

Traditional Testing Techniques:  
bacterial inhibition,  
enzyme/fluorescence  
immunoassay, isoelectric focusing

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Isns  
&  
AFPDE



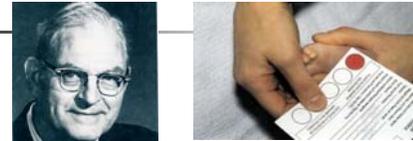
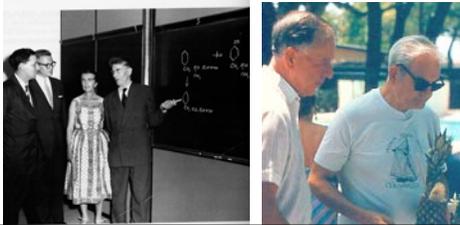
Marrakech, novembre 2006

A systematic newborn screening is recommended for a disorder where:

- 1) there is considered to be a direct benefit to the newborn from early diagnosis;
- 2) the benefit is reasonably balanced against financial and other costs (at individual and population levels);
- 3) **there is a reliable test suitable for neonatal screening;** and
- 4) there is a satisfactory system in operation to deal with diagnostic testing, treatment and follow-up of identified babies.

1934 A. Folling ;  
the PKU disease

1954 H. Bickel :  
the PKU diet  
1962 R. Guthrie : the BIA  
assay

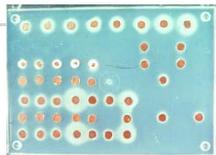


« While I recognize how pivotal the phenylalanine assay was for the development of newborn screening, I have always considered the filterpaper blood specimen to be my most important contribution »

Guthrie R. The origins of newborn screening. Screening 1992;1: 5-15

### The Guthrie test

- microbiological inhibition assay (beta2-thienylalanine, *Bacillus subtilis*)
- in worldwide use
- semi-quantitative and difficulty for full automation
- but cheap and reliable



### The Guthrie test

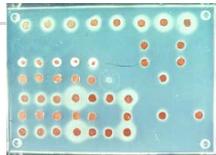
The « quadratic punch indexer machine »  
of R. Phillips



## The Guthrie test

Applicable to other analytes

- Leucine
- Methionine
- Galactose
- ...



## Methods in use : phenylalanine

Linear up to (at least) 20 mg/dl (1.2 mmol/L)

CV% of about 5% around the cut-off level

Enables the handling of large number of samples

## Methods in use : phenylalanine

Fluorometry

automation

AutoAnalyser @ Technicon™ (Hill, 1965)



Alpkem® RFA 300™

Centrifugal analyzer

semi-automation

Microplate adaptation



## Methods in use : phenylalanine

Spectrophotometry

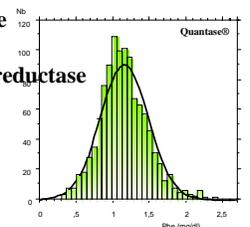
L-aminoacid oxidase

Phenylalanine ammonia lyase

Phenylalanine oxidase

Phenylalanine : NAD<sup>+</sup> oxidoreductase

(Microplate adaptation)



## Methods in use : phenylalanine

Chromatographic methods



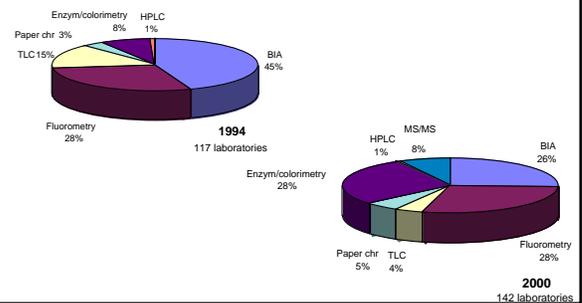
thin layer chromatography  
automated amino acid analysis (ion exchange,  
Stein-Moore 1958)

HPLC methods : isocratic, UV  
gradient, derivatisation

Tandem mass spectrometry



## In Europe



## Methods in use : phenylalanine

The endogenous protein catabolism is the main mechanism responsible for the increase of blood phenylalanine concentration in PKU infants, even in premature ones

phenylalanine (mg/dl)

classic PKU

3 mg/dl

days

## Methods in use : isoelectric focusing

*Screening for hemoglobinopathies*

+

— difficulty for full automation  
qualitative method  
but still the recommended method

## Methods in use : isoelectric focusing

*Screening for hemoglobinopathies*

2005: out of 48453 tests  
135 profiles cannot be interpreted (BW < 1500 g)

birth: reciprocal switch in production of HbF and HbA

118 newborns

blood transfusions in the early weeks of life

## Methods in use : enzyme determinations

Glucose-6-phosphate dehydrogenase

Biotinidase

.../...

## immunoassays : TSH, T4, 17OHP, IRT,...

- Radioimmunity
- Time resolved fluorescence (« Microplate technology »)
- Enzyme immunoassay (« Microplate technology »)

## immunoassays : TSH, T4, 17OHP, IRT,...

Commercial kits use :

- different antibodies (different cross-reactivities)
- different molecular forms of the analyte for calibration

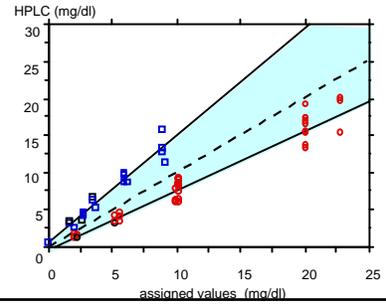
TSH



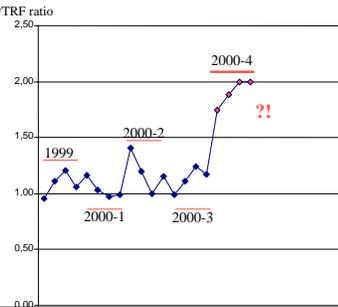
Reliable quantitative measurements require reliable calibrators.



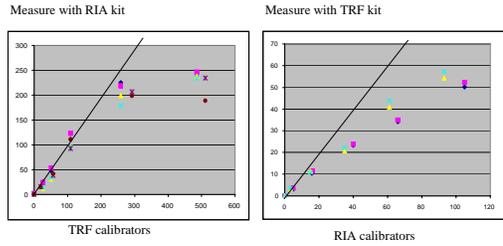
### Calibrators Phe



### Calibrators TSH

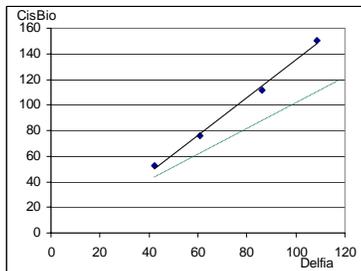


### Calibrators IRT

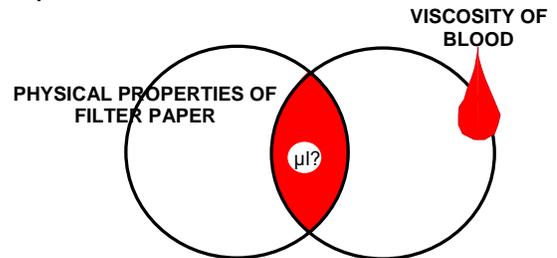


### Calibrators 17OHP

CisBio/Delfia = 1,24 à 1,40



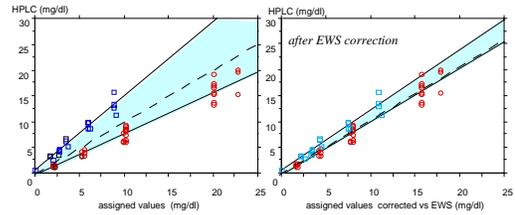
"the paper disk punched is a volumetric measurement for analysis"



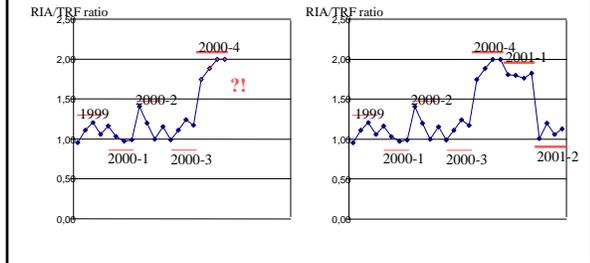
## Need of « reference materials »



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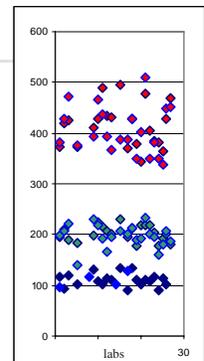
## Need of « reference materials »



## recommendations

- Set up of an internal quality control system
- Participate to external quality control schemes

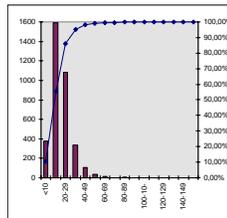
Phe ( $\mu\text{mol/L}$ )



## recommendations

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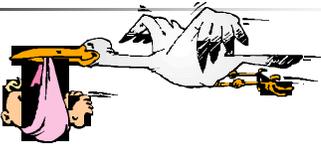
•Periodic check of the « normal » distribution of the « screened » population: the stability of analytical systems can be assessed by following population parameters: mean and median of normals, positive rates at different levels of concentration



## recommendations

- Set up of an internal quality control system
- Participate to external quality control schemes
- Periodic check of the « normal » distribution of the « screened » population

**The main characteristic of a screening program is to rely on a single assay to « detect » babies with a suspicion of a disease !**



Thank you for your attention