LABORATORY DIAGNOSIS
OF INBORN ERRORS OF METABOLISM IN CROATIA

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15th International Conference on Rare Diseases
„Rare Diseases - Open Your Heart And Mind”

CROATIA - BASIC INFORMATION

Area: 56,594 km^2
Population: 4,437,460
Births per year: 39 000

- **Rare disease patients ?**
- **Inborn errors of metabolism: ?**

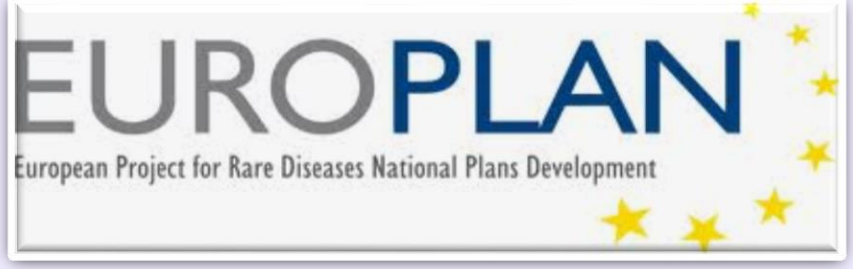
- **NBS laboratory: 1**
- **Metabolic laboratory:1**

INBORN ERRORS OF METABOLISM ?

- **Registry is essential to show the real situation regarding rare diseases**
- **It provides a source of real-time information to develop guidelines for monitoring and to support patient management**
- **It assists the health care strategists in the country to devise a long-term strategic plan to ensure resources for diagnostics and treatment**

Without the International Classification of Diseases (ICD-11) and national register –

the diseases are not visible !!!



The Croatian national plan for rare diseases has been developed around of the following nine priority areas:

1. Promotion of the knowledge and availability of information on rare diseases;
2. Support of rare disease registries and securing their sustainability;
3. Establishment of referral centres and centres of expertise activities;
4. Improvement of the availability and quality of health services for rare disease patients (prevention, diagnosis, treatment);
5. Improvement of access to treatment with orphan medicinal products;
6. Securing the availability of special social services for rare diseases patients;
7. Empowerment of patient's organisations;
8. Encouraging research activities in the field of rare diseases;
9. International networking in the field of rare diseases.

THE CROATIAN ALLIANCE FOR RARE DISEASES

Croatian Alliance for Rare Diseases

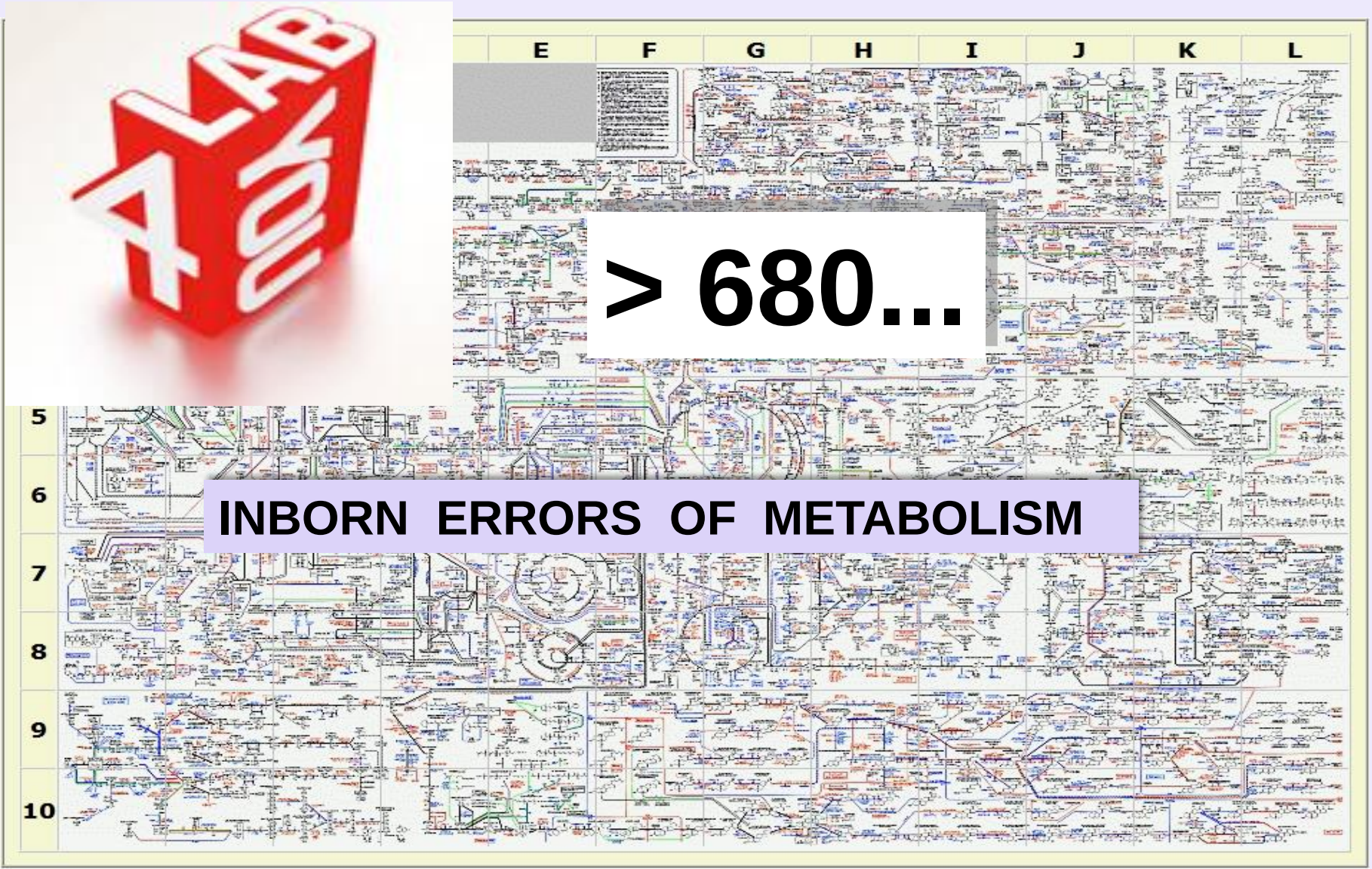
- The umbrella organisation for rare diseases that gathers **20 non-profit organisations**
- At present there are more than **350 different rare diseases** registered in the association.
- The Society are taking care for more than 1500 patients

Diagnosis of Rare Diseases

Patients living with rare diseases **visit an average of 7.3 physicians** before receiving an accurate diagnosis, according to a recent survey of patients, family members, physicians and health care professionals.

The Journal of Rare Disorders; March 6, 2014

RARE DISORDERS
6,000 - 8,000



> 680...

INBORN ERRORS OF METABOLISM

ORGANIZATION OF LABORATORY DIAGNOSTICS OF INBORN ERRORS OF METABOLISM IN CROATIA

NATIONAL SCREENING PROGRAM

SELECTIVE SCREENING

Department of Laboratory Diagnostics, University Hospital Center Zagreb

Department of Laboratory Diagnostics, University Hospital Center Zagreb

Laboratory is accredited by medical laboratories ISRN EN ISO 15189

15189 - HAA 7466

Hrvatska akreditacijska agencija (HAA)/Croatian Accreditation Agency is an independent and non for profit public institution that acts as the national accreditation service in the Republic of Croatia

NATIONAL SCREENING PROGRAM

- Neonatal screening is centralised in Croatia and is an obligatory part of health care.

- Neonatal screening is provided for

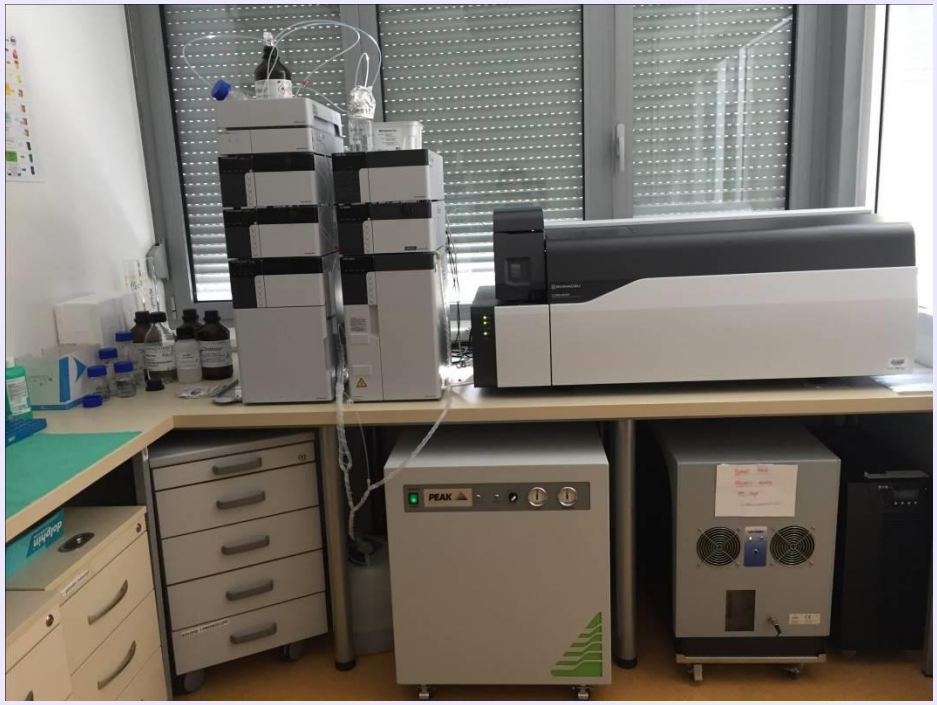
phenylketonuria and **hypothyroidism**

EXPANDED NBS - CROATIA

Republika Hrvatska
Ministarstvo zdravlja

Isovaleric acidemia
Glutaric acidemia type I
Carnitine uptake defects

MCAD deficiency
VLCAD deficiency
LCHAD deficiency



NEWBORN SCREENING

32 Birth clinics

Screening laboratory

Primary contact physician

Metabolic specialist

Metabolic center

Dietitians...

Parents

Scientific evaluation

Effective NBS requires a close working relationship between hospitals, newborn screening program, and follow-up program

DURHAM, N.C., February 3, 2017 – Baebies, Inc., announced today it has received *de novo* clearance from the U.S. Food and Drug Administration (FDA) for SEEKER

SEEKER is the first and only platform in newborn screening for lysosomal storage disorders authorized by the FDA!

SEEKER is a high throughput laboratory solution that quantitatively measures the activity of lysosomal enzymes from newborn dried blood spot specimens. Reduced activity of these enzymes may be indicative of Mucopolysaccharidosis Type I (MPS I), Pompe, Gaucher or Fabry disease.

Piero Rinaldo, MAYO CLINIC:

Pompe Disease- *March 2015* **… US implementation 35%**
MPS I-*February 2016***…….. US implementation 21%**
X-ALD- *February 2016***……. US implementation 62%**

More conditions are coming…

ACID LIPASE DEFICIENCY
AMINO ACIDS DISORDERS (9+)
DUCHENE MUSCULAR DISTROPHY
GAMT
FRAGILE X
HYPERBILIRUBINEMIA
INFECTIOUS DISEASES
G-6-PDH
METAL DISORDERS
NCL2
SPINAL MUSCULAR ATROPHY

LYSOSOMAL DISORDERS

Fabry
Gaucher
Krabbe
NPAB
Galactosialidosis
MLD
MPS II, III, IV, VI

TREATMENT OPTIONS FOR INBOR ERRORS OF METABOLISM

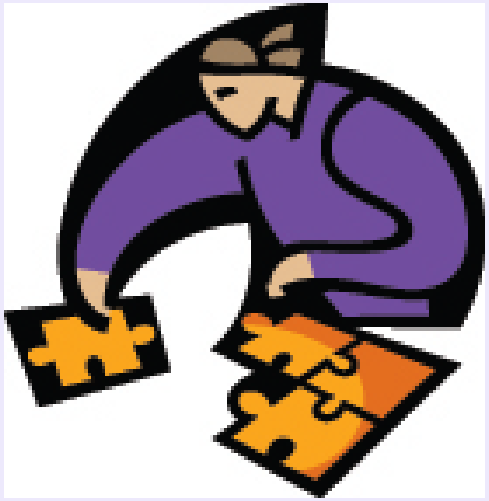
- Hematopoietic stem cell transplantation
- Enzyme replacement therapy
- Substrate reduction therapy
- Human cell and gene therapy

…

SUPORTIVE THERAPY

SPECIFIC THERAPY

One should be aware:



The efficacy of many current therapies relies on

early detection

and treatment

prior to the onset of irreversible pathology!!!

INBORN ERROR OF METABOLISM ?

> 680

SELECTIVE SCREENING APPROACH

CLINICAL SUSPICION

clinical observations should accompany the samples

SPECIALIST MANAGEMENT

LABORATORY DIAGNOSTICS

DIAGNOSTICS OF INHERITED METABOLIC DISORDERS - SELECTIVE SCREENING

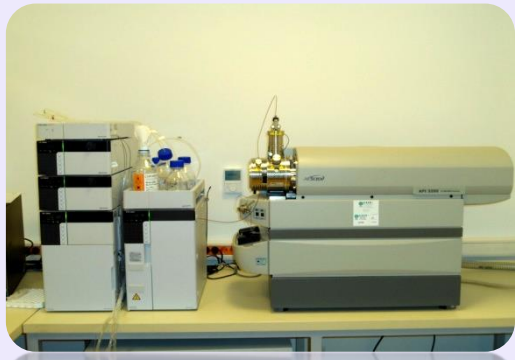
☑ Rational approach to diagnostics

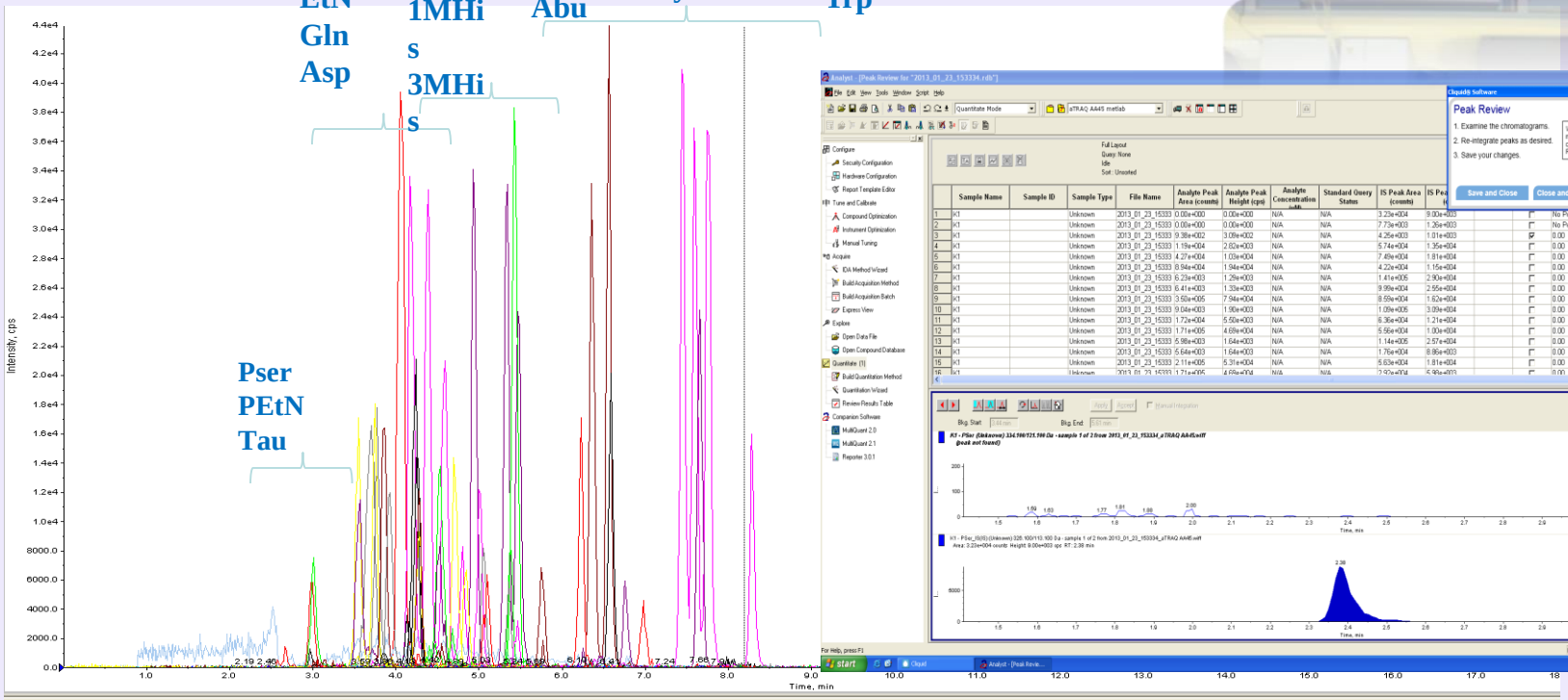
basic metabolic tests: glucose, ammonium, lactate, pyruvate, reducing substances...

special metabolic tests:
amino acids, organic acids, carnitine, acyl carnitine, galactose, oligosaccharides, glycosaminoglycans, CDG, VLCFA ...

☑ **confirmation tests:**
lysosomal storage disorders (enzyme activityes)

SPECIAL METABOLIC TESTS- AMINO ACIDS



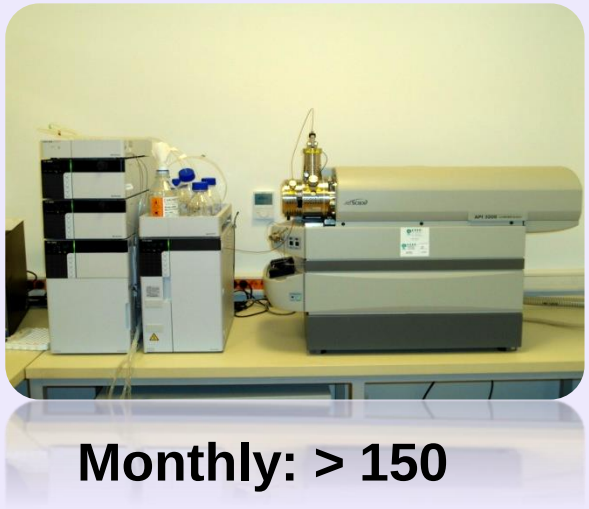


Monthly:

- **150 qualitative**
- **80 quantitative**

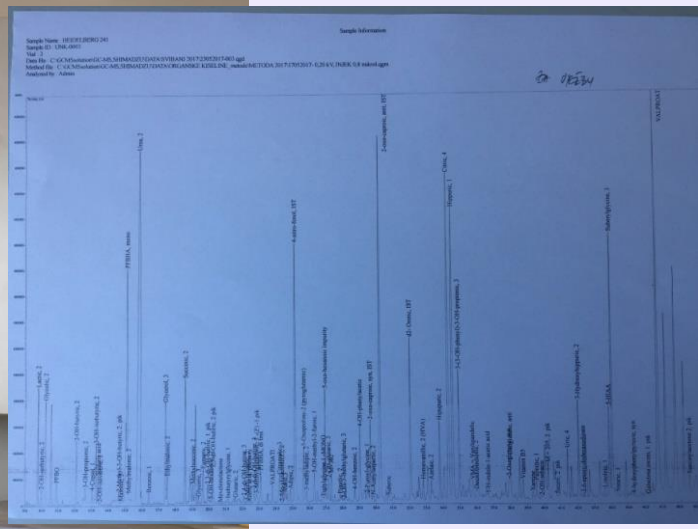
18 min

SPECIAL METABOLIC TESTS- Acyl carnitine profile



Monthly: > 150

SPECIAL METABOLIC TESTS- Organic acids



Monthly: > 100

DIAGNOSTICS OF INHERITED METABOLIC DISORDERS - SELECTIVE SCREENING

☑ Rational approach to diagnostics

basic metabolic tests: glucose, ammonium, lactate, pyruvate, reducing substances...

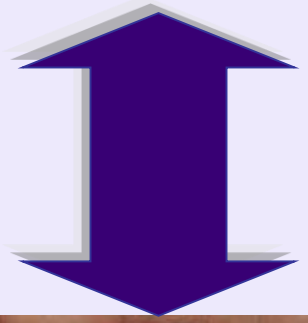
special metabolic tests:
amino acids, organic acids, carnitine, acyl carnitine, galactose, oligosaccharides, glycosaminoglycans, CDG, VLCFA ...

☑ **confirmation tests:**
lysosomal storage disorders (enzyme activityes)

http://www.kbc-zagreb.hr/klinicki-zavod-za-laboratorijsku-dijagnostikupopis-pretrag/

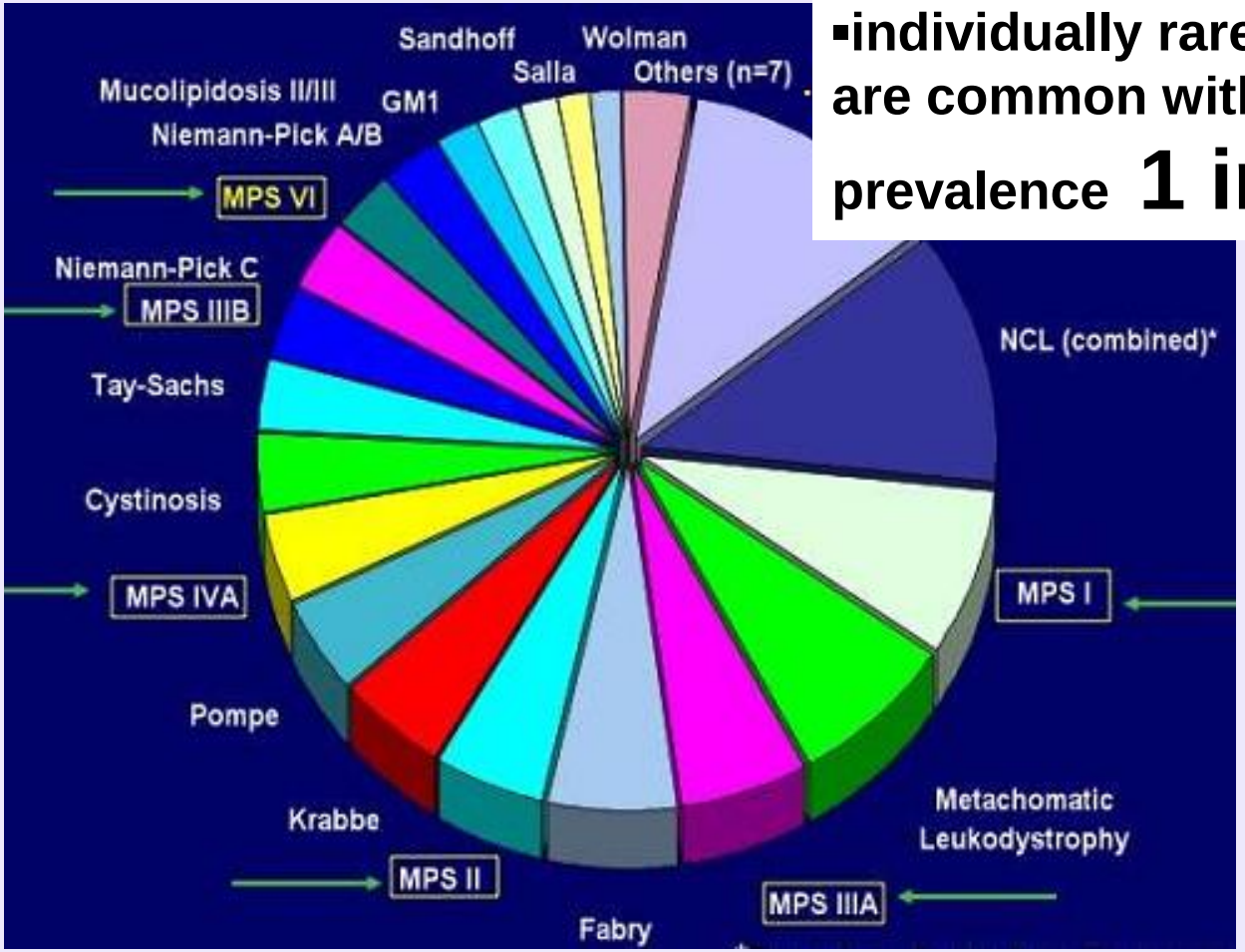
Genetic testing is available for the most common genetic conditions in laboratories of clinical hospitals or research institutes. Genetic testing is covered by the Croatian Institute for Health Insurance: when a certain test is not available in Croatia, a second medical opinion from 2-3 medical professionals is needed before a sample can be sent abroad.

However, there are still some problems with these types of cross-border services.



LYSOSOMAL STORAGE DISORDERS

►The need for better recognition...



- individually rare, but as a group they are common with overall prevalence **1 in 7500** live births!

?

(For Australia 1980/1996; Meikleet et al, 1999)

Identification of Infants at Risk for Developing Fabry, Pompe, or Mucopolysaccharidosis-I from Newborn Blood Spots by Tandem Mass Spectrometry

C. Ronald Scott, MD1, Susan Elliott, BS2, Norman Buroker, PhD1, Lauren I. Thomas, MS1, Joan Keutzer, PhD4, Michael Glass, MS2, Michael H. Gelb, PhD3, and Frantisek Turecek, PhD3

These estimates of prevalence are 2 to 4 times greater than the prevalence estimated by clinical diagnosis..

(*J Pediatr 2013)*

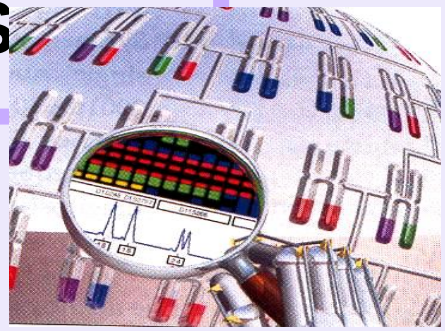
LSDs- LABORATORY APPROACH

"unspecific tests"

Preliminary screening tests

LYSOSOMAL ENZYME ACTIVITY

MOLECULAR ANALYSIS



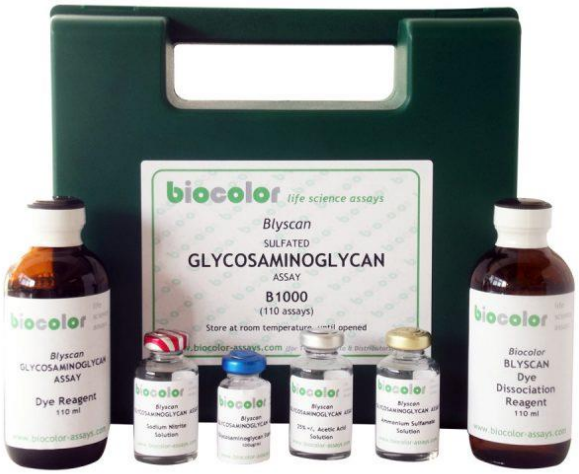
MPS- PRELIMINARY SCREENING TESTS

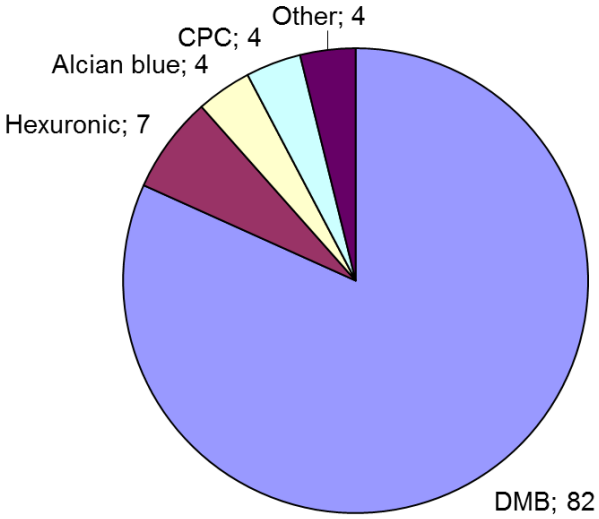
ERNDIM Urine MPS: an External Quality Assurance scheme for diagnostic testing of mucopolysaccharidoses in urin

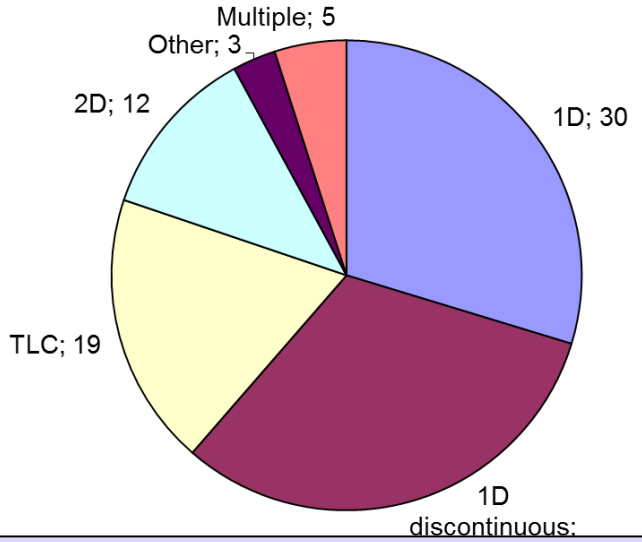
Quality of quantitative MPS screening

Proficiency of urine MPS screening

Methods used to analyse GAG



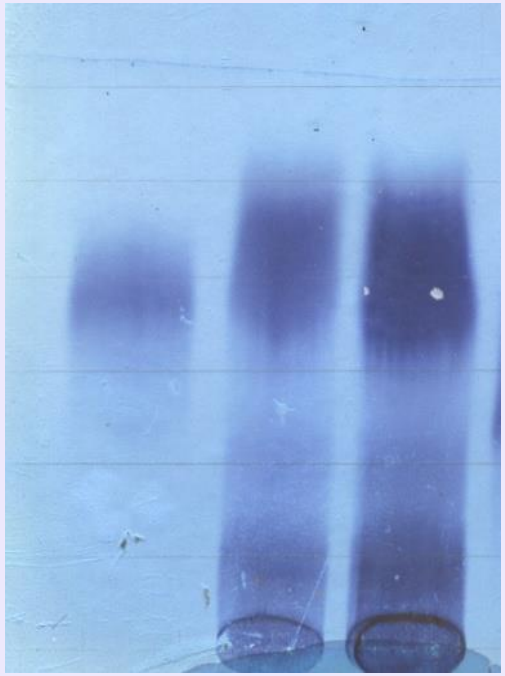




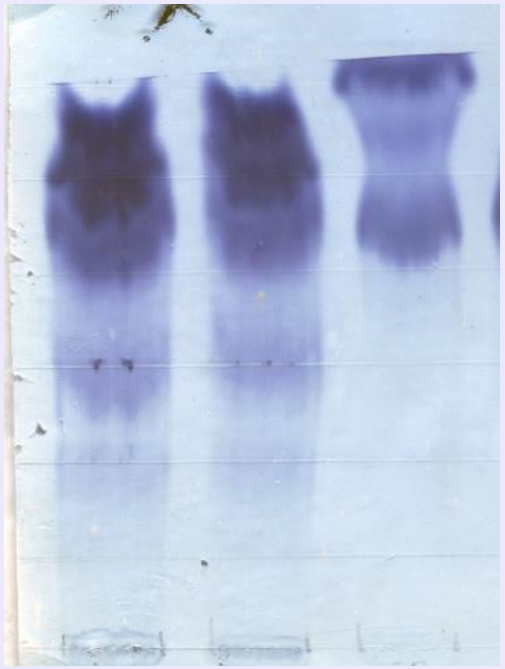
1,9-dimethylmethylene blue (*DMB*)-based colorimetric method

TLC - GLYCOSAMINOGLYCANS

Dermatan sulphate



5
4
3
2
1



Keratan sulphate

MPS type I, II, VI

MPS type IV

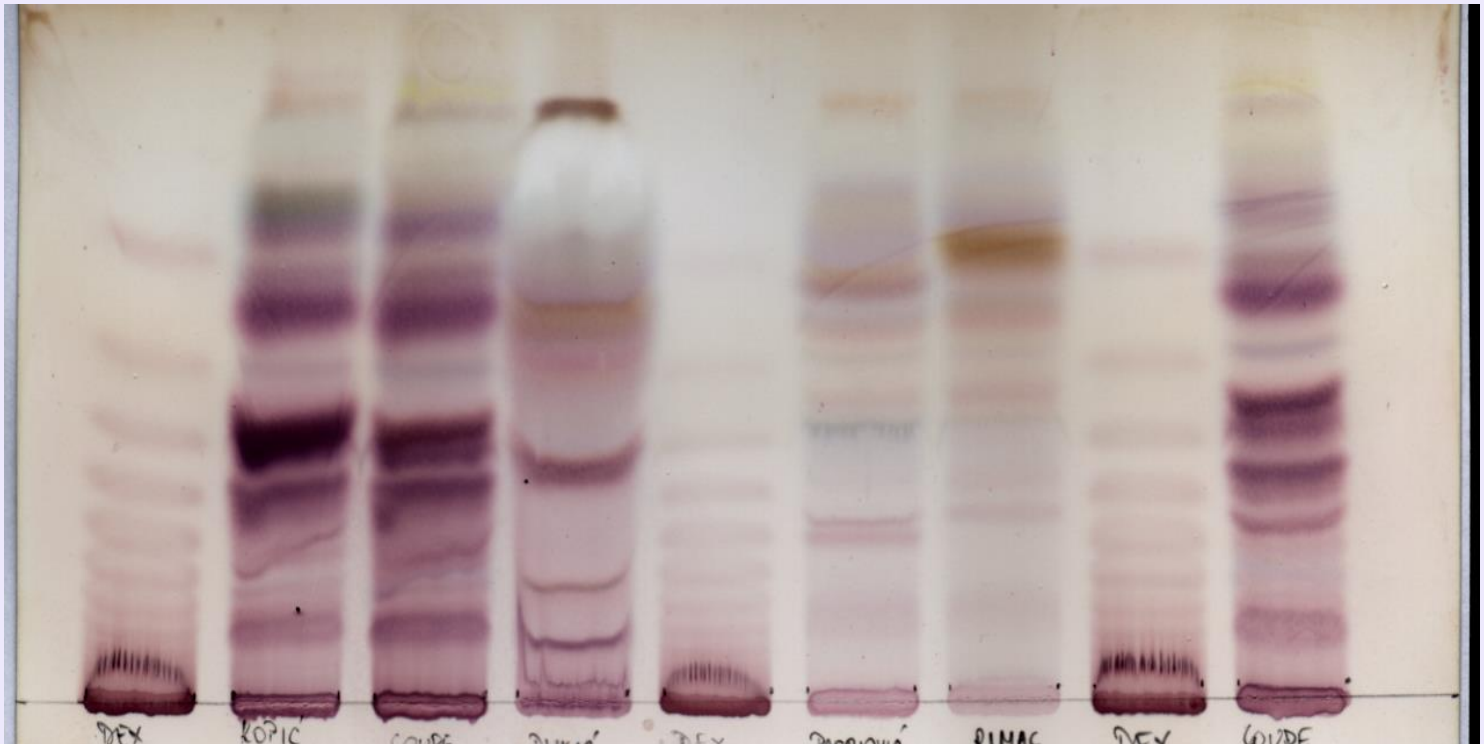
1 2 3 4 5

URIN

OLYGOSACCHARIDES

TLC

- GM1-GANGLIOSIDOSIS
- M.POMPE
- β-MANNOSIDOSIS
- MPS IVB
- GM2-SANDHOFF
- α-FUCOSIDOSIS
- α -MANNOSIDOSIS
- SIALIDOSIS

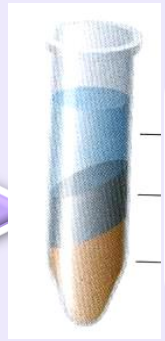


LYSOSOMAL ENZYME ACTIVITY ANALYSIS

SERUM

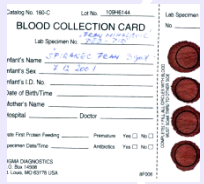
☞ **MUCOLIPIDOSES**

LEUKOCYTES



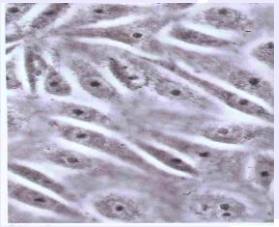
☞ **ALL LYSOSOMAL HYDROLASES**
except
-sialidase (ML I)
-α-glucosidase (M.Pompe)-lymphocites
-phosphotransferase (ML II i III)

DBS



☞ **SOME LYSOSOMAL HYDROLASES**

FIBROBLASTS



☞ **CONFIRMATION OF DIAGNOSIS**

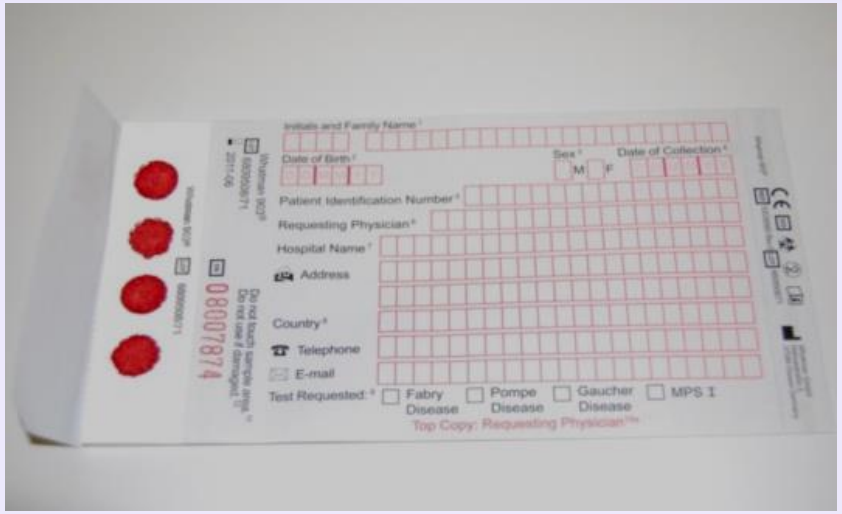
DRIED BLOOD SPOT

- α-galactosidase (FABRY DISEASE)
- α-glucosidase (POMPE DISEASE)
- β-glucosidase (GAUCHER DISEASE)
- α-L-iduronidase (MPS I DISEASE)

- Lysosomal acid lipase (CHOLESTERYL ESTER STORAGE DISEASE; WOLMAN D.)

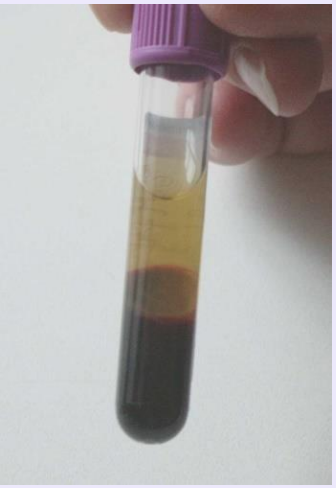
- Sphingomyelinase (NPAB)
- Palmytoyl protein thioesterase 1 (BATTEN DISEASE)

DBS –SELECTIVE SCREENING LSD FOR THE REGION



2016	B i H -Tuzla	B i H -Banja Luka	B i H -Sarajevo	Macedonia -Skopje	Albania - Tirana	Serbia -Beograd	Serbia -Novi Sad	Serbia -Niš	Kosovo	Monte negro -Podgorica	Slovenia - Ljubljana	Slovania Maribor
POMPE -DBS	135	97	103	52	66	157	139	152	121	90	155	89
FABRY -DBS	111	35	234	66	89	212	234	167	89	68	167	111
MPS I -DBS	30	12	43	24	27	89	56	32	44	12	54	23
GAU CHER -DBS	43	32	54	66	75	67	78	27	44	30	47	20

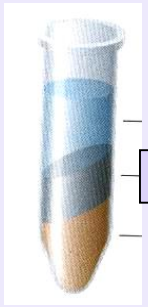
Enzyme activity in DBS is below the cut-off value...

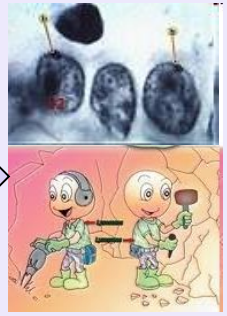


~8 ml EDTA-blood

Leukocytes

Lymphocytes





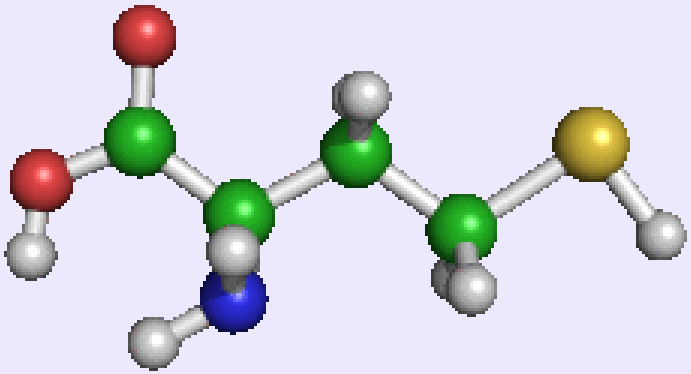
DNA molecular genetic testing was performed in commercial clinical international labs including CENTOGENE, Bioscientia and Nijmegen Medical Center...

All of the parents of the patients with IEMs were tested for carrier status

ERNDIM

QUALITY ASSURANCE IN LABORATORY TESTING FOR IEM

UOI 119
ERNDIM SCHEMES-2017

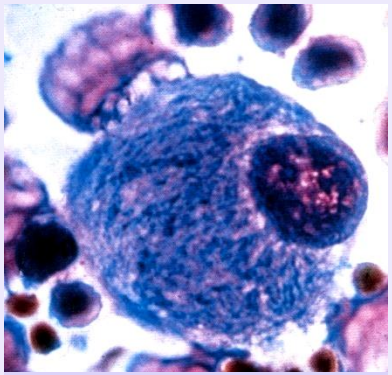


Amino Acides
Spec.Assays in Urine
Spec.Assays in Serum
Quant Org Acids in Urine
Lysosomal Enzymes
Acylcarnitine DBS

Qual.Org Acids Urine Heidelberg
Prof.Test Czech Republic
Urine Mucopolysaccharides

Purines and Pyrimidines in Urine
Cyst.in white Blood Cells

LSDs – THE ROLE OF A LABORATORY



M.GAUCHER

Gaucher- Clinical case

Aghion- Glucocerebrosidase accumulation

De Duve- Lysosome

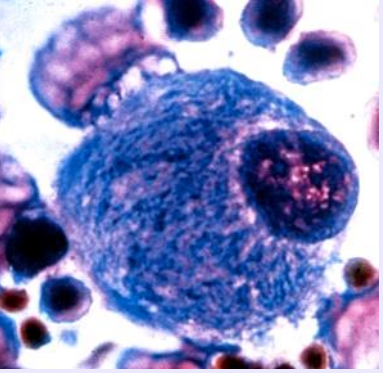
Brady- Glucocerebrosidase deficiency

Beutler- Glucocerebrosidase gene

Barton- Placental ERT (Ceradase)

Brady- Recombinant ERT (Cerezyme)

1882 1932 1955 1965 1985 1991 1994



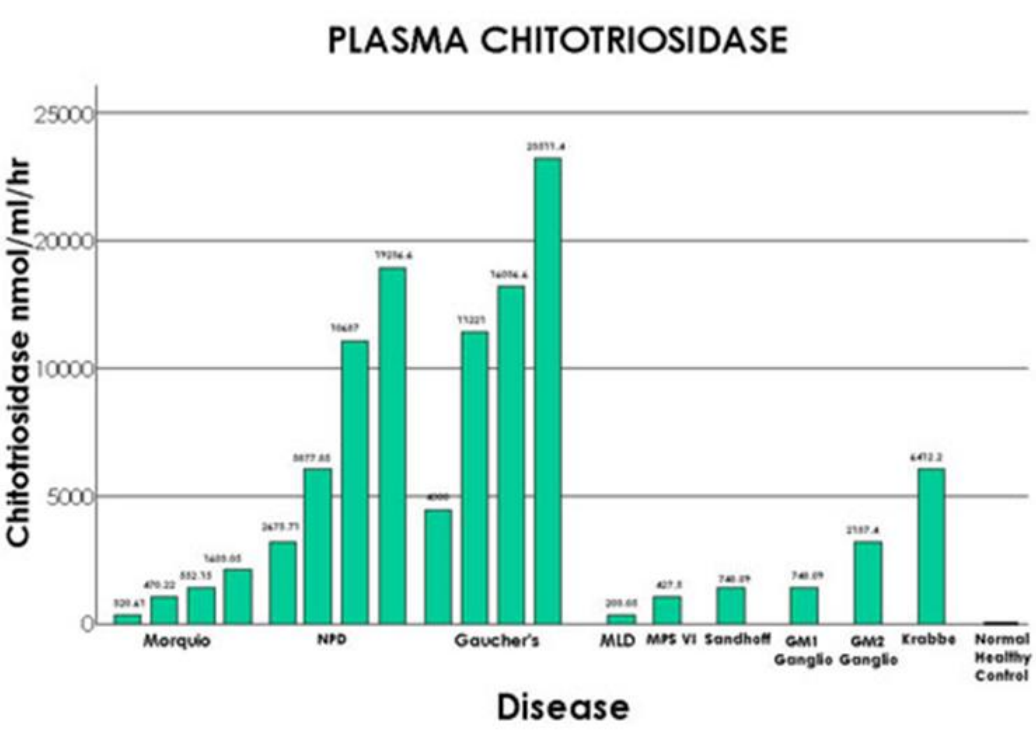
CHITOTRIOSIDASE - GOOD, BUT NOT AN IDEAL BIOMARKER

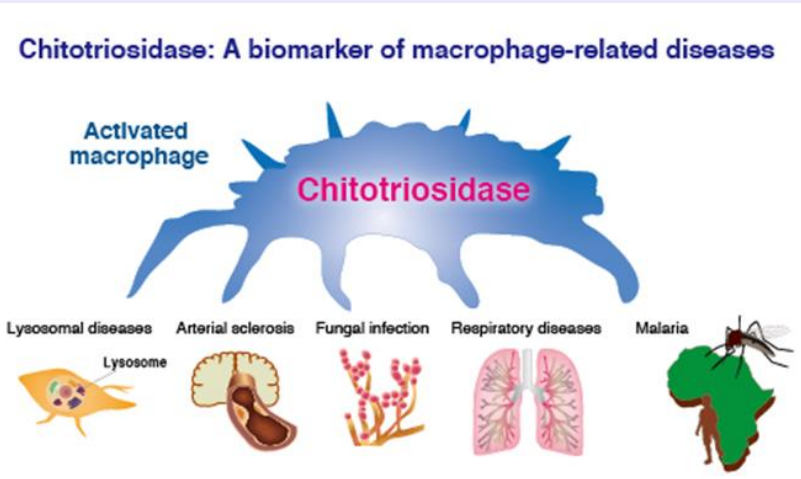
☹

Approximately 25% od us carry two null mutations for Chitotriosidase – we cannot monitor these patients!!!
-HETEROZYGOTES – about 50% activity !!!

GAUCHER PATIENTS 24

☹



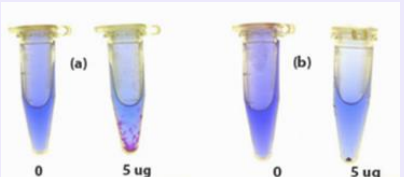


SARCOIDOSIS, ARTRITIS, MS…

Chitotriosidase - a putative biomarker for sporadic amyotrophic lateral sclerosis-
Clinical Proteomics, 2013

Chitotriosidase as a possible marker of clinically evidenced atherosclerosis in dyslipidemic children-
Journal of Pediatric Endocrinology and Metabolism,2014

BIOMARKERS – MPS



MPS I	**5 patients**
MPS II	**4 patients**
MPS VI	**1 patient**

DMB must be considered with caution for use in patients with proteinuria/hematuria

UPLC-MS/MS multiplex method allows quantification of GAGs

- **detection of subtle alteration in urine of MPS patients with disease-specific biomarkers (DS,KS,HS,CS)**
- **monitoring the ERT dose response for treated patients**
- **no interfering components + high specificity**

Andalusian Agency for Healthcare Quality
Independent Assessment Body

Healthcare Provider's Assessment Report

University Hospital Center Zagreb

Network: MetabERN

Subnetworks:

1. **Aminoacid and organic acids related disorders**
4. **Lysosomal disorders**

THE RARE INHERITED METABOLIC DISEASES EUROPEAN REFERENCE NETWORK: MetabERN

Coordinator
Prof. Maurizio Scarpa MD PhD
Helios Dr. Horst Schmidt Klinik Wiesbaden, Germany

69 HCPs from 18 COUNTRIES

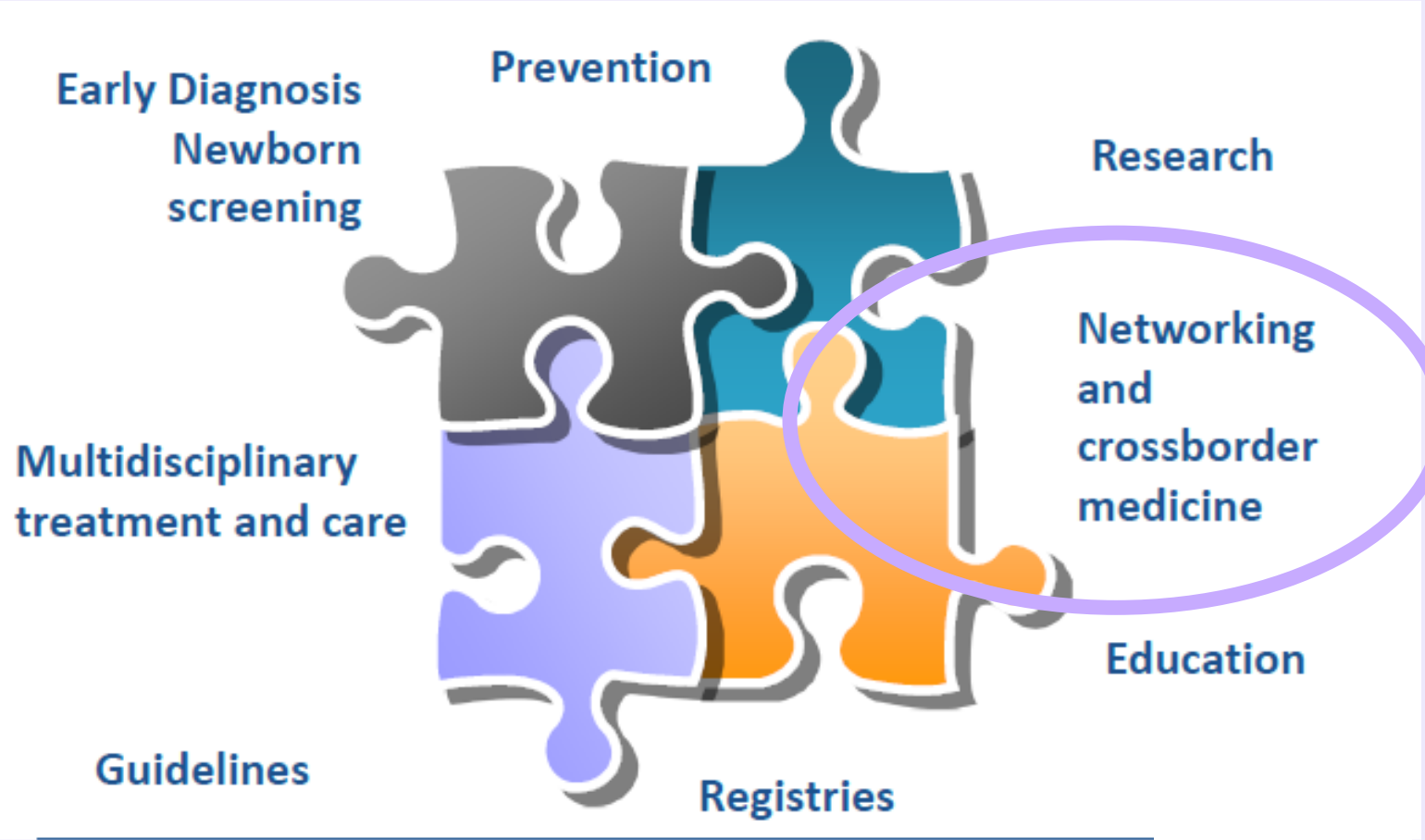
The MetabERN is endorsed by and partners with the Society for the Study of the Inborn Errors of Metabolism (SSIEM)

BE	BG	CZ	DE	DK	ES	FR	HR	HU	IT	LT	NL	NO	PL	PT	SE	SI	UK
6	1	1	10	1	5	9	1	1	11	1	5	2	1	5	2	1	6

MESSAGE TO TAKE HOME

LABORATORY DIAGNOSIS OF INBORN ERRORS OF METABOLISM

ON THE JOURNEY FROM CLINICAL SUSPICION TO TREATMENT
we cannot act, and we are not, alone ...



15th International Conference on Rare Diseases

„Rare Diseases –

Open Your Heart And Mind”