

## Part III: Integration of X-linked Adrenoleukodystrophy and SEEKER® Workflows for Same-Day Referrals

As new disorders are added to the Recommended Uniform Screening Panel (RUSP), public health laboratories must determine the best strategy to incorporate the tests for these disorders into their already busy workflows. We previously examined basic metabolite and enzyme activity assay workflows in [Part II of this white paper series](#). The recent additions of two lysosomal storage disorders (LSDs), Pompe and Mucopolysaccharidosis Type I (MPS I), and X-linked Adrenoleukodystrophy (X-ALD) to the RUSP have triggered discussions in laboratories and state legislatures on the best approach to implement these three tests. Baebies' SEEKER digital microfluidic platform is the only FDA authorized platform for newborn screening of enzymatic activity indicative of LSDs, including Pompe and MPS I. For those laboratories that are considering adding Pompe, MPS I and X-ALD to their screening panels, this white paper outlines the differing screening methodologies for LSDs and X-ALD and describes how SEEKER can easily be incorporated into any laboratory to optimize workflow.

[Click Here to Read Part I: Synthetic Substrates](#)

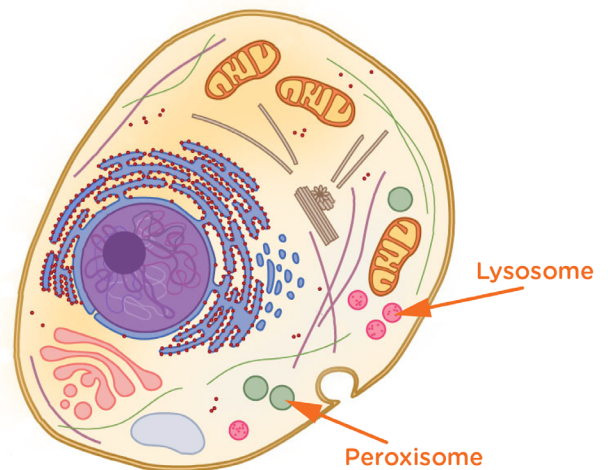
[Click Here to Read Part II: How to Measure Product Formation](#)

### LSDs and X-ALD: The Differences

Lysosomal storage disorders are a group of approximately 60 rare inherited metabolic disorders that are caused by lysosomal enzyme dysfunction, usually as a consequence of a deficiency in a single enzyme required for the metabolism of lipids, glycoproteins or mucopolysaccharides. X-ALD, commonly mistaken as an LSD, is a peroxisomal metabolic disorder caused by a deficiency of the peroxisomal membrane transporter protein ALDP, which is responsible for transporting very long chain fatty acids (VLCFA) to the peroxisome for degradation<sup>1</sup>. X-ALD is an X-linked disease and therefore primarily affects males, but female heterozygous carriers can develop symptoms later in life.

**Because LSDs are caused by enzyme deficiencies, and X-ALD is caused by a deficiency in a transport protein, there are fundamental differences in how screening assays for these disorders are performed in a newborn screening laboratory.** Deficiencies in enzyme activity, which are characteristic of LSDs, are measured using enzyme activity assays, where **reduced** product formation measured as concentration of product/unit time (*micromoles/liter/hour*) is a biomarker for the disease. On the other hand, X-ALD is identified by measuring the accumulation of the VLCFA C26 (due to the deficient transport protein), which is reported as **increased** concentration of a C26 lysophosphatidyl choline derivative (*micromoles/liter*).

### Lysosome and Peroxisome Locations in the Cell



### Implementing Newborn Screening for Pompe, MPS I and X-ALD

Currently, only a handful of states are screening for Pompe, MPS I and X-ALD<sup>2</sup>. The early experiences of these pioneering programs demonstrate that multiple methods can be used to screen for each of these three conditions. **While the Pompe and MPS I screening assays are both based on enzyme activity, which requires a separate incubation step prior to measurement, X-ALD screening assays use direct metabolite measurement that is**

compatible with the tandem mass spectrometry (MS/MS) metabolite screening methods currently already in use in U.S. screening laboratories (reviewed in [Part II of this series](#)). The C26 LPC measurement used in X-ALD screening is usually performed in a two-tier MS/MS process where samples over a cutoff concentration in the first tier FIA-MS/MS reflex to a second tier LC-MS/MS with different cutoff concentrations and can be combined with either the non-derivatized or derivatized MS/MS metabolite methods without the need to purchase additional equipment.

	Pompe and MPS I (LSDs)	X-ALD
Subcellular Location	Lysosome	Peroxisome
Deficiency	Lysosomal enzyme activity	Transport protein
Screening Test	Reduced turnover of artificial substrate	Elevated C26 LPC
Unit of Measure	Enzymatic Activity ( <i>micromoles/liter/hour</i> )	Concentration ( <i>micromoles/liter</i> )
Substrate Required	Yes	No
Time to Result	Up to 3.5 hours total for 4 LSDs with SEEKER	2 - 3 hours

**Table 1.** This table illustrates the fundamental differences between screening for LSDs and X-ALD.

Baebies does not offer a newborn screening test for X-ALD, however SEEKER can help streamline implementation of Pompe and MPS I screening assays for public health laboratories that are looking to stay current with the RUSP. The SEEKER platform has a self-contained instrument that does not require any facility modifications and can be installed and ready to analyze samples in minutes. Validation of the FDA authorized SEEKER LSD panel, which includes enzymatic activity screening assays for Pompe, MPS I, Gaucher and Fabry, is also significantly faster than existing laboratory developed test options for these assays as shown in [Part II of this series](#). X-ALD is technically not an LSD nor is it measured through enzyme activity. Because the test for X-ALD is a metabolite assay performed on MS/MS, it has been demonstrated with amino acids and acylcarnitine profiling using existing MS/MS equipment<sup>3</sup>. With SEEKER implementation for Pompe and MPS I and MS/MS implementation for X-ALD, laboratories can have screening results in under 4 hours with high sensitivity, providing the potential to go from punch to referral in as little time as one work day. This quick and easy turnaround time is especially important for disorders such as Pompe, where days matter with newborns diagnosed with infantile onset forms of the disease<sup>4,5</sup>. Moreover, same-day reporting of these three additional conditions by integrating SEEKER workflow for LSDs with metabolite MS/MS workflow for X-ALD would enable the U.S. labs to meet the timeliness goal of 7 days from birth to reporting of results<sup>6,7</sup>.

## References

- <sup>1</sup> Kemper AR, et al. 2015 (updated 2017). <https://www.hrsa.gov/advisorycommittees/mchbadvisory/heritabledisorders/nominatecondition/reviews/alddecisionletter.pdf>.
- <sup>2</sup> NewSTEPs 2017 Meeting Summary. [https://www.newsteps.org/sites/default/files/new20disorders20meeting20summary202017\\_meetingsummary\\_july2017\\_ss.pdf](https://www.newsteps.org/sites/default/files/new20disorders20meeting20summary202017_meetingsummary_july2017_ss.pdf).
- <sup>3</sup> Haynes CA, De Jesus VR. Clinical Biochemistry. 2016; 49(1).
- <sup>4</sup> Bodamer OA, Scott RC, Giugliani R. Pediatrics. 2017; 140(s1).
- <sup>5</sup> What is infantile onset Pompe disease versus late onset Pompe disease? Interview with Dr. Priya Kishnani (Baebies whitepaper; <http://baebies.com/infantile-onset-pompe-disease-vs-late-onset-pompe-disease-baebies-interviews-dr-priya-kishnani/>)
- <sup>6</sup> U.S. Government Accountability Office. Newborn screening timeliness. 2016. <https://www.gao.gov/assets/690/681635.pdf>.
- <sup>7</sup> Understanding Enzyme Assays for Lysosomal Storage Disorders – Part II: How to Measure Product Formation (Baebies whitepaper: <http://baebies.com/understanding-enzyme-assays-lysosomal-storage-disorders-measure-product-formation-part-ii/>)