РЕКОМЕНДАЦІЇ ДЛЯ ВПРОВАДЖЕННЯ В ПРАКТИКУ

EXPANDED NEWBORN SCREENING: A CHANCE FOR THE HEALTH OF EACH BABY

EXPANDED NEWBORN SCREENING (NBS)

- Identifies the risk of possible inborn errors of metabolism
- Generally no clinical symptoms are present in the first hours after birth
- Important: the screening of all babies, no matter their health condition
- Most of the inborn metabolic disorders can be treated if detected in due time
- Cause: genetic, most often recessive autosomal transmission

Newborn screening implies to check every baby in a short time after birth in order to identify the risk of possible inborn errors of metabolism, that could be treated but they are not clinically evident in the first days of life, and that have a genetic trigger.

Until clinical symptoms occur various organs and systems of the baby could be severely and irreversibly affected.

Early identification of the metabolic disorder is leading to fast and proper treatment, therefore further complications can be avoided!

TESTING METHOD

Newborn Screening (NBS) – limited tests initially (due to the lack of a multiplex method able to analyze a large number of rare disorders).

Late ’90s – mass spectrometry (MS) was introduced in the NBS labs the number of detectable disorders whose negative evolution can be corrected increases considerably.

MS - High performance, fast, reliable and non-expensive method the test becomes accessible to larger categories of patients

A lot of European countries have expanded the mandatory newborn screening programs after introducing MS.

The development of mass spectrometry permitted the analysis of aminoacids and acylcarnitines by a rapid multianalyse method.

Before this, NBS was limited because of the lack of a multiplex method able to detect a large number of markers for rare disorders at the same time.

Immunoenzymatic methods for each disorder were used prior to MS à long analysis timeà high costsà small number of detected disorders.

MS proved to be very effective due to the fast-response time and multianalyte measurements, resulting in reduced costs.

THE INCIDENCE OF INBORN ERRORS OF METABOLISM

According to the statistics, the overall incidence of inborn errors of metabolism is around 1:2000 babies, with variations from country to country and geographical region.

Phenylketonuria (PKU), as an example, has an average incidence of 1:4500 births.

Most babies look healthy at birth, but some could be affected by rare metabolic disorders.

This disorders have severe impact on babies’ health, like mental retardation and in some cases even death.

Therefore the implications of late diagnosis are huge, both as human grief and as financial effort.

THE NEED OF TESTING EVERY NEWBORN

National programmes of newborn screening have been implemented in USA and most of the European; the screening is mandatory.

From 5 up to more than 50 inherited metabolic disorders are detected by the national screening, depending on the country.

Despite the severity, the clinical evolution of the disease can be reasonably controlled and complications avoided by treatment or special diet, if the diagnosis was established shortly after birth.

In USA, parents’ refusal of the newborn screening test of their baby rises major problems

that involve counselling before the final decision is made.

NBS SCOPE

Early diagnosis and treatment starting.

~ 20 disorders can lead to death of irreversible damage of the nervous system in the first 2 weeks of life.

Only 10-20% of the newborn show clinical symptoms in the first week 5-10% could die in the first week.

Newborn affected by PKU and congenital hypothyroidism could lose a significant percentage of their intellectual capacity when not treated in the first three weeks.

Metabolic disorders that can be detected by this test.

Type	Examples	Analysis method
17 aminoacid metabolism disorders	PKU, MSUD, citrullinemia, tyrosinemia, homocystinuria	LC-MS/MS
30 fatty acid and other organic acid metabolic disorders	MCAD, SCAD, VLCAD, metilmalonic, propionic, glutaric acidemia, betaketothiolase deficiency	LC-MS/MS
Endocrine disorders	Congenital hypothyroidism Congenital adrenal hyperplasia	ELISA, Fluorometry LC-MS/MS,
Other	Cystic fibrosis Biotinidase defficiency Galactosemia	ELISA, Fluorometry LC-MS/MS

PHENYLKETONURIA (PKU)

Phenylalanine (Phe) – neurotoxic in high concentrations 2% of the cases: - dihydropterin reductase defect (DHPR) + biopterin deficiency

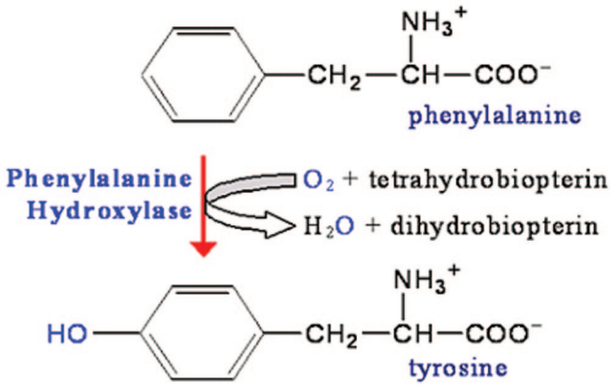
Marker: Phe

Untreated in due time à mental retardation, convulsions

Permanent treatment: Low Phe diet à normal development

Symptoms:

- Microcephaly
- Epilepsy
- Behavioral disorders
- Cardiac defects



MAPLE SYRUP URINE DISEASE (MSUD)

Defect of branched-chain aminoacid metabolism.

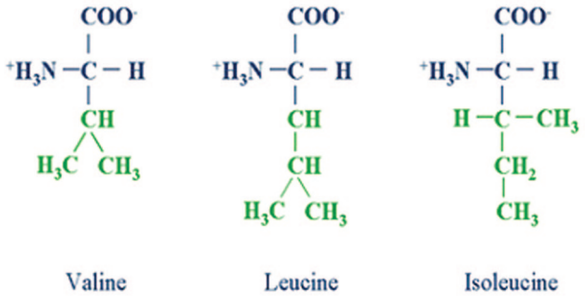
Aminoacids and ketoacids accumulation in blood

Markers: Leucine + Isoleucine

Clinical signs: lethargy, coma, convulsions, specific smell of the urine, encefalopathy, autistic behavior, sometimes even sudden death.

The baby seem normal at birth; if not detected in time the disease can produce irreversible brain damage.

Early diagnosis and careful diet à normal development.



HOMOCISTEINURIA

Deficiency: cistationin-b sintase.

Methionine (Met)àCysteine (Cys) conversion affected.

Cys, homocisteine, Met accumulation.

Marker: Met

Affected children – apparently normal

Clinical symptoms appear after 1-2 years when various systems are irreversibly affected

Symptoms:

- Ectopia lentis
- Glaucoma
- Thromboembolic events
- Osteoporosis
- Stroke
- Mental retardation

Treatment: special diet, without Met; pyridoxine.

FATTY ACIDS AND ORGANIC ACIDS DISORDERS

Deficiency in fatty acids (FA) mitochondrial b-oxidation.

The cells cannot process the fatty acids.

Not enough energy is produced.

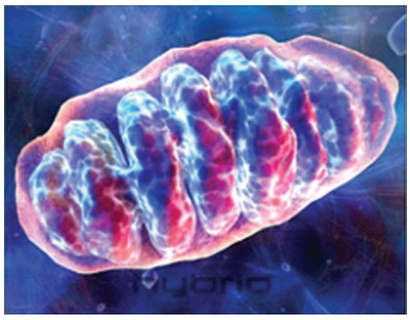
Toxic accumulation of un-oxidated FA as associated with hypoglycemia and tissue lesion.

Approx. 10% of the affected babies die in the first week.

Most of the disorders in this category – detected by MS/MS (acylcarnitine accumulation measurement).

MCAD, SCAD, VLCAD, methylmalonic, propionic, glutaric acidemias.

Treatment – differs from condition to condition (glucose, carnitine).



CONGENITAL HYPOTHYROIDISM

Incidence - 1:3000.

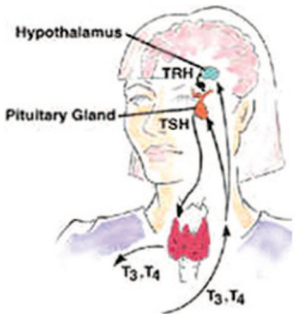
Reduced thyroid hormones synthesis.

Screening Test: TSH and T4.

The baby looks healthy at birth.

The symptoms occur usually after 3 months when the brain is irreversibly affected à Severe consequences (mental retardation, growth deficit) if not diagnosed and treated in the first 2-3 weeks of life.

Hormonal treatment - very effective, prevents installation of the clinical condition.



CYSTIC FIBROSIS

More than 1000 identified gene mutations – a lot of them are fatal.

CFTR protein(Cl- channel) affected à

Viscous mucus production in the lungs

Difficult diagnosis: unspecific, multiple symptomatology.

The reduced life expectancy in this cases is

extended by early diagnosis and proper treatment.

Actions:

Respiratory infections treatment.

Reducing mucus secretion.

Digestive enzymes, vitamins, nutrient supplements.

SAMPLE COLLECTON

Ideally, the blood for newborn screening is collected in the maternity, 48-72 h after birth.

For preterm babies the first sample is collected before an eventual transfusion or hyperalimentation.

A second test is required after 1 month.

For newborn in intensive care units, blood and urine sample collection is recommended.

Blood spots can be sometimes the unique source of DNA, useful in further diagnostic studies, and could offer valuable information to

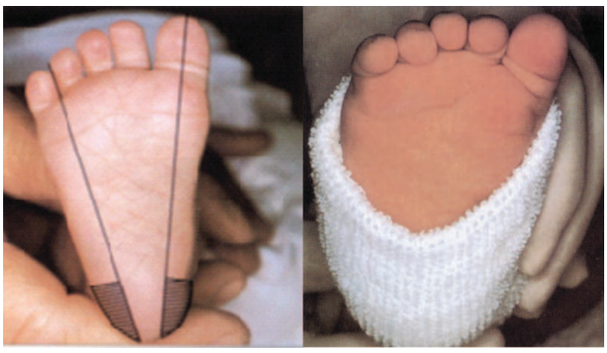
the family (for genetic counselling).

STANDARD SAMPLING PROCEDURE

Sample collection: 48 Hours after birth, and after the baby was fed.

Simple sampling procedure:

- Only a few drops of blood are needed
- The sampling should be performed from the heel of the newborn (medial and lateral areas).



STEPS

1. Special filter paper; antiseptic conditions (clean the heel with warm tissue).
2. On each circle marked on the paper a single large blood drop is applied.
3. Drying time (at room temperature): 3-4 h.
4. Minimum 2 valid spots are required.
5. Sample storage: room temperature or 40C, light and humidity protected.
6. The samples should be submitted to the laboratory as soon as possible.

INCORRECT SAMPLES

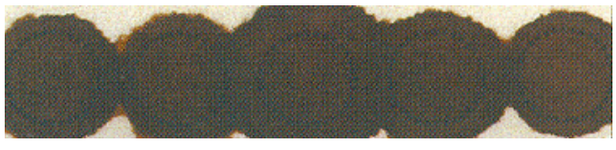
Incorrect sampling

- Agglutination
- Application on both sides of the paper
- Blood not absorbed
- Overfilling
- Insufficient sample

Incorrect drying:

- Serum separated from blood cells- halo effect
- Closing the paper card before drying

Overfilling



Insufficient sample

Coagulation

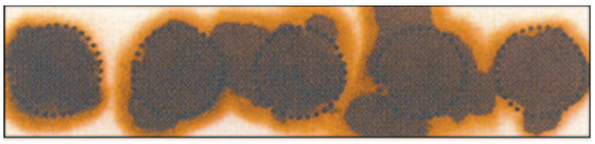
Incorrect drying:

- Serum separated from blood cells- halo effect
- Closing the paper card before drying

Incorrect or incomplete screening form

Contamination

Halo



SECOND SAMPLING

It is required when:

- The sampling was incorrect (see before).
- The sample was collected before 48h from birth.
- Pre-term babies.
- Babies that undergone transfusion prior to sampling.
- Babies showing clinical signs of metabolic disorders.
- Positive result in the first screening.
- If confirmed, for further diagnostic other biological samples will be collected (serum, plasma, urine etc).

SAMPLE ANALYSIS– LC-MS/MS

Liquid chromatography-mass spectrometry (LC-MS/MS).

High performance technique, developed in the last two decades.

The most sensitive technique also offering structural information (compared to NMR).

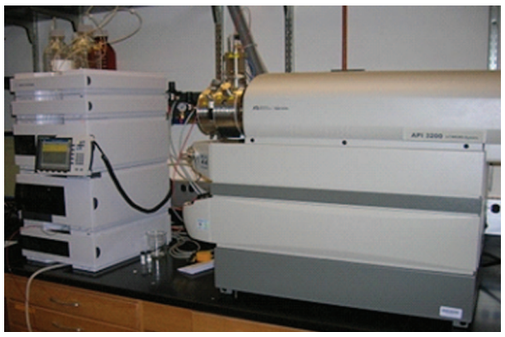
Much higher accuracy, specificity and sensitivity compared to ligand-binding assays (ELISA, RIA).

Quasi-universal (can be applied in the analysis of any type of compounds).

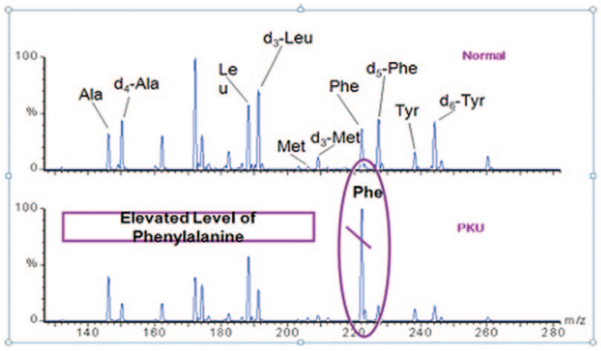
Advantages of LC-MS/MS

- Simultaneous and sensitive analysis of a large number of markers.
- Fast and cost-effective.
- A series of aminoacids and acylcarnitines are evaluated in only one analytical step.
- The risk of a disease is detected in due time avoiding developmental damage.
- Most tests use a few drops of blood from pricking the baby's heel.

If a screening test suggests a problem, baby's doctor will follow up with further testing. If those tests confirm a problem, the doctor may refer you to a specialist for treatment. Following doctor's treatment plan can save your baby from lifelong health and developmental problems.

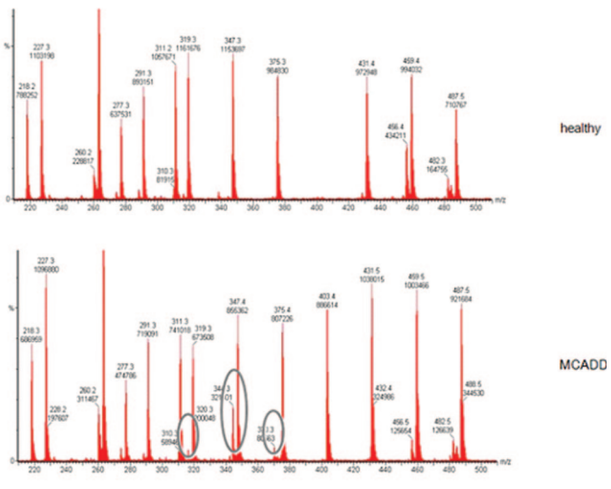


First example: PKU evaluation



The signal corresponding to Phe is 3 times higher than in a normal baby.

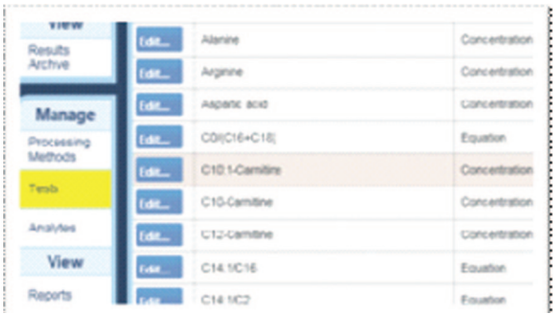
Second example: MCAD evaluation

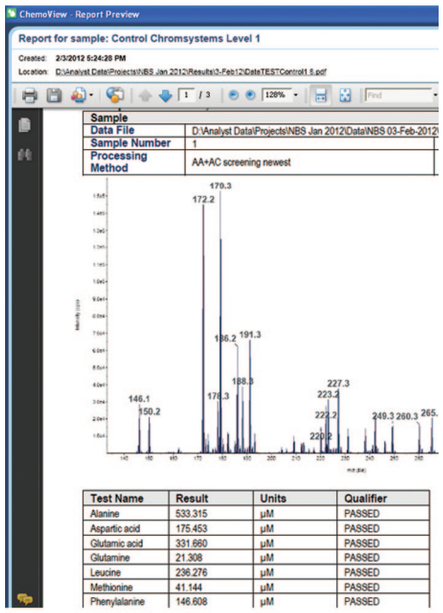


Medium chain acylcarnitines in high concentration compared to a normal test.

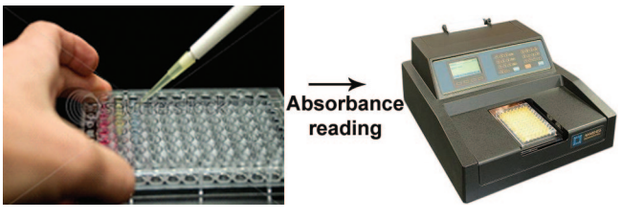
Semi-quantitative calculation

- Special software (Chemoview)
- The concentrations of aminoacids and acylcarnitines are calculated by comparing with the signal of the deuterated homologues (Internal standards), added in known amount.





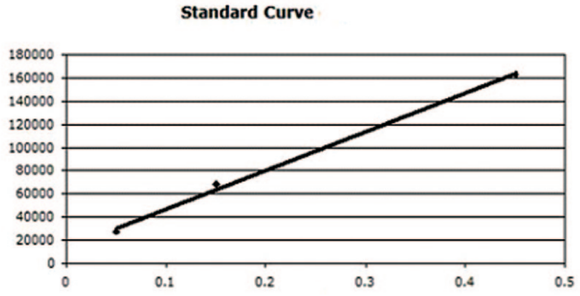
SAMPLE ANALYSIS – ELISA



Imunoassay à the Ac-Ag complex.

Results extrapolation à Concentration calculation.

- Cystic fibrosis
- TSH
- Galactosemia



REFERENCE CONCENTRATION

The reference concentrations – established on newborn analyzed in our lab using standard statistical methods.

More than 2000 samples from newborn have been analyzed and reference intervals estimated for each parameter (aminoacids, acylcarnitines, TSH, IRT, TGAL).

The reference concentrations are periodically adjusted with adding the last results to the statistics.

Example: reference concentrations for aminoacids

Marker	Ala	Arg	Asp	Cit	Glu	Gly	Leu	Met	Orn	Phe	Pro	Val	Tyr
Max	1722.1	99.1	686.5	341.8	986.2	3148.6	1234.2	94.0	1364.2	542.9	878.7	1506.1	823.8
Min	65.7	1.746	28.0	3.3	102.6	105.3	53.7	1.8	29.1	16.2	26.5	30.7	13.9
Median	307.0	7.0	81.3	10.9	420.1	333.1	157.9	9.6	104.6	45.9	104.4	78.8	67.4
Percentile 98%	612.2	24.6	208.1	23.6	693.9	735.8	296.9	20.5	228.5	78.7	186.0	160.4	178.3

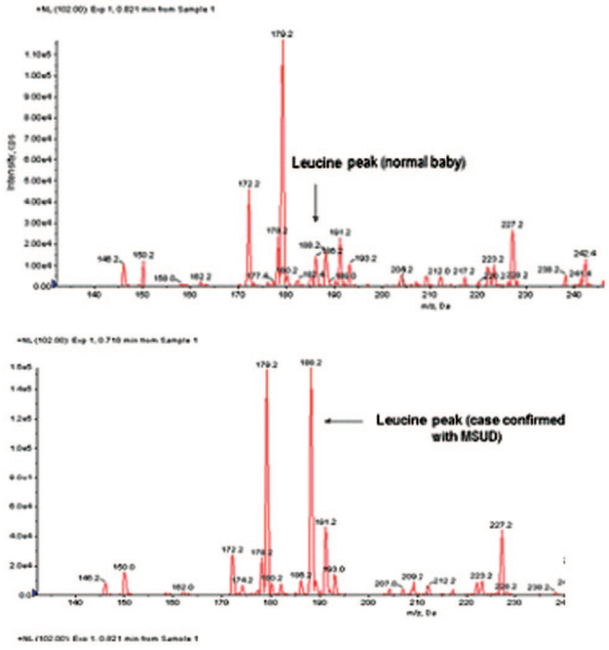
SPECIAL CASES

- Newborn with severe health issues at birth.
- Preterm babies.
- Children of other age than neonatal.
- Already diagnosed patients needing regular monitoring of the evolution.

These situations require an interpretation for each particular case.

The reference concentrations statistically calculated on newborn are only indicative.

Sometimes a second sampling is needed.



BENEFITS OF NEWBORN SCREENING

- Early detection of inborn errors of metabolism.
- Rapid intervention (retest, diagnostic methods, adequate treatment in due time).
- Reduced morbidity and mortality (the evolution of most of these diseases could be stopped or at least slowed down).
- Familial planning – the parents are informed about the possible genetic issues and counselled to perform prenatal investigations if they want another baby.

NEWBORN SCREENING IN BRIEF

The first objective of Cytogenomic Medical Laboratory:

All customers can take advantage of the best services for their health.

The laboratory is equipped with a new LC-MS/MS system for Expanded Newborn Screening (47 disorders of aminoacids, fatty acids and other organic acids).

Also: screening for congenital hypothyroidism, cystic fibrosis, galactosemia, congenital adrenal hyperplasia.

Quality control through participation in the US Newborn Screening Quality Assurance Program.

Every newborn should be tested!

Even if there are no familial records of genetic disorders, there is still a risk to give birth to an affected baby. The clinical symptoms occur too late but the newborn screening highlights the problem and the treatment is applied at the right time.

To whom is the test addressed?

- Normal newborn
- Preterm babies
- Already diagnosed babies
- Newborns in intensive care units