False negatives. The presence of maternal antibodies in the baby's blood may be below the threshold for detection. Thus a negative direct antiglobulin test result does not rule out the possibility of anemia while a positive result does not necessarily mean that the baby will develop anemia.

Chapter 10 Advances in Newborn Screening using MS/MS

Mass spectrometry as an analytical technique has been used for many years in both qualitative and quantitative research applications, Typically the applications for bilological compounds involved the use of Gas chromatogaraphy to separate the compounds of interest, prior to injection into and analysis by the mass spectrometer. GC MS is typically a slow process that does not lend itself well to mass screening applications. With the development of Tandem Mass spec, these difficulties were overcome and the specialty analysis became available that was both fast and sensitive. It was initially used for specialized clinical testing to measure carnitine esters in the blood and urine of children suspected of inborn errors of metabolism.

Mass spectrometry separates and measures the mass to charge ratio of ions that have been produced from fragmentation of parent molecules in the ionization chamber of the mass spectrometer. The most common techniques consists of separation of the substances to be measured in a gas chromatograph, followed by fragmentation and measurement in a single mass spectrometer. .

The tandem mass spectrometer, abbreviated MS/MS usually consists of a pair of analytical quadrupole mass spectrometers. Separated by a reaction chamber or collision cell. (In most instruments the collision cell is actually a third quadrupole.)
The substance to be analyzed undergoes a *soft* ionization procedure (e.g., fast atom bombardment or electrospray) to create quasimolecular ions, and is injected into the first quadrupole, which separates the *parent ions* from each other. The ions then pass (in order of m/z ratio) into the reaction chamber or collision cell, where they are subjected to controllable fragmentation by collisions with inert gases like argon or helium;) These fragments of the parent ions then pass into the second analytical quadrupole where they are analyzed according to the m/z ratios of the fragments.
Electrospray ionisation is a 'soft ionisation' technique which enables the direct analysis of biological high molecular weight substances like proteins previously considered non-candidates for mass spectrometry. Compounds can be detected and quantified directly from solution; there is no need to volatalise the sample. It offers excellent low sensitivity (femtomole detection limits). Because separation of compounds in the mixture is by mass

spectrometry instead of chromatography, the entire process, from ionization and sample injection to data acquisition by computer, takes only seconds.

The computer data can be analyzed in several ways. One can use a *parent ion* mode to obtain an array of all parent ions that fragment to produce a particular daughter ion, or a *neutral loss* mode to obtain an array of all parent ions that lose a common neutral fragment. Further, these *scan functions* can be changed many times during analysis, so that one can detect and measure butyl esters of acylcarnitines (by the signature ion at m/z 85) and the butyl esters of a-amino acids (by loss of a neutral 102 fragment) in the same sample.

MS/MS permits very rapid, sensitive and, with appropriate internal standards, accurate measurement of many different types of metabolites with minimal sample preparation and without prior chromatographic separation. Because many amino acidemias, organic acidemias, and disorders of fatty acid oxidation can be detected in 1 to 2 minutes, the system has adequate throughput to handle the large number of samples that are processed in newborn screening programs. Some conditions that can be diagnosed by MS/MS are listed in Table 1, together with the compound(s) on which diagnosis is based.

Table 1 Some disorders detectable by tandem mass spectrometry

Disorder	Diagnostic metabolite
Amino acidemias	
Phenylketonuria	Phenylalanine & tyrosine
Maple syrup urine disease	Leucine 1 isoleucine
Homocystinuria (CBS deficiency)	Methionine
Citrullinemia	Citrulline
Hepatorenal tyrosinemia	Methionine & tyrosine
Organic acidemias	
Propionic acidemia	C3 acylcarnitine
Methylmalonic acidemia(s)	C3 acylcarnitine
Isovaleric acidemia	Isovalerylcarnitine
Isolated 3-methylcrotonylglycinemia	3-Hydroxyisovalerylcarnitine
Glutaric acidemia (type I)	Glutarylcarnitine
Hydroxymethylglutaric acidemia	Hydroxymethylglutarylcarnitine
Fatty acid oxidation disorders	
SCAD deficiency	C4,6 acylcarnitines
MCAD deficiency	C8,10:1 acylcarnitines
VLCAD deficiency	C14,14:1,16,18 acylcarnitines

LCHAD and trifunctional protein deficiency	C14,14:1,16,18 acyl- and 3-hydroxy acylcarnitines
Glutaric acidemia type II	Glutarylcarnitine
CPT-II deficiency	C14,14:1,16,16:1 acylcarnitines

It is important to note that MS/MS cannot replace current programs to screen for biotinidase deficiency, hypothyroidism, hemoglobinopathies, virilizing adrenal hyperplasia, and galactosemia; these conditions cannot be identified by MS/MS at this time and must be detected by other means.