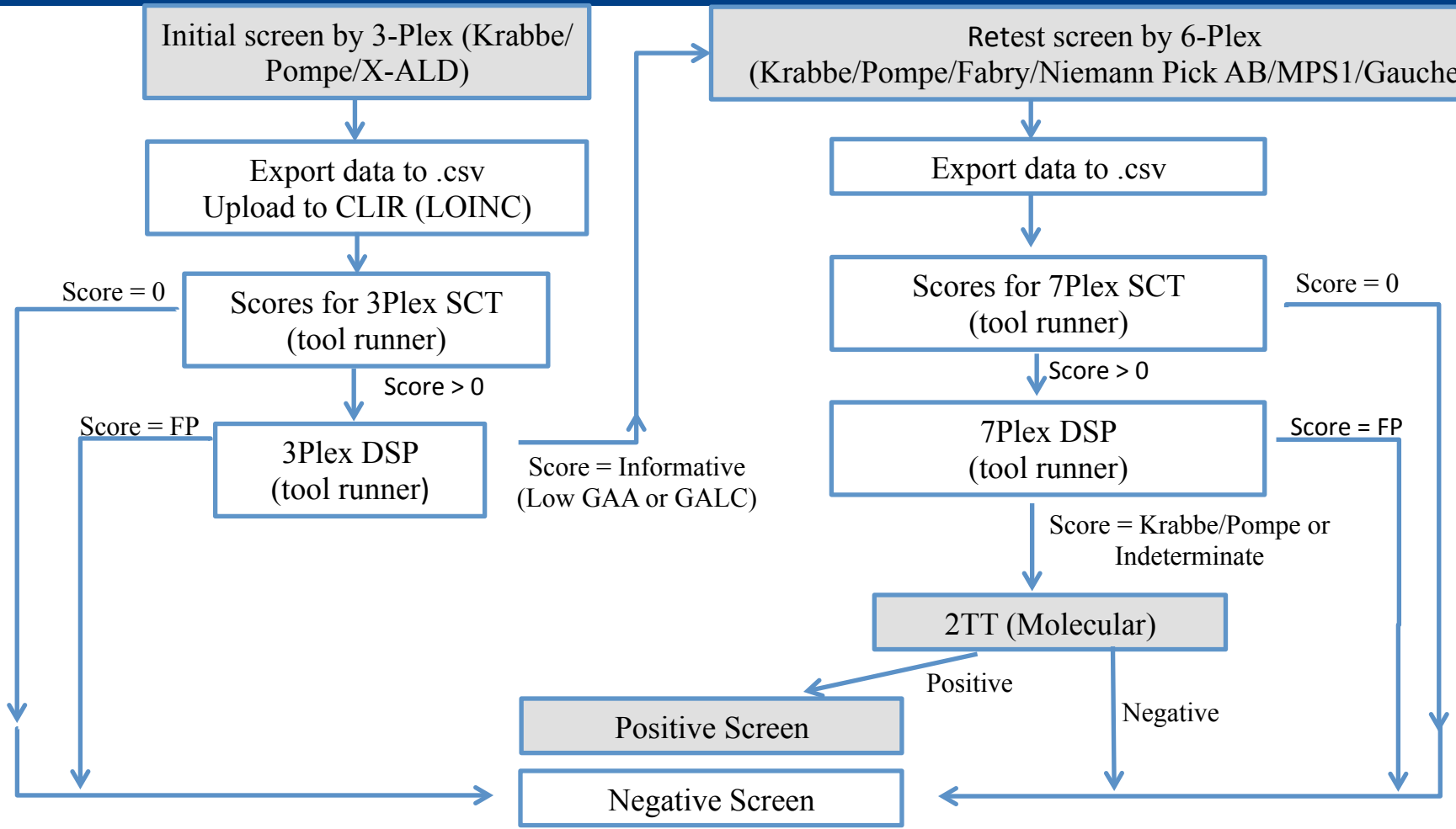
To date, none of the infants with a possible case have developed symptoms

Reminder :Krabbe/Pompe Screening Algorithm

1. Screen enzymes (run CLIR)
Low IDUA or GAA activity (<20% daily mean)
2. Retest in duplicate using same assay and also with 6-plex LSDs (CLIR analysis)

- ≤ 10% (GALC) ≤ 12 % (GAA) → DNA testing
  - 1 or more variants* → Referral
  - 0 variants → Screen negative
- ≥ 10.0 -12.0 % (GALC) ≥ 12-15% (GAA) → Compare to other enzymes Adjust if others low → Screen negative
- > 12% (GALC) of daily mean >15% (GAA) → Screen negative

Sample flow using CLIR



Limitations of Study

- Retrospective data:
  - Ran all samples through a 3 marker tool (GALC, GAA, C26-LPC
  - did not run six-plex enzyme tool on all samples that tested low for GALC and GAA.
- We tested many, but not all important positive samples (limited sample quantities)
- Affects how we look at numbers: had to project numbers based on results from a subset of samples that had full testing

CLIR: Retrospective Case Analysis

Disease	# Positives tested	# False Positives	#infantile cases	# Possible Late onsets
Krabbe	131	84	6 of 6	13 of 14*
Pompe	39	8	2 of 2	14 of 14

- All true Krabbe cases detected
- Case definitions are still very important
- In CLIR, can see location specific controls

CLIR Results compared to Cutoffs

Date	NY4 3-Plex	CLIR Retest Two enzymes*		NY (retest)		# of Spec Run
	Cases	Krabbe	Pompe	Krabbe	Pompe	with 7-Plex tool
June 2015- Aug 2017	586,763	555	298	5,026	743	289 of 853
Retest rate		0.09%	0.05%	0.86%	0.12%	~33% of data

* Projected number –based on reduction rate of subset data(33%)

Disease	CLIR RT*	CLIR second tier 6 enzymes*	NY Cutoffs second tier	% change
Krabbe	555	113	248	-45%
Pompe	298	183	111	+165%
Pompe (hybrid)	111 NY (retest)	68	111	-61%

Objectives of Study

- Can CLIR be easily added to lab work flow ✓
- Compare performance of cutoffs versus CLIR ✓
- Reduce number of required retests ✓
- Reduce number of required second tier tests ✓
  - Big reduction for Krabbe
  - Pompe can use some work/currently "hybrid" approach works better – tool will be re-evaluated
- Reduced false positives, especially for Krabbe with no false negatives ✓

Next Steps

- Continue with prospective study
- Adjust tool to lower number of Pompe retest versus “hybrid” approach
- Evaluate MPS I and other LSDs with CLIR

Questions

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