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Review

Expanded newborn screening of inherited metabolic disorders by tandem mass spectrometry: Clinical and laboratory aspects

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Abstract

Newborn screening started in the 1960s for the purpose of identifying phenylketonuric patients to begin early intervention and to prevent mental retardation in these patients. Soon thereafter, screening programs expanded to include additional genetic disorders added individually one at a time. In the 1980s, tandem mass spectrometry (MS/MS) was introduced in clinical laboratories, and in the 1990s, the technique was used for newborn screening. Unlike measuring one analyte at a time, MS/MS allows measurement of >40 analytes, in a few minutes with the use of a single assay. Currently, MS/MS is being used for the identification of several amino acid, organic acid and fatty acid disorders. Several states in the United States and many other countries are using MS/MS in newborn screening. However, there is a significant disparity among different newborn screening programs for disorders being screened by MS/MS and many other challenges are faced by the expanded newborn screening. It is anticipated that in the future the use of MS/MS in newborn screening will expand both at the analyte and geographic levels. Clinicians and laboratory scientists should become familiar with MS/MS, disorders being screened in their patients' population and the future of this emerging technology.

Keywords: Newborn screening; Metabolic disorder; Tandem mass spectrometry; Inborn error of metabolism

Contents

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Introduction

The primary goal of any screening program is the early detection of clinically important disorder(s) in order to minimize morbidity and mortality. Mass newborn screening began in the 1960s when Guthrie and Susi developed a method for estimation of phenylalanine in blood samples collected on a filter paper for the detection of phenylketonuria (PKU) using a bacterial inhibition assay [1,2]. As desired for mass screening, the sample collection and analysis methods were very simple, rapid and reliable [3]. The approach was so successful that soon thereafter, the screening for PKU was instituted in the entire US and many other developed countries [3]. Until the early 1990s, few other diseases, "though one at a time," were added to the newborn screening programs. The process of adding one disease at a time was slow and laborious. It required the analysis of one or more analyte(s) for each of the disorders added for metabolic screening. In the 1990s, with the introduction of tandem mass spectrometry (MS/MS) into the metabolic screening laboratories, the paradigm of analyzing one analyte per disorder changed. With a single and "2–3 min" long analysis of a small blood spot, MS/MS allows the determination of multiple analytes characteristic of several (>40) metabolic disorders [4–12]. Currently, most states in the United States and many other countries have implemented or are in the process of implementing newborn screening by MS/MS [13]. As there are significant variations among "state and commercial" newborn screening programs, the pediatricians and laboratory scientists should become familiar with the capabilities and limitations of MS/MS testing performed in their pediatric population.

Sample collection

Through a heel stick, sample is collected from a newborn on a special filter paper commonly called the "newborn screening" or Guthrie card [3]. To increase the blood flow, the heel is first warmed up to ~42°C for a few minutes, followed by cleaning and puncturing the skin by a sterile lancet no longer than 2.5 mm. After wiping the first drop, several filter paper circles are filled completely with blood by gently touching the filter paper against the oozing blood. The filter paper is then dried at room temperature for several hours and mailed to the testing laboratory by regular mail at room temperature. For good quality results, it is important not to squeeze the heel to avoid hemolysis, dab the filter paper during blood collection, over saturate the filter paper and touch the filter paper to avoid contamination.

To allow adequate dietary challenge for the newborn, sample should be collected after at least 24 h of oral feeding and before discharge from the hospital, generally by less than 5 days of age. If the newborn is discharged before 24 h, to avoid the risk of no sample at all, a filter paper sample should be collected before discharge and then repeated when the infant is seen by the pediatrician at about 1 week of age.

Fundamentals of MS/MS and sample analyses

Mass spectrometry is a technique of identifying and quantifying analytes based on their molecular mass and charge. For analysis by a mass spectrometer, the analyte of interest must be converted into a gas phase ion, i.e., vaporized and ionized. Before analysis, analytes are generally derivatized to change their chemical properties for better volatilization and ionization to increase sensitivity and specificity. Also, before analysis by mass spectrometry, the analyte(s) of interest are separated from a mixture of compounds. In an instrument with a single mass spectrometer, separation is generally achieved by gas or liquid chromatography. Several ionization techniques such as electron impact, chemical and electrospray are available.

In electron impact ionization, the analyte molecules enter the ionization chamber and are exposed to high energy electrons (generally 70 eV). This causes destabilization and rearrangement of the analyte molecules resulting in positively charged, negatively charged and neutral fragments. Depending upon the analysis, the mass spectrometer analyzes either the positively or negatively charged fragments, whereas neutral fragments are pumped out of the system. Also, depending on the mass spectrometer setting and based on the mass to charge ratio (m/z), only the desired fragments will reach the detector; others will strike the magnetic poles and be destroyed.

In chemical ionization, high energy electrons ionize a reagent gas (e.g., methane, ammonia, isobutane) which then ionizes the analyte molecules. The most commonly used chemical ionization technique is positive ion in which generally there is a transfer of a proton from the ionized reagent gas to the analyte molecule. The ion produced from the analyte molecule by addition or removal of one electron is called the molecular ion. In contrast to electron impact ionization, chemical ionization leaves the analyte(s) relatively intact.

To ionize a sample using electrospray (ES), sample is first dissolved in a mixture of water and organic solvent (e.g., methanol, isopropanol or acetonitrile) then sprayed through a small tube into a strong electric field in the presence of a flow of warm gas (nitrogen) to assist desolvation and formation of ions. As above, based on their m/z, the mass spectrometer separates and selects desired ions and presents them to the detector for identification and quantification of the analyte(s). The mass spectrometer can monitor ions either by scanning ions in a particular mass to charge ratio (m/z) range or by selecting single or multiple ions (selected ion monitoring). When standard operating conditions are used, the fragments formed and their relative proportions are reproducible and can be used for the identification and quantification of the analytes. Using gas or liquid chromatography coupled with a single mass spectrophotometer, a complex analysis of several dozen analytes generally takes >30 min.

As the name implies, the tandem mass spectrometer has 2 or more mass spectrometers arranged in tandem. The most commonly used instrument for the newborn screening is a triple quardrupole mass spectrometer. It consists of two mass spectrometers (hence the term MS/MS) separated by a collision cell. The collision cell is another mass spectrophotometer and,