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Review

# Mass spectrometry in clinical chemistry: the case of newborn screening

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ABSTRACT

Newborn screening (NBS) program is a complex and organized system consisting of family and personnel education, biochemical tests, confirmatory biochemical and genetic tests, diagnosis, therapy, and patient follow up. The program identifies treatable metabolic disorders possibly when asymptomatic by using dried blood spot (DBS). During the last 20 years tandem mass spectrometry (TMS) has become the leading technology in NBS programs demonstrating to be versatile, sensitive and specific. There is consistent evidence of benefits from NBS for many disorders detected by TMS as well as for congenital hypothyroidism, cystic fibrosis, congenital adrenal hyperplasia by immune-enzymatic methods.

Real time PCR tests have more recently been proposed for the detection of some severe combined immunodeficiences (SCID) along with the use of TMS for ADA and PNP SCID; a first evaluation of their cost-benefit ratio is still ongoing. Avoiding false negative results by using specific biomarkers and reducing the false positive rate by using second tier tests, is fundamental for a successful NBS program. The fully integration of NBS and diagnostic laboratories with clinical service is crucial to have the best effectiveness in a comprehensive NBS system.



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## 1. Introduction

Newborn screening (NBS) is known to be a biochemical test enabling the identification of many inborn errors of metabolism (IEM) few days after birth. If they are not diagnosed and early treated, most of them can cause mental and/or growth retardation, severe permanent sequelae and in some case death. Considering that NBS requires expert lab technicians, chemists, biologists, nutritionists, medical specialists for metabolic disorders, it cannot be considered only an useful biochemical test, but it should be considered as a complex and integrated program. The objective of a newborn screening program is to detect some IEM before the clinical manifestation of the associated symptoms. As a consequence, medical doctors can start the best available treatment and have the best prognosis by modifying the natural course of the disease. The history of NBS as population-based test dates back from the beginning of 1960s when the microbiologist Robert Guthrie developed a simple and inexpensive bacterial inhibition assay (DBS based) able to identify the most frequent aminoacidopathia: the phenylketonuria [1]. In the following decade, some other clinical labs both in the United States and in Europe, added the congenital hypothyroidism (CH) to their panel, again by using a single drop of whole blood on paper. The following development of electrospray tandem mass spectrometry in more recent years (1990s) has permitted the introduction of this new technology in clinical chemistry laboratories, in particular for newborn screening purposes [2]. MS/MS is a versatile, specific and sensitive technology giving technicians the possibility to measure many biomarkers in a single and fast analytical run. People working in newborn screening field understood the possibility to pass from one DBS for one test and one disorder to one DBS for one multiplex test for many disorders. In fact, today MS/MS can easily identify and quantify in a 2 or less minutes' run – several metabolites such as acylcarnitines, aminoacids, succinylacetone [3,4] and more recently some purines [5–8]. Nowadays, including pilot projects regionally ruled and structured national NBS programs, many labs all around the world screen for more than 30 or more IEM with a single test. The number of potential identifiable disorders is not a technological hamper but it depends on regional or national public health strategies.

Some expanded newborn screening programs are now screening not only for PKU, CH, more recently for cystic fibrosis (CF), congenital adrenal hyperplasia (CAH) and galactosemia, but also for others aminoacidopathias, beta oxidation fatty acid defects, organic acidurias, urea cycle defects and since 2011 for some severe combined immunodeficiences (SCID). One the fundamental worldwide-approved criterion is that one IEM can be screened only if a related treatment is available. For all disorders included in the newborn screening programs a therapy should be possible even if, in some cases, not completely curative. The term "not completely" is one relevant reason why newborn screening panels are not all the same worldwide.

The opinion of this writer is that even if a therapy is not completely curative, the early detection of the disorder and a following immediate correct treatment should give babies the possibility to have better quality of life, to extend life expectancy and when required, to allow a suitable genetic counseling (especially for future prenatal diagnoses). Moreover, an early diagnosis relieves families with a severe ill child from difficult diagnostic iter. These simple criteria represent the basal evaluation reported from Wilson and Jungner in 1963 to World Health Organization [9].

Therefore, the NBS cannot be considered only an efficient and isolated lab test of preventive medicine but it is a more integrated public health system involving many different categories such as lab technicians, chemists, biologists, biochemical geneticists (for both primary and confirmatory tests), administrative personnel, nurses, dieticians and/or pharmacologists, medical doctors expert in metabolic disorders and pediatricians. As consequence, only if laboratory NBS procedures, laboratory confirmatory procedures, clinical service and continue education are fully integrated, a real effectiveness of a NBS program will be reached.

## 2. Materials and methods

### 2.1. Sample collection

Blood samples were normally obtained by heel stick or hand-prick, spotted on filter paper (903, Whatmann, Milan, Italy or 226 grade paper, Ahlstrom, Helsinki, Finland) often referred as "Guthrie cards", dried at room temperature and sent daily by courier or mail to the newborn screening laboratory. In most part of European countries as well as in the USA and Australia a formal guideline for the sampling procedure is available. In about half the countries these guidelines have been developed by health authorities and in the other half by professional groups, either locally or nationally.

The number of drops is variable (minimum 3–4, maximum 12) and it is generally depending on the number of tests (both first and second tier tests).

The expansion of blood on the paper depends on haematocrit level and drying conditions.

Blood collection for newborn screening in Italy (as in most part of countries) is recommended between 48 and 72 h of life. In Europe, the collection ranges from the 36 h in Austria and Croatia to 168 h of Greece, Netherlands and Luxembourg [10]. Timing of the collection is a critical issue because marker levels could vary physiologically in the neonatal period [11].

In some countries, such as Finland and Malta, cord blood sampling is used for newborn screening testing according to early maternal and newborn discharge [12,13], but it has been evaluated that better results are obtained from samples collected by heel/hand prick at a later stage [14,15].

Use of urine collected onto an absorbent paper placed in the baby's nappy could be a very useful tool as additional but not alternative NBS test. Even if not common, some NBS programs continued the practice of urine screening [16] and in some cases the urine DBS has significantly contributed to the IEM detection up to 44% of total cases [17].

An important parameter for the quality of the program is the completeness of sampling, preferably 100% but reaching this value is practically impossible because in most countries it should be possible for parents to refuse participation of their children to NBS programs. If informed consent is taken seriously, the option to stay out will sometimes take place.

### 2.2. Blood collection in premature infants and in newborns on parenteral nutrition or transfused

Prematurity, birth weight, parenteral nutrition, transfusions and type of feeds can all potentially influence NBS results.

In order to decrease parental stress related to retesting and to ensure that important information for interpreting screening results is given, established protocols are useful. These protocols should provide repeated tests during the early postnatal period for infant requiring blood/plasma transfusion, for premature babies or term/preterm babies on parenteral nutrition at the time of screening sample collection.

In some countries [18,19] for premature infants (birth weight <1800 g), the first DBS sample is collected on the 3rd–5th day of life, then two additional samples at 15 and 30 days. For babies on parenteral nutrition, including premature babies, a second sample at 48 h after the ending of parenteral nutrition is collected. In all