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Digital microfluidics (DMF), a new technology for newborn screening of Lysosomal Storage Disease: the first experience in Tuscany

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INTRODUCTION

Lysosomal storage disorders (LSDs) are a group of more than 70 different diseases caused by genetic defects in genes encoding proteins involved in the lysosomal degradation. Newborn screening for Lysosomal Storage Disorders (LSD) has spread worldwide thanks to the advances in diagnostic technologies. Digital microfluidics (DMF) is an alternative method to mass spectrometry (MS/MS) for LSD screening. In the routine newborn screening program of LSD performed in Tuscany with FIA-MS/MS about 3464 DBS samples were assayed for GAA, GLA and IDUA enzyme activities using both methods. The results were compared in order to evaluate the performances of the two methods.

METHODS

The DMF technique is based on electrowetting effect able to transport, merge, split, mix and dispense nanodroplet samples using surface energy gradients by activating a pattern of control electrodes. In DMF devices an electric field allows submicroliter droplets to move independently on an electrode-plate chip through voltage variations under software control. The droplets are sandwiched between two parallel plates; the top plate contains the droplets whereas the bottom one an array of electrodes (Millington at al, 2010). The space between the plates is filled with silicone oil to prevent evaporation of the samples during incubation process. Data acquisition occurs by fluorometer detection system (Fig.1).

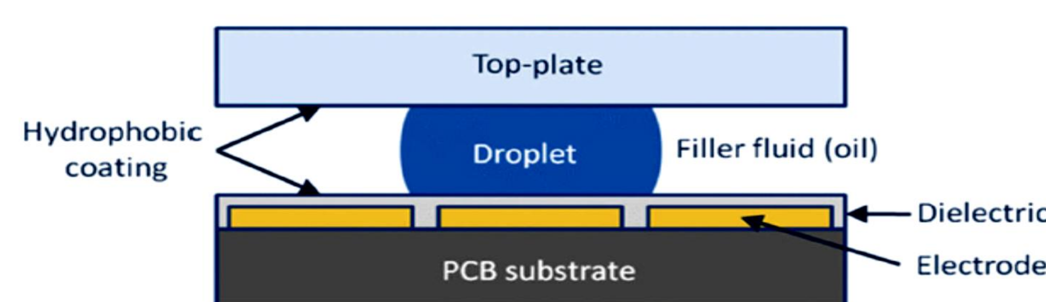

Fig. 1 Principles of electowetting-based digital microfluidics (Millington DS et al, 2018)

RESULTS

During newborn screening program of LSD performed in Tuscany with FIA-MS/MS, 3464 newborns were also analysed for IDUA, GAA, and GLA enzyme activity using digital microfluidics on Baebies' SEEKER platform.

The distribution of the data obtained by DMF assay was established for each newborn; both birth weight and gestational age were considered. The enzyme activity for each lysosomal enzyme was approximately lognormal when considering only full term births (Fig.2).

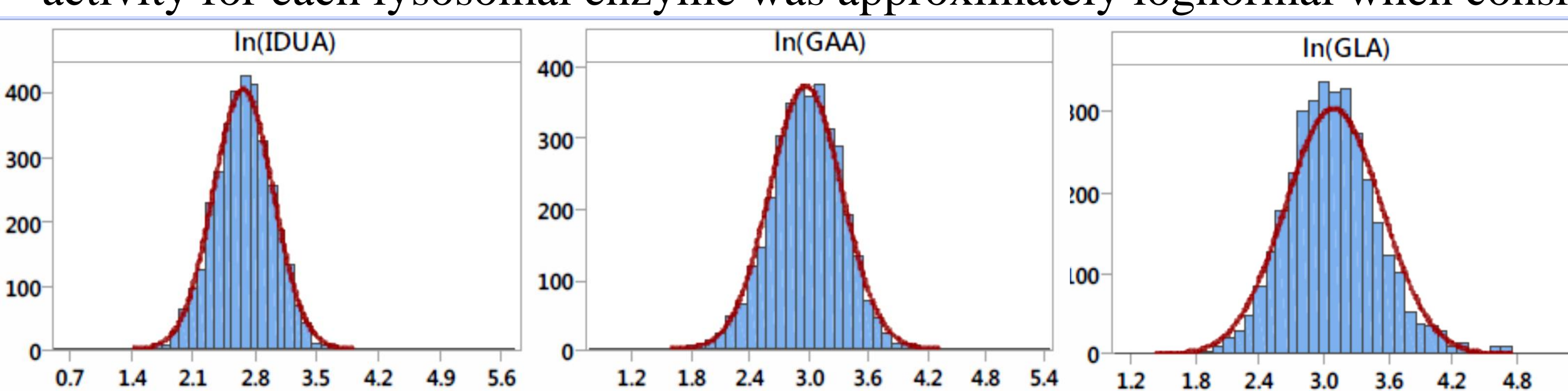

Fig. 2 Activity distributions of enzymes activity. GLA activity had a slight skew to the normal fit comparing the other enzymes, most likely due to elevated activity in low birth weight newborns.

Sample	Enzyme	NBS by FIA-MS/MS	NBS by DMF	Retested sample by FIA-MS/MS	Molecular analysis results
1	IDUA	● (red)	● (green)	● (green)	not performed
2	IDUA	● (red)	● (green)	● (green)	not performed
3	IDUA	● (red)	● (green)	● (green)	not performed
4	IDUA	● (red)	● (green)	● (green)	not performed
5	IDUA	● (red)	● (green)	● (green)	not performed
6	IDUA	● (red)	● (red)	● (green)	not performed
7	IDUA	● (red)	● (red)	● (red)	Pseudodeficiency variants
8	IDUA	● (red)	● (red)	● (red)	Compound heterozygote for a pseudodeficit variant and VUS*
9	IDUA	● (red)	● (red)	● (red)	Compound heterozygote for a pathogenic variant and VUS*
10	IDUA	● (red)	● (red)	● (red)	Compound heterozygote for a pathogenic variant and VUS*
11	GLA	● (red)	● (green)	● (green)	not performed
12	GLA	● (red)	● (green)	● (green)	not performed
13	GLA	● (red)	● (green)	● (green)	not performed
14	GLA	● (red)	● (green)	● (green)	not performed
15	GLA	● (red)	● (green)	● (green)	not performed
16	GLA	● (red)	● (green)	● (green)	not performed
17	GLA	● (red)	● (red)	● (red)	Late onset mutation
18	GLA	● (red)	● (green)	● (red)	Neutral variant with high residual enzymatic activity and normal level of plasma LysoGb3 (Burlina et al, 2017)
19	GLA	● (red)	● (green)	● (red)	Wilde type female

*VUS: variant of unknown significance

● (green) **Normal enzymatic activity, higher than preliminary cut-off value calculated on 0.1 percentile of tested samples.**

● (red) **Deficient enzymatic activity less than preliminary cut-off value calculated on 0.1 percentile of tested samples.**

Tab.1 Comparative analysis results

After categorizing, the mean activity for both IDUA and GAA was increased in very low birth weight samples (rounded down to nearest 500g). The elevation is much more significant for GLA enzyme, when the enzymatic activity can increased until 400% of full term for very low birth weight newborns.

A comparison between the two methods occurred after DMF data were elaborated to define a preliminary threshold for each enzyme and after the screening process by reference method has been completely completed. According to the algorithm used for the LSD' NBS program in Tuscany, newborns presenting with an enzymatic activity below the cut-off using FIA-MS/MS method, are recalled for a second dried blood spot. During the comparison period, the number of newborns requiring a repeat specimen by routinely MS/MS based method, was nine for low GLA activity, ten for low IDUA activity and zero for GAA. Twelve (all these samples resulted with normal enzymatic activity at DMF at first tier test) out of nineteen resulted normal at the second analysis by MS while seven confirmed with a low enzymatic activity required to be submitted to molecular analysis (Tab1).

Four additional dried blood spots resulted abnormal (low enzymatic activity: two for IDUA and two for GLA) at only DMF and not at FIA-MS/MS tests but no further investigation was performed.

DISCUSSION

One of the most important feature of DMF method is the overall shorter time to obtain screening results and an easier analytical process than mass spectrometry. Moreover we have conducted a short evaluation to catch some preliminary results on performances in term of sensitivity and specificity.

Based on our preliminary results, the DMF technology showed an optimal specificity at the last comparable to the FIA-MS/MS method. These results should be reproduced and extended to a bigger number of tests, however the theoretical specificity looks like higher than MS. The low number of false positives seems to be acceptable for both technologies.

We were not able to measure the sensitivity of the seeker compared to MS because of the short comparison study design. The only real Fabry patient with late onset mutations presented with low enzymatic activity to both the technologies. Moreover we have tested 27 neonatal and not neonatal DBS samples from 10 MPSI, 11 Pompe and 6 Fabry patients (the DBS were stored at -20°C until the analysis) from our database and for all of them the enzymatic activity measured by DMF resulted lower than cut-off, confirming a theoretical optimal sensitivity.

CONCLUSION

Based on our preliminary results it looks like that the DMF method is suitable to be used for routinely screening of GLA, IDUA and GAA activity. The overall performances are at the least comparable to the FIA-MS/MS method even though they should be confirmed on more extensive and prospective study.