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Newborn Screening for the Full Set of Mucopolysaccharidoses in Dried Blood Spots Based on First-tier Enzymatic Assay Followed by Second-Tier Analysis of Glycosaminoglycans

Zackary Herbst[1], **Xinying Hong**[2], **Martin Sadilek**[1], **Maria Fuller**[3,4], **Michael H. Gelb**[1,5]

[1]Dept. of Chemistry, University of Washington, Seattle, WA 98195 USA

[2]Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA, 19104, USA

[3]Genetics and Molecular Pathology, SA Pathology at Women's and Children's Hospital, North Adelaide 5006, Australia;

[4]School of Biological Sciences and Adelaide Medical School, University of Adelaide, Adelaide 5005, Australia

[5]Dept. of Biochemistry, University of Washington, Seattle, WA 98195 USA

Abstract

Newborn screening (NBS) for the full set of mucopolysaccharidoses (MPSs) is now possible by either measuring all of the relevant enzymatic activities in dried blood spots (DBS) using tandem mass spectrometry followed by measurement of accumulated glycosaminoglycans (GAGs) or the vice-versa approach. In this study we considered multiple factors in detail including reagent costs, time per analysis, false positive rates, instrumentation requirements, and multiplexing capability. Both NBS approaches are found to provide acceptable solutions for comprehensive MPS NBS, but the enzyme-first approach allows for better multiplexing to include numerous additional diseases that are appropriate for NBS expansion. By using a two-tier NBS approach, the false positive and false negatives rates are expected to acceptably low and close to zero.

1. Introduction

MPS disorders result from deficiency of one of several enzymes that are required for degradation of GAGs in lysosomes. NBS for a subset of MPSs is ongoing worldwide. In the United States, MPS-I was added to the Recommended Uniform Screening Panel (RUSP), and several states have started MPS-I newborn screening. The Netherlands also screens for MPS-I. More recently, MPS-II was added to the RUSP, and Illinois [1] and Missouri [2]

maria.fuller@adelaide.edu.au .

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have started to screen for this disorder. Taiwan screens for MPS-I and MPS-II but also MPS-IVA, and MPS-VI [3].

NBS for these MPSs is warranted because of the availability of treatment options (enzyme replacement therapy and haematopoietic stem cell transplantation) that work best if carried out as early in life as possible. Enzyme replacement therapy also is approved for MPS-VII but this MPS is not included in any NBS programs. Development of NBS methods for the other MPS substrates is of interest because of the ongoing clinical development of novel therapies including enzyme replacement therapy and gene therapy.

2. Multiplexed LC-MS/MS assays of enzymatic activities relevant to MPSs.

LC-MS/MS assays of MPS enzymes was developed by the Gelb laboratory, and the most recent work includes all MPS subtypes except MPS-X [4]. A cassette of substrates, each specific for the enzyme relevant to each MPS disorder, are incubated in assay buffer together with a DBS punch. To cover all of the MPS subtypes, 2 DBS punches and 2 assay cocktails are used. After incubation to allow enzymatic product formation, both mixtures are combined for a single injection into the mass spectrometer for LC-MS/MS analysis [4]. Detection of the MPS-IIID enzyme was limited to leukocytes and fibroblasts; use of dried blood spots (DBS) did not lead to detectable enzymatic activity [4]. However, recent studies have shown that DBS extract contains endogenous inhibitory factors that reduce the activity of the MPS-IIID enzyme and that adequate activity can be detected if the extracted is diluted (Liu and Gelb, submitted). MPS-X is the most recently discovered MPS subtype due to deficiency of arylsulfatase K, and only a few patients have been identified [5]. Presumably a suitable enzymatic activity assay could be developed and multiplexed with the other MPSs.

3. GAG analysis.

In all MPS subtypes, one or more GAG molecular species accumulates due to deficiency of one of the enzymes required for GAG degradation in lysosomes. GAGs occur as multiple classes (heparan sulfate, dermatan sulfate, chondroitin sulfate, keratan sulfate and hyaluronan). Each class contains a complex mixture of molecular species owing to semi-random additional modification of the disaccharide repeat units (sulfation, acetylation, and C5 epimerization). Given the structural complexity and the high molecular weight of the full-length GAGs, these polymers are typically enzymatically degraded in vitro to give a manageable number of disaccharide repeat units that can be analyzed by LC-MS/MS. This is the classical GAG analysis method, referred to in this article as the Internal Disaccharide method [6][7]. A related method is to degrade the polymers by heating in methanol or butanol to give alkyl glycoside units that can also be analyzed by LC-MS/MS [8]. Both the enzymatic and non-enzymatic degradation methods are surrogates for measuring the amounts of the full-length GAG polymers as the amounts of disaccharides should be proportional to the amounts of the polymers.

Two additional GAG analysis methods involve the quantification of the non-reducing end (NRE) of the GAG polymer. In one method, Sensi-pro [9], full-length GAG polymers are first digested with bacterial enzymes to give many internal disaccharides per GAG polymer

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and a single copy of the non-reducing end. The mixture is chemically derivatized, and the non-reducing end is quantified by LC-MS/MS. The second non-reducing end method is the Endogenous NRE method which uses LC-MS/MS to detect non-reducing end GAG fragments that exist endogenously in biological samples [10]. These markers are also chemically derivatized prior to LC-MS/MS analysis.

4. Two-tier analysis for minimizing false positives in NBS of MPSs.

It is well established that false positives are common in NBS of MPSs when only a single method is used (enzymatic activity or GAG analysis). Thus, high precision NBS requires one of 2 possible 2-tier approaches. One method is to first measure the enzymatic activity in DBS and to pass all below-cutoff DBS to a second-tier GAG analysis using the same DBS so it can be done as part of NBS. The alternative is to measure GAG levels first and to explore the deficiency of one of the relevant enzymes if one or more GAG molecular species is elevated. Here, we consider these two options in detail with an effort to consider all factors including reagent costs, time per analysis, rates of false positives, instrumentation requirements, and multiplexing capability.

Consideration of the two NBS options first requires further discussion of GAG analysis methods. We have carried out a detailed comparison of the Internal Disaccharide and Endogenous NRE methods using newborn DBS from MPS-I [7], MPS-II [11] and MPS-IIIA, -IIIB, -IIIC, -IIID, -IVA, -VI, and -VII [12]. We also included GM1-gangliosidosis because GAG biomarkers are well known to accumulate in urine from these patients. Comparative studies with Sensi-pro were carried out for MPS-I only [7], and since it did not outperform the Internal Disaccharide method and is a much more laborious method, we did not study Sensi-pro for the other MPS types. Results show that the Endogenous NRE method greatly outperforms the other methods in terms of the number of false positives. To date there is no evidence for false positives using the Endogenous NRE method with DBS. Despite this success of the Endogenous NRE method, it is very unlikely to be used for first-tier NBS of MPSs. Because there is only 1 NRE per GAG polymer versus hundreds of internal disaccharides per polymer, NRE methods require a mass spectrometer that is much more sensitive than those typically used for first-tier NBS in NBS laboratories. Even if top-end machines could be installed in NBS laboratories, they would not hold up to hundreds of samples per day as they would require extensive cleaning on a schedule that is not appropriate for first-tier NBS. Sensi-pro would present the same issues, and also is not the method of choice as noted above. This leaves the Internal Disaccharide method as the only viable GAG-based, first-tier option for NBS of MPSs.

Table 1 compares a number of features of NBS for all MPSs except MPS-X based on enzymatic activity assay followed by GAG analysis or the vice-versa approach. For GAG analysis, two 3 mm DBS punches are needed per newborn, 1 for digestion with Heparinases I, II, and III, and chonroitinase B for MPS-I, -II, -IIIA, -IIIB, -IIIC, -IIID, and -VI and one for digestion with chondroitinase ABC for MPS-VI and -VII [7][11][12]. Keratanase II can be omitted for DBS analysis since keratan sulfate-derived GAG internal disaccharides are not informative for MPS-IVA [12]. Enzymatic assays also require 2 punches because two buffers are required to cover all the MPSs [4].

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Cost is a factor in considering first-tier NBS methods. The Internal Disaccharide method requires a collection of commercial bacterial enzymes for GAG degradation. To minimize costs, we report for the first time in this chapter a study of the minimum amounts of bacterial enzymes that support full GAG degradation. We started with the amounts of bacterial enzymes reported in the literature and systematically reduced each enzyme in increments while following the LC-MS/MS signals for each GAG-derived disaccharide. Results in Supplemental Figure S1 establishes the minimum amounts of GAG degrading enzymes that can be used without a observed decrease in the MS/MS signal for the biomarker. Table 1 gives the estimated cost for GAG digestion enzymes on a per newborn basis and with the established minimal enzyme amounts used. We also give the estimate cost for the set of MPS enzyme substrates and internal standards required for the multiplex, LC-MS/MS, enzymatic activity assay. Costs for GAG analysis reagents is about 10-fold less than for enzymatic activity analysis (Table 1). Full details of the cost estimations are given in Supplemental Material S1 and S2.

The NBS analysis time is compared for the first-tier GAG versus first-tier enzymatic assay methods (Table 1). GAG analysis requires overnight incubation with bacterial enzymes, and the enzymatic activity assay requires overnight incubation with substrates. Thus, in both cases LC-MS/MS could be started in the afternoon of Day 2 (after the few hours of post-incubation workup time). The LC-MS/MS analysis time per newborn is 2.1 min for the multiplex enzymatic activity analysis [13][4]. Published LC-MS/MS run times per newborn for the GAG Internal Disaccharide method are about 5 min per newborn [14]. Based on our own non-published studies, the LC-MS/MS run time can be reduced to 2.5 min without compromising biomarker quantification (Supplemental Material S3). Thus, for both methods, NBS results would be available in the morning of day 3 with a throughput of about 429 newborns per LC-MS/MS instrument (Table 1) (Supplemental Material S3).

Table 1 also provides typical examples of LC-MS/MS instruments that have sufficient sensitivity to detect either the GAG-derived biomarkers or the enzymatic products. This is based on actual experimentation in our laboratory. GAG analysis by the Internal Disaccharide requires mass spectrometers that are more sensitive than those used for enzymatic activity assays (Table 1). Non-reducing end, GAG methods require even more sensitive machines that those listed in Table 1 (as noted above).

False positive rates for first-tier GAG analysis using the Internal Disaccharide method are shown in Table 1. Details of the estimates are given in Supplemental Material S4. For GAG analysis, the false positive rates are sufficiently high such that reasonably accurate estimates can be made from published analysis of only ~200 newborn DBS [7][11][12]. False positive rates for first-tier enzymatic activity assays (Table 1) come from a study of ~100,000 random newborn DBS [15]. Results of this study are similar to those from two other laboratories [3][16]. The total false positive rate for all MPSs using the GAG method is 13.5%, 192-fold higher than the total false positive rate for the enzymatic activity assay (0.07%) (Table 1). The GAG method gives a very high false positive rate of 7% for MPS-IVA (Table 1), and this value is similar to the rate reported by Tomatsu and colleagues using plasma from non-newborn MPS-IVA patients [17]. While keratan sulfatide-derived GAG biomarkers are useful for diagnosis of MPS-IVA using urine, they are not useful fr

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DBS due to massive overlap to the reference range [12]. Values for MPS-I, -II, and MPS-III are similar between our studies [11][7][12] and those reported in a pilot study by Tomatsu and colleagues [14].

The Internal Disaccharide GAG and enzymatic activity assays use completely different LC columns. The former uses a HyperCarb porous graphitic carbon column, which functions as a hybrid reverse-phase/normal-phase matrix, and the latter uses a reverse-phase column. It may be possible to add a hydrophobic derivative to the GAG biomarkers so that they could be analyzed with a reverse column and thus possibly in the same multiplex LC-MS/MS run as the enzymatic activity assay. However, the GAG biomarkers must be analyzed in negative ion mode whereas most of the analytes in the enzymatic activity assay require positive ion mode. Because of the large number of analytes, it is highly unlikely that on-the-fly polarity switching of the electrospray ionization source would be feasible. Thus, it seems certain that first-tier GAG analysis would require a separate set of LC-MS/MS instruments from those used for the enzymatic activity panel (Table 1).

The Endogenous NRE GAG method works well using DBS for NBS of GM1-gangliosidosis, but the Internal Disaccharide GAG method does not work with DBS [12]. This is because the Internal Disaccharide markers are derived from keratan sulfate, and the overlap with the reference range is massive (as noted above for MPS-IVA). Thus, any NBS program that includes GM1-gangliosidosis will have to include first-tier measurement of betagalactosidase activity as part of the multiplex enzymatic activity LC-MS/MS run. As noted above, first-tier analysis using the Endogenous NRE GAG method is not feasible.

5. Concluding remarks.

NBS for the full set of MPSs is feasible. Furthermore, multiplexing allows for NBS of all MPSs with essentially no additional demands on time per analyses and staffing requirements beyond what is already used for NBS of several other non-MPS diseases. This is only feasible with the use of the LC-MS/MS. Fluorescence enzymatic activity assays provide a platform for NBS of a subset of these diseases but cannot be used for GAG analysis.

One can consider first-tier NBS for MPSs and second-tier DNA sequencing of the relevant gene instead of GAG analysis. The recent study from the California NBS laboratory for MPS-I shows that DNA sequencing is much less useful than GAG analysis for reducing false positives arising from first-tier assay of enzymatic activity [18].

After careful consideration of all the factors associated with first-tier enzymatic activity assay followed by second-tier GAG analysis versus the vice-versa approach (Table 1) we conclude that both methods provide good solutions to NBS for the full set of MPSs with essentially complete elimination of false positives. However, based on the integration of all factors, the most logical method is first-tier enzymatic assay followed by second-tier GAG analysis. The main driver of this conclusion is that first-tier GAG analysis would require a second set of LC-MS/MS instruments on top of the LC-MS/MS instruments being used for multiplex analysis of other diseases including several non-MPS lysosomal storage diseases now part of NBS panels worldwide. Doubling of the number of LC-MS/MS instruments and

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associated staff would essentially negate the savings in costs afforded by avoiding relatively expensive enzyme substrates (Table 1). On the other hand, with first-tier enzymatic activity assay, only a single additional LC-MS/MS workstation would be needed for second tier GAG analysis on the relatively small number of samples where a below-cutoff enzymatic activity is observed.

The power of LC-MS/MS for multiplexing is obvious in our report of an 18-plex assay that includes enzymatic activity assay for several diseases as well as lipid biomarker assay of X-linked adrenoleukodystrophy and metachromatic leukodystrophy [13]. This method is fast enough for population NBS with a sample inject-to-inject time of 2.1 min. This is the method that is currently being used in the New York NBS laboratory as part of the ScreenPlus program for prospective piloting of 14 diseases that represent an expansion of NBS [19].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References:

[1]. Burton BK, Barbar, MPS-II NBS in IL, the first \textasciitilde1 yr, WORLD. (n.d.).

[2]. Bilyeu H, Washburn J, Vermette L, Klug T, Validation and Implementation of a Highly Sensitive and Efficient Newborn Screening Assay for Mucopolysaccharidosis Type II, Int. J. Neonatal Screen 6 (2020) 79. 10.3390/ijns6040079. [PubMed: 33124617]

[3]. Chien Y-H, Lee N-C, Chen P-W, Yeh H-Y, Gelb MH, Chiu P-C, Chu S-Y, Lee C-H, Lee A-R, Hwu W-L, Newborn screening for Morquio disease and other lysosomal storage diseases: results from the 8-plex assay for 70,000 newborns, Orphanet J. Rare Dis 15 (2020) 38. 10.1186/s13023-020-1322-z. [PubMed: 32014045]

[4]. Khaledi H, Gelb MH, Tandem Mass Spectrometry Enzyme Assays for Multiplex Detection of 10-Mucopolysaccharidoses in Dried Blood Spots and Fibroblasts., Anal. Chem 92 (2020) 11721–11727. [PubMed: 32786498]

[5]. Verheyen S, Blatterer J, Speicher MR, Bhavani GS, Boons G-J, Ilse M-B, Andrae D, Sproß J, Vaz FM, Kircher SG, Posch-Pertl L, Baumgartner D, Lübke T, Shah H, Al Kaissi A, Girisha KM, Plecko B, Novel subtype of mucopolysaccharidosis caused by arylsulfatase K (ARSK) deficiency, J. Med. Genet 59 (2022) 957–964. 10.1136/jmedgenet-2021-108061. [PubMed: 34916232]

[6]. Kubaski F, Mason RW, Nakatomi A, Shintaku H, Xie L, van Vlies NN, Church H, Giugliani R, Kobayashi H, Yamaguchi S, Suzuki Y, Orii T, Fukao T, Montaño AM, Tomatsu S, Newborn screening for mucopolysaccharidoses: a pilot study of measurement of glycosaminoglycans by tandem mass spectrometry., J. Inherit. Metab. Dis 40 (2017) 151–158. [PubMed: 27718145]

[7]. Herbst ZM, Urdaneta L, Klein T, Fuller M, Gelb MH, Evaluation of Multiple Methods for Quantification of Glycosaminoglycan Biomarkers in Newborn Dried Blood Spots from Patients with Severe and Attenuated Mucopolysaccharidosis-I, Int. J. Neonatal Screen 6 (2020) 69. 10.3390/ijns6030069. [PubMed: 33123640]

[8]. Auray-Blais C, Lavoie P, Zhang H, Gagnon R, Clarke JTR, Maranda B, Young SP, An Y, Millington DS, An improved method for glycosaminoglycan analysis by LC-MS/MS of urine samples collected on filter paper., Clin. Chim. Acta Int. J. Clin. Chem 413 (2012) 771–778.

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[9]. Lawrence R, Brown JR, Al-Mafraji K, Lamanna WC, Beitel JR, Boons G-J, Esko JD, Crawford BE, Disease-specific non–reducing end carbohydrate biomarkers for mucopolysaccharidoses, Nat. Chem. Biol 8 (2012) 197–204. [PubMed: 22231271]

[10]. Saville JT, McDermott BK, Fletcher JM, Fuller M, Disease and subtype specific signatures enable precise diagnosis of the mucopolysaccharidoses., Genet. Med. Off. J. Am. Coll. Med. Genet 21 (2019) 753–757.

[11]. Herbst ZM, Urdaneta L, Klein T, Burton BK, Basheeruddin K, Liao H-C, Fuller M, Gelb MH, Evaluation of Two Methods for Quantification of Glycosaminoglycan Biomarkers in Newborn Dried Blood Spots from Patients with Severe and Attenuated Mucopolysaccharidosis Type II, Int. J. Neonatal Screen 8 (2022) 9. 10.3390/ijns8010009. [PubMed: 35225932]

[12]. Herbst ZM, Hong X, Urdaneta L, Klein T, Waggoner C, Liao H-C, Kubaski F, Giugliani R, Fuller M, Gelb MH, Endogenous, non-reducing end glycosaminoglycan biomarkers are superior to internal disaccharide glycosaminoglycan biomarkers for newborn screening of mucopolysaccharidoses and GM1 gangliosidosis, Mol. Genet. Metab (2023) 107632. 10.1016/j.ymgme.2023.107632. [PubMed: 37407323]

[13]. Hong X, Sadilek M, Gelb MH, A highly multiplexed biochemical assay for analytes in dried blood spots: application to newborn screening and diagnosis of lysosomal storage disorders and other inborn errors of metabolism., Genet. Med. Off. J. Am. Coll. Med. Genet 22 (2020) 1262–1268.

[14]. Kubaski F, Osago H, Mason RW, Yamaguchi S, Kobayashi H, Tsuchiya M, Orii T, Tomatsu S, Glycosaminoglycans detection methods: Applications of mass spectrometry., Mol. Genet. Metab 120 (2017) 67–77. [PubMed: 27746032]

[15]. Scott CR, Elliott S, Hong X, Huang J-Y, Kumar AB, Yi F, Pendem N, Chennamaneni NK, Gelb MH, Newborn Screening for Mucopolysaccharidoses: Results of a Pilot Study with 100 000 Dried Blood Spots, J. Pediatr 216 (2020) 204–207. 10.1016/j.jpeds.2019.09.036. [PubMed: 31732130]

[16]. Burton BK, Hoganson GE, Fleischer J, Grange DK, Braddock SR, Hickey R, Hitchins L, Groepper D, Christensen KM, Kirby A, Moody C, Shryock H, Ashbaugh L, Shao R, Basheeruddin K, Population-Based Newborn Screening for Mucopolysaccharidosis Type II in Illinois: The First Year Experience, J. Pediatr 214 (2019) 165–167.e1. 10.1016/j.jpeds.2019.07.053. [PubMed: 31477379]

[17]. Tomatsu S, Okamura K, Maeda H, Taketani T, Castrillon SV, Gutierrez MA, Nishioka T, Fachel AA, Orii KO, Grubb JH, Cooper A, Thornley M, Wraith E, Barrera LA, Laybauer LS, Giugliani R, Schwartz IV, Frenking GS, Beck M, Kircher SG, Paschke E, Yamaguchi S, Ullrich K, Haskins M, Isogai K, Suzuki Y, Orii T, Kondo N, Creer M, Okuyama T, Tanaka A, Noguchi A, Keratan sulphate levels in mucopolysaccharidoses and mucolipidoses, J. Inherit. Metab. Dis 28 (2005) 187–202. 10.1007/s10545-005-5673-3. [PubMed: 15877208]

[18]. Fillman T, Matteson J, Tang H, Mathur D, Zahedi R, Sen I, Bishop T, Neogi P, Feuchtbaum L, Olney RS, Sciortino S, First Three Years' Experience of Mucopolysaccharidosis Type-I Newborn Screening in California, J. Pediatr 263 (2023) 113644. 10.1016/j.jpeds.2023.113644. [PubMed: 37516270]

[19]. Wasserstein MP, Orsini JJ, Goldenberg A, Caggana M, Levy PA, Breilyn M, Gelb MH, The future of newborn screening for lysosomal disorders, Neurosci. Lett 760 (2021) 136080. 10.1016/j.neulet.2021.136080. [PubMed: 34166724]

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Table 1.

NBS for MPSs by first-tier enzymatic activity versus GAG biomarker analysis.

MPS First-Tier NBS Method	Number of 3 mm DBS punches per newborn	Reagent Costs per Newborn[2]	Analysis Time[2]	LC-MS/MS instrument models required[2]	False positive rate after first-tier NBS[2]	Additional comments
Internal Disaccharide GAG LC-MS/MS Analysis	2	$0.46	Results are ready in the morning of day 3 at a throughput of 429 newborns per LC-MS/MS instrument	PerkinElmer QSight 400 series, Waters TQ-S, Sciex 5500	MPS-I and -II (1.6%) MPS-IIIA-D (4.9%) MPS-IVA (7%) MPS-VI and -VII (0%) TOTAL 13.5%	GAG analysis requires a dedicated LC-MS/MS station with chromatography appropriate for gAg fragments.
Multiplex LC-MS/MS Enzymatic Activity	2	$6.00 (including GM1-gangliosidosis)	Similar for first-tier GAG NBS	Waters TQD or Xevo TQ	MPS-I (0.004%) MPS-II (0.017%) MPS-IIIA (0.017%) MPS-IIIB (0%) MPS-IIIC (0.017%) MPS-IVA (0.0076%) MPS-VI (0.0038%) MPS-VII (0%) TOTAL 0.07%	Multiplexable with many other non-MPS diseases.

[1] GAG first-tier analysis also finds multiple sulfatase deficiency but not GM1- gangliosidosis, but enzymatic activity first-tier analysis finds both.

[2] Full details of the estimation is provided as Supplemental Material.