EXTERNAL QUALITY ASSESMENT FOR ENZYMATIC ANALYSIS OF LYSOSOMAL DISORDERS. Comparison of enzymatic performance in Fibroblasts and Dry Blood Spots

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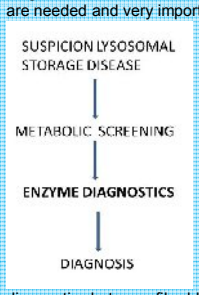
BACKGROUND:

Enzyme diagnostics is acknowledged as a key component in the diagnostics of LSD's. External quality assurance (EQA) schemes are needed and very important for improvement of the reliability of diagnostics. Since 2010 ERNDIM offers an EQA scheme for lysosomal enzymes in EBV cells. In 2012 the EQA scheme started with fibroblasts, the gold standard in enzyme diagnostics. During the last decade enzyme diagnostics in dry blood spots (DBS) was developed and used in several laboratories. To obtain a good picture of the performance of the enzyme diagnostics we compared the reliability of the enzyme diagnostics between fibroblasts and DBS for all participating laboratories and within the same Laboratories(DBS labs) all over the world.



OBJECTIVES

- **Robust EQA Scheme lysosomal enzymes**
- **Reliability enzyme diagnostics fibroblasts?**
- **Reliability enzyme diagnostics DBS?**

LYSOSOMAL ENZYMES IN EQA SCHEME FIBROBLASTS AND DBS

DISEASE	ENZYME	FIBRO'S	DBS
HURLER	α-L-iduronidase(AIDU)	Patient(P)	P
HUNTER	Iduronate sulphatase(IDU2S)	Control(C)	P
MPS VI	Arylsulphatase B(ASB)	Patient	P
GAUCHER	β-Glucosidase(BGLU)	Patient	P
POMPE	α-Glucosidase(AGLU)	Control	P
GM1	β-Galactosidase(BGAL)	Control	C
GM2	β-Hexosaminidase(BHEXT)	Control	
FABRY	α-Galactosidase(AGAL)	Patient	P
KRABBE	Galactocerebrosidase(GALC)	Control	
NIEMANN-PICK	Sphingomyelinase(SM)	Control	

RESULTS:

- **Lysosomal Enzymes are stable for at least 3 weeks(Figure 1).**
- **Satisfactory intra-assay repeatability in fibroblasts compared to the EBV cells used in 2010-2011(Figure 2)**
- **Interlaboratory reproducibility fibroblasts about 50%(Figure 3)**
- **Intra-assay repeatability DBS compared to fibroblasts is low (50% vs 15%)(Fig 4)**
- **Poor interlaboratory reproducibility in DBS(Figure 5)**
- **0 - 15 % of the participants measured an enzyme activity not corresponding with a patient value in fibroblasts (Figure 6)**
- **5-40 % of the participants measured an enzyme activity not corresponding to a patient value in DBS(Figure 6)**

CONCLUSION:

- **EQA Scheme for lysosomal enzymes in fibroblasts is robust**
- **Enzyme diagnostics in fibroblasts is reliable**
- **Enzyme diagnostics in DBS is much less reliable, especially for Gaucher, Hunter and MPS VI**

ERNDIM (European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism) www.erndim.org
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STABILITY LYSOSOMAL ENZYMES FOR TRANSPORT FIBROBLASTS

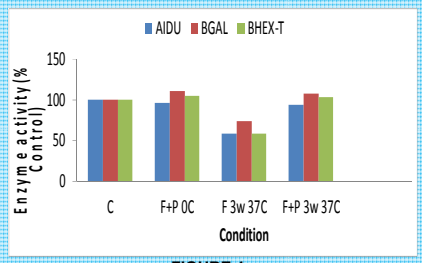

FIGURE 1

(C) Fibroblast homogenates
(F+P) Fibroblast homogenates freese dried with cryoprotectant
(F 3w 37C) Fibroblast homogenates freese dried, kept for 3 weeks 37°C
(F+P 3w 37C) Fibroblast homogenates freese dried with cryoprotectant , kept for 3 weeks 37°C

INTERLABORATORY REPRODUCIBILITY ABSOLUTE VS RELATIVE ACTIVITY

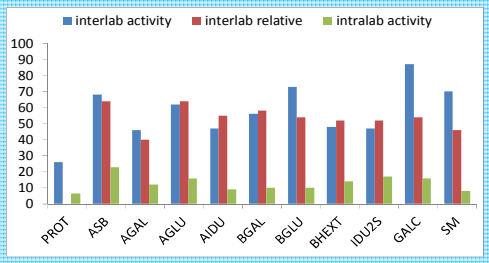

FIGURE 3

Interlaboratory activity represents the standard deviation as % of the mean enzyme activity found in fibroblasts by all participants.
Interlab relative represents the standard deviation as % of the mean relative enzyme activities found by all participants compared to their own control fibroblasts .
Intralaboratory activity represents the difference in the activity measured in duplicate samples as % of the mean activity of the duplicate sample

INTERLABORATORY REPRODUCIBILITY FIB vs DBS

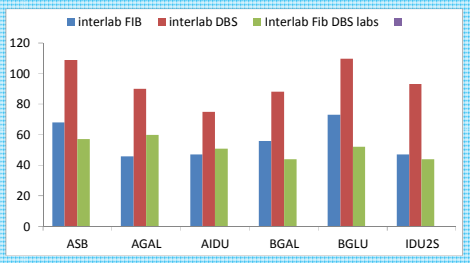

FIGURE 5

Interlab Fib represents the interlaboratory reproducibility as % of the mean activity measured in fibroblasts by all participants
Interlab DBS represents the interlaboratory reproducibility as % of the mean activity measured in DBS by all DBS participants
Interlab Fib DBS labs represents the interlaboratory reproducibility as % of the mean activity measured in fibroblasts by all DBS participants

INTRALABORATORY REPEATABILITY FIBROBLASTS vs EBV cells

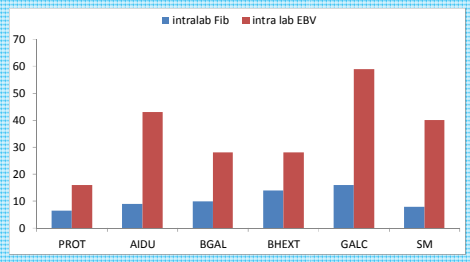

FIGURE 2

INTRALABORATORY REPEATABILITY FIB vs DBS

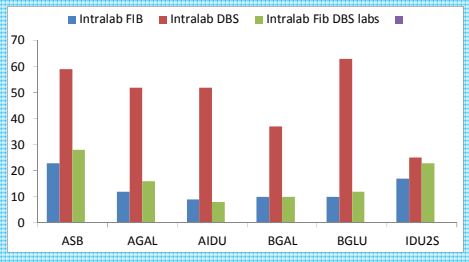

FIGURE 4

Intralab Fib represents the intralaboratory repeatability measured as the difference in the activity in duplicate samples as % of the mean activity of the duplicate sample measured in fibroblasts for all participants for the indicated enzymes.
Intralab DBS represents the intralaboratory repeatability in DBS for all DBS participants.
Intralab Fib DBS labs represents the intralaboratory repeatability in fibroblasts measured by all DBS participants

FAILURE OF ENZYME DIAGNOSTICS FIB vs DBS

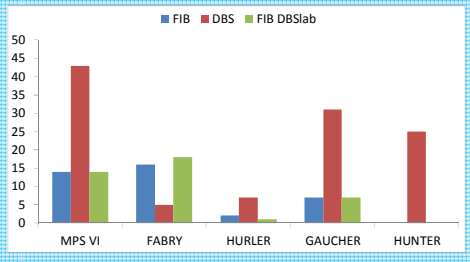

FIGURE 6

FIB, DBS, FIB DBSlab represents the % of participants measuring an enzyme activity in patient samples not different from control samples or an enzyme activity in control samples resembling a patient sample in respectively fibroblasts, DBS or fibroblasts by DBS lab for the indicated diseases/enzymes

METHODS:

Freeze dried samples of fibroblast homogenates from patients or controls were shipped to about 64 participants in about 28 countries all over the world. Dry blood spots (DBS) on Whatman filter paper were sent to about 20 participants.
Enzyme activities of 6-10 lysosomal enzymes were measured in fibroblasts and DBS from patients and controls. Intralaboratory repeatability was determined by the difference in the activity measured in duplicate samples divided by the mean activity*100%.

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