



**REGION 6 PRIORITY ACTIVITY 1 PROJECT  
Laboratory Quality Assurance  
(HRSA 07-016)**

**SUMMARY OF RESULTS OF EDUCATIONAL CHALLENGE #1**

The blood spots used in this educational challenge, (EC), were prepared and stored at -20 °C under vacuum until shipment. The variability of the preparation was evaluated by analyzing the blood spots at different times and on different instruments prior to shipping. A summary of these results are shown in Table 1 and Figures 1 and 2.

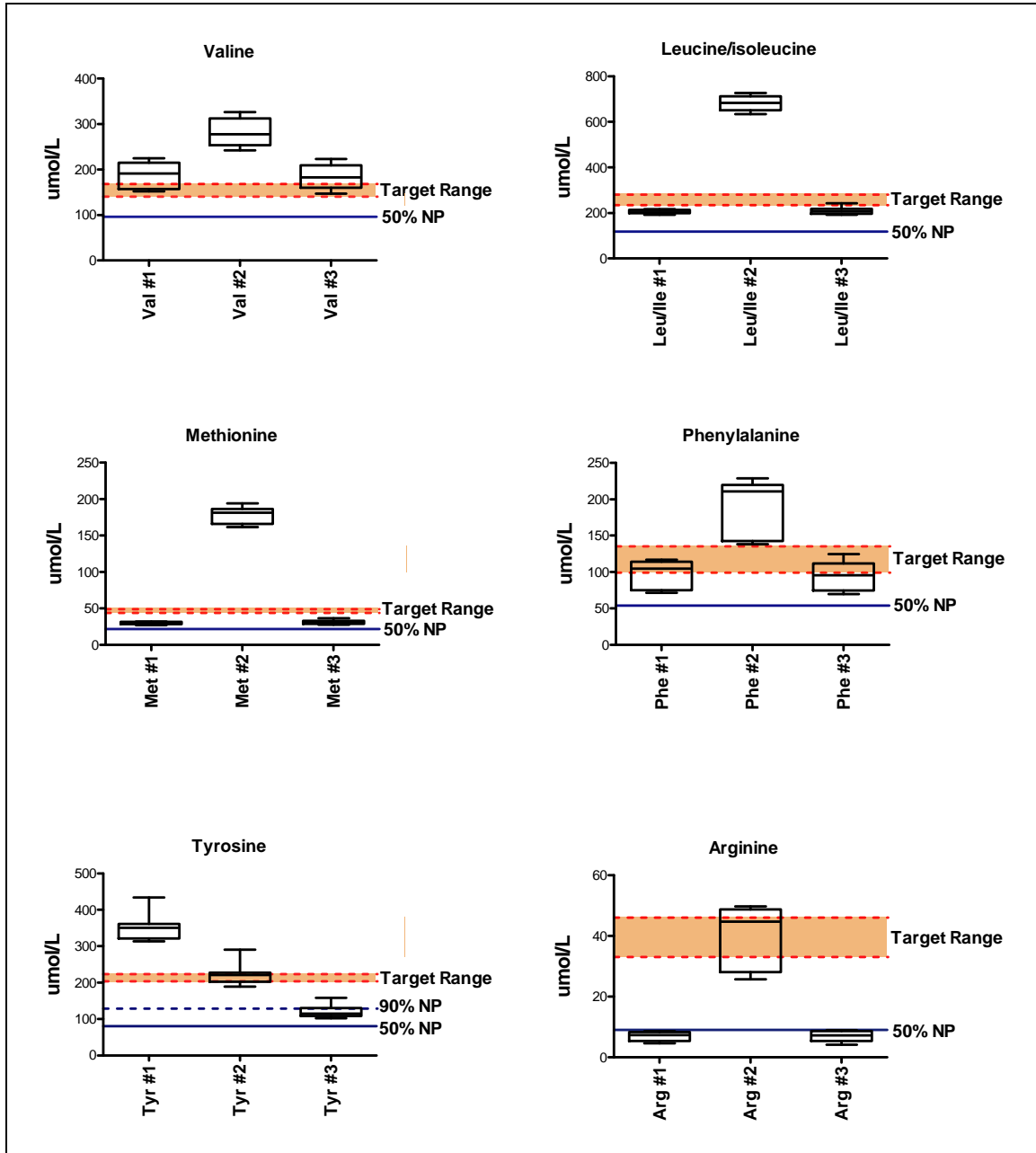
The focus of the educational challenge was the amino acid tyrosine. Three sets of blood spots were prepared. The first set reproduced the pattern of an infant with tyrosinemia type II (10.08.EC#1); the second set reproduced the pattern of an infant receiving intravenous hyperalimentation (10.08.EC#2); and the third set reproduced the pattern of an infant with tyrosinemia type I (10.08.EC#3).

Three blood spots, one of each pattern, were sent to the participating laboratories and a questionnaire for each spot was distributed electronically.

SPOT	AMINO ACID/RATIO	MEAN	SD	
<b>10.08.EC#1</b>	Valine (Val)	186	30	
	Leucine/Isoleucine (Leu/Ile)	205	8.6	
	Methionine (Met)	30	1.7	
	Phenylalanine (Phe)	95	20	
	<b>Tyrosine (Tyr)</b>	<b>352</b>	<b>36</b>	
	Arginine (Arg)	6.8	1.5	
	Valine / Phenylalanine (Val/Phe)	2.0	0.17	
	Leucine/Isoleucine / Phenylalanine (Leu/Phe)	2.3	0.46	
	Leucine/Isoleucine / Alanine (Leu/Ala)	0.65	0.20	
	Methionine / Phenylalanine (Met/Phe)	0.33	0.79	
	Phenylalanine / Tyrosine (Phe/Tyr)	0.27	0.060	
	<b>Succinylacetone</b>	<b>0.31</b>	<b>0.16</b>	
	<b>10.08.EC#2</b>	Valine (Val)	282	32
		Leucine/Isoleucine (Leu/Ile)	681	33
Methionine (Met)		178	11	
Phenylalanine (Phe)		185	41	
<b>Tyrosine (Tyr)</b>		<b>222</b>	<b>29</b>	
Arginine (Arg)		39	11	
Valine / Phenylalanine (Val/Phe)		1.6	0.20	
Leucine/Isoleucine / Phenylalanine (Leu/Phe)		3.8	0.78	
Leucine/Isoleucine / Alanine (Leu/Ala)		2.2	0.75	
Methionine / Phenylalanine (Met/Phe)		1.1	0.27	
Phenylalanine / Tyrosine (Phe/Tyr)		0.84	0.21	
<b>Succinylacetone</b>		<b>0.31</b>	<b>0.16</b>	
<b>10.08.EC#3</b>		Valine (Val)	184	27
		Leucine/Isoleucine (Leu/Ile)	209	16
	Methionine (Met)	31	2.8	
	Phenylalanine (Phe)	95	19	
	<b>Tyrosine (Tyr)</b>	<b>120</b>	<b>17</b>	
	Arginine (Arg)	7.0	1.7	
	Valine / Phenylalanine (Val/Phe)	2.0	0.19	
	Leucine/Isoleucine / Phenylalanine (Leu/Phe)	2.3	0.45	
	Leucine/Isoleucine / Alanine (Leu/Ala)	0.63	0.21	
	Methionine / Phenylalanine (Met/Phe)	0.34	0.074	
	Phenylalanine / Tyrosine (Phe/Tyr)	0.79	0.13	
	<b>Succinylacetone</b>	<b>6.1</b>	<b>1.2</b>	

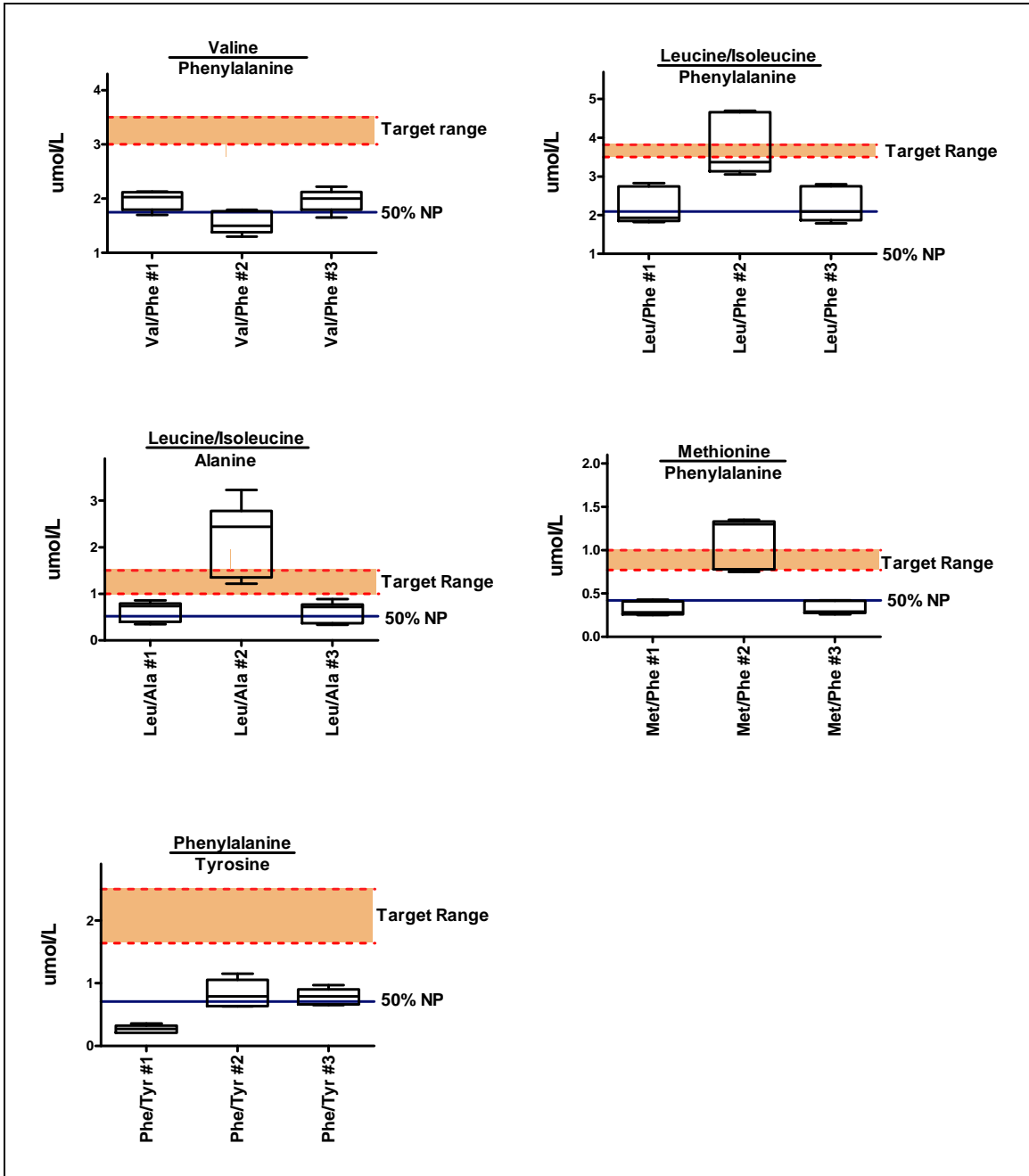
*Table 1. Concentration of amino acids, expressed in  $\mu\text{mol/L}$ , and ratios of amino acids measured in the three EC blood spots. The values are the mean of 9 replicates.*

## AMINO ACIDS



**Figure 1.** Min to Max box and whisker plots of amino acids measured in the three EC blood spots (9 replicates). Spot #1 = Tyrosinemia type II; spot #2 = Intravenous hyperalimentation; spot #3 = Tyrosinemia type I. The blue solid line represents the 50% of the Normal Population, the blue dotted line, in the Tyrosine panel, represents the 90% of the Normal Population, and the pink shaded area represents the Target Range of the cut-off for the specific analyte (values obtained from Region 4 MS/MS Collaborative Project ([www.region4genetics.org/msms\\_data\\_project/data\\_project\\_home.aspx](http://www.region4genetics.org/msms_data_project/data_project_home.aspx)))

## RATIOS OF AMINO ACIDS



**Figure 2.** Min to Max box and whisker plots of ratios of amino acids measured in the three EC blood spots (9 replicates). Spot #1 = Tyrosinemia type II; spot #2 = Intravenous hyperalimentation; spot #3 = Tyrosinemia type I. The blue line represents the 50% of the Normal Population and the pink shaded area represents the Target Range of the cut-off for the specific analyte (values obtained from Region 4 MS/MS Collaborative Project ([www.region4genetics.org/msms\\_data\\_project/data\\_project\\_home.aspx](http://www.region4genetics.org/msms_data_project/data_project_home.aspx))).

### GENERAL INFORMATION

Five laboratories participated in this educational challenge.

The following tables list the amino acids and acylcarnitines that can be identified by MS/MS and the percentage of laboratories participating in this project monitoring the different species.

AMINO ACID	% OF LABORATORIES MONITORING
Cit, Leu/Ile, Met, Phe, Tyr	100 %
Arg, Val	80 %
Ala, ASA, Gly, Homocit, Orn	60 %
Pro, SUAC	40 %

ACYLCARNITINES	% OF LABORATORIES MONITORING
C0, C3, C5, C5OH, C5DC, C6, C8, C10, C14, C14:1, C16, C16OH, C18, C18:1, C18OH, C18:1OH	100 %
C4, C3DC, C4DC, C6DC, C10:1, C14OH, C16:1, C16:1OH	80 %
C2, C4OH, C8:1, C10:2, C12, C12OH, C14:2, C18:2	60 %

Forty percent of the laboratories perform second tier tests, included are second tier tests for succinylacetone, methylmalonic acid, methylcitric acid, ethylmalonic acid, homocysteine, allo-isoleucine. Sixty percent of the laboratories do not perform any 2nd tier tests.

### EDUCATIONAL CHALLENGES 10.08.EC# 1-3

The focus of the first set of educational challenges was on tyrosine.

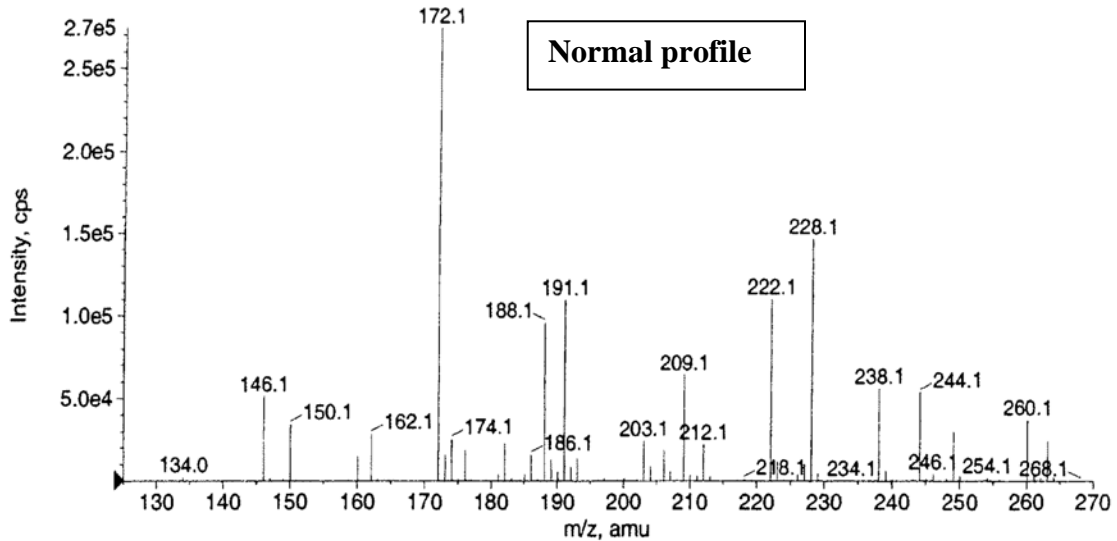
Spot #10.08.EC#1 was from a tyrosinemia type II case

Spot #10.08.EC#2 was from an infant receiving intravenous hyperalimentation

Spot #10.08.EC#3 was from a tyrosinemia type I case

The disorders of tyrosine metabolism and the other causes of elevated tyrosine in the newborn period are discussed in the Educational Piece (see attached).

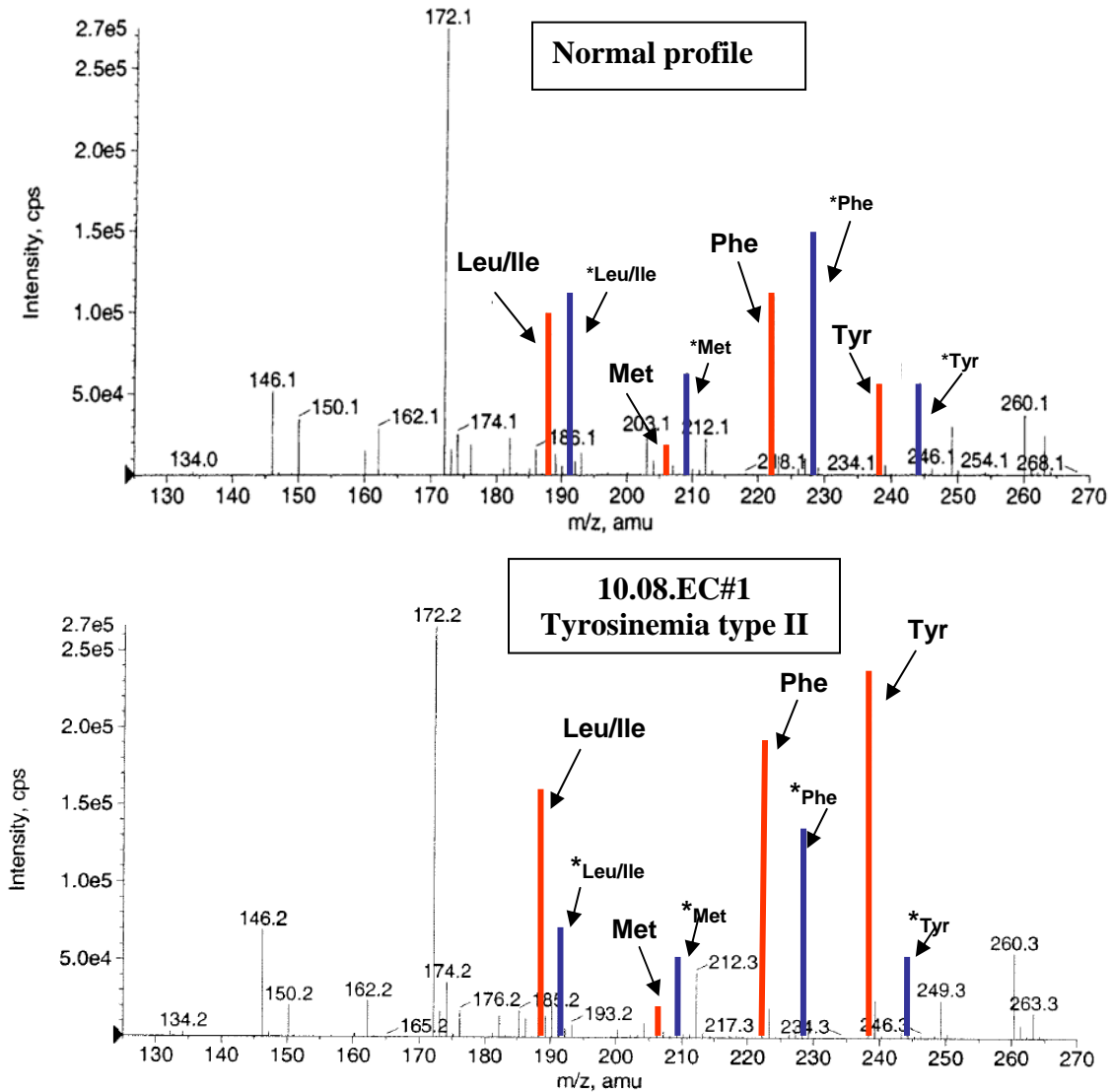
Each profile will be discussed separately. A normal amino acid profile (neutral loss of 102) is shown in Figure 3.



**Figure 3.** Normal amino acid profile acquired using NLS (102) from a blood spot. The m/z ratio corresponds to the butyl esters of the amino acids:

<i>m/z</i>	<i>Amino acid</i>
146	Alanine
150	<i>d4</i> -Alanine
162	Serine
172	Proline
174	Valine
188	Leucine/Isoleucine
191	<i>d3</i> -Leucine
206	Methionine
209	<i>d3</i> -Methionine
222	Phenylalanine
228	<i>d6</i> -Phenylalanine
238	Tyrosine
244	<sup>13</sup> C6-Tyrosine

## SPOT 10.08.EC#1 - TYROSINEMIA TYPE II



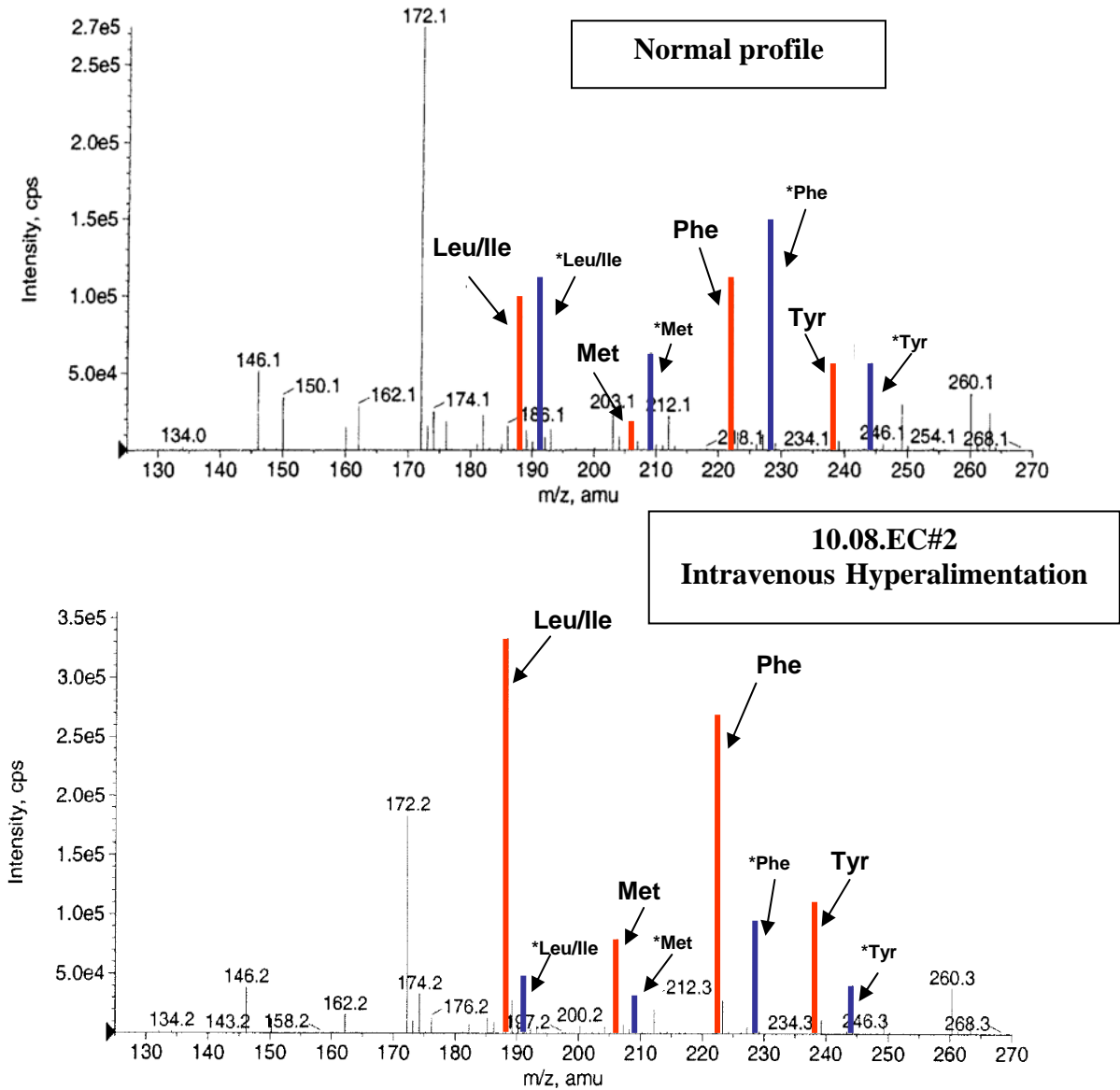
**Figure 4.** MS/MS amino acid profile of a normal control and of a tyrosinemia type II (10.08.EC#1) case. Red lines indicate the most informative amino acids, blue lines are their respective deuterated Internal Standards (IS); m/z corresponds to the butyl esters of the amino acids.

The primary marker in newborn blood spots for tyrosinemia type II is tyrosine. The elevation of tyrosine is quite significant, even in a newborn, and no other amino acids are elevated (Fig.4). Succinylacetone was not elevated in this spot, excluding tyrosinemia type I. Using a cut-off for tyrosine within the target range suggested by the Region 4 Collaborative Project, allows the identification of infants with tyrosinemia type II (Fig. 1) ([www.region4genetics.org/msms\\_data\\_project/data\\_project\\_home.aspx](http://www.region4genetics.org/msms_data_project/data_project_home.aspx)). The same pattern of analytes observed in this blood spot, can also be seen in transient tyrosinemia of the newborn. In this condition tyrosine usually decreases with time, while in

tyrosinemia type II the tyrosine level increases with time. Although the ratio Phe/Tyr is not used as a secondary marker for this condition, it is evident from Figure 2 that this ratio is reduced compared to the other spots. This is also observed in the plots by marker posted in the Region 4 MS/MS collaborative project web-site where the lowest Phe/Tyr ratios are observed in tyrosinemia type II, III and transient tyrosinemia of the newborn.

Requesting plasma amino acids or a repeat screen are both adequate follow-up actions in this case, with more targeted tests (urine organic acids to formally exclude tyrosinemia type I and confirm the presence of other metabolites, DNA analysis [not currently available in the USA]) depending on the result of the plasma amino acids or of the repeat screen. Use of adequate cut-offs is critical in detecting tyrosinemia type II. In states mandating two screens, the laboratory performing the newborn screening test should always refer to the results of the first screen when an elevated tyrosine is identified on a second screen test, and follow-up accordingly.

**SPOT 10.08.EC#2 - INTRAVENOUS HYPERALIMENTATION**



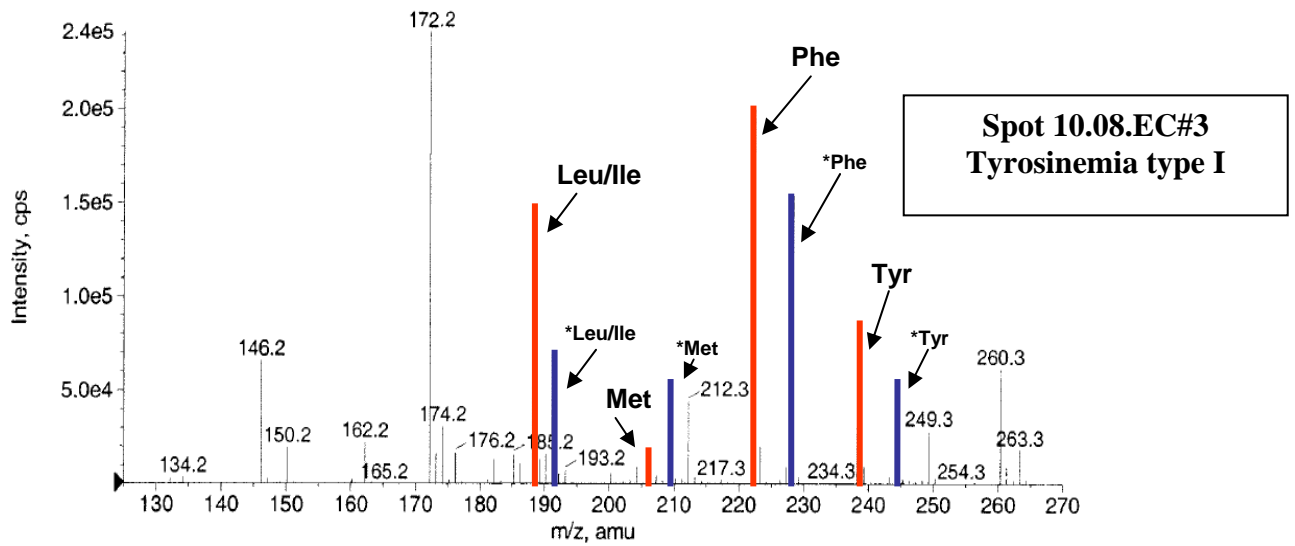
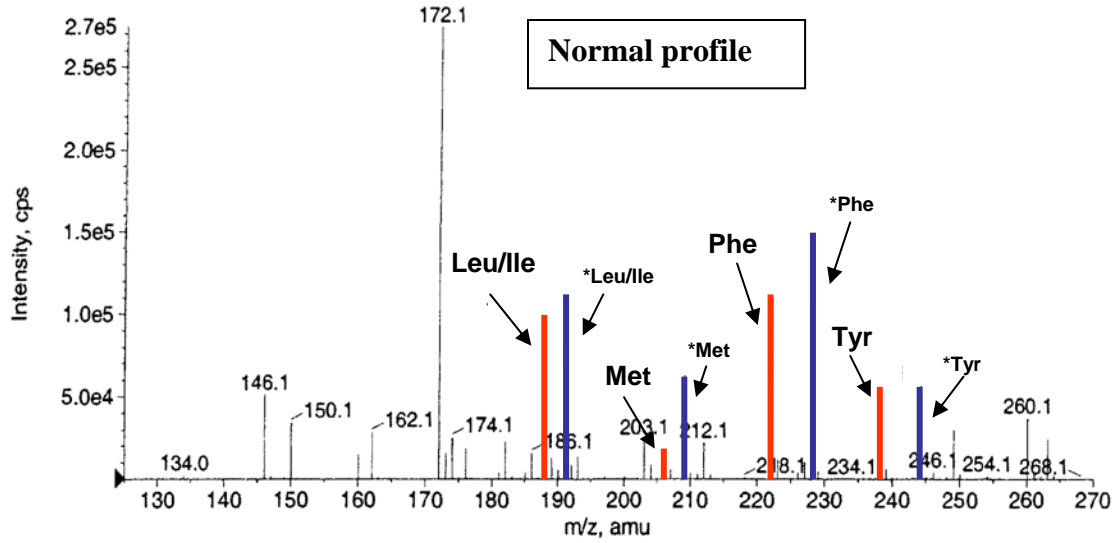
**Figure 5.** MS/MS amino acid profile of a normal control and of an infant receiving intravenous hyperalimentation (10.08.EC #2). Red lines indicate the most informative amino acids and blue lines are their respective deuterated Internal Standards (IS); m/z corresponds to the butyl esters of the amino acids.

This blood spot was from a very-low birth weight infant (670 g). Several amino acids were present at a concentration above the recommended target range of their respective cut-off (Fig.5). Although Leu/Ile has the highest concentration and several of the ratios typically used as secondary markers for MSUD (Leu/Ala, Leu/Phe, Val/Phe) are

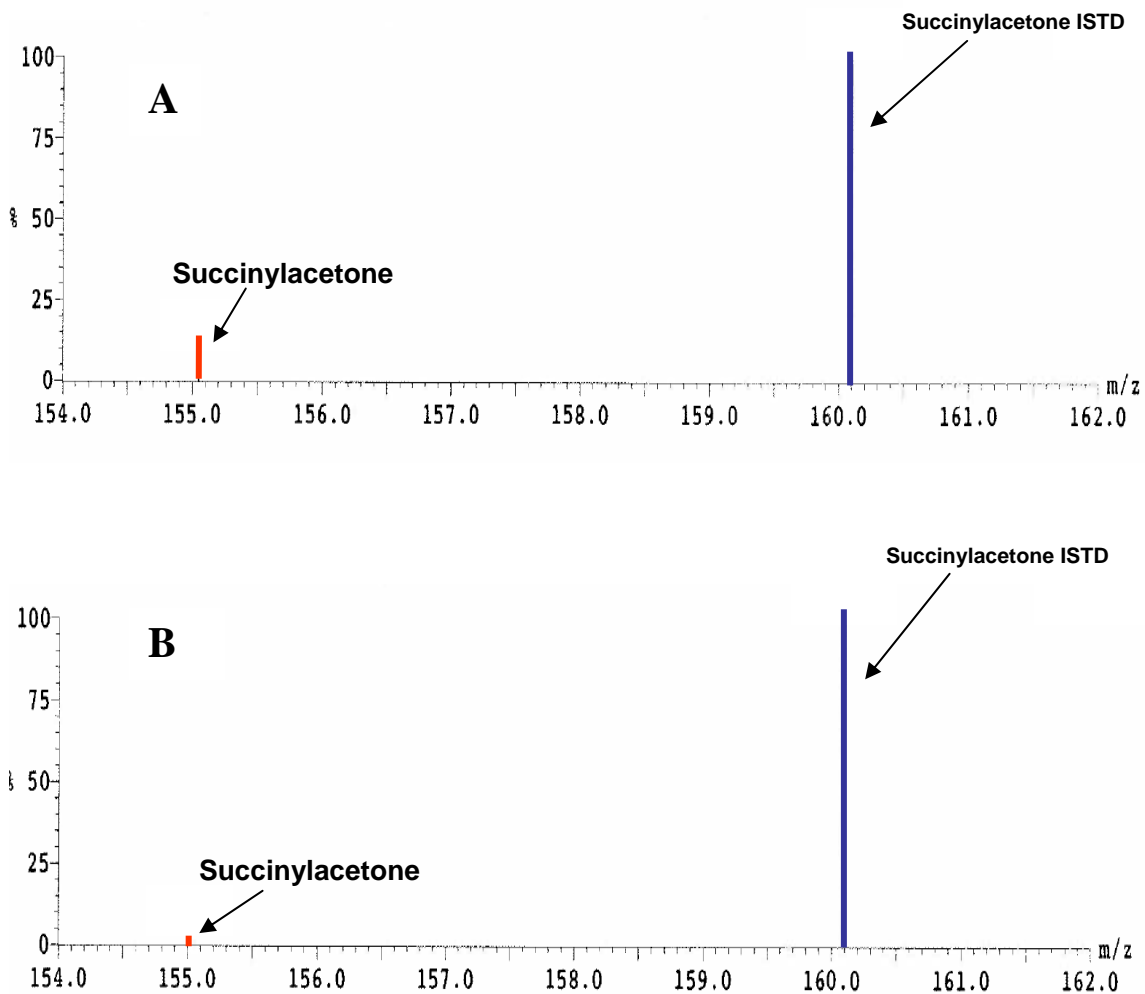
increased (Fig. 2), the birth weight and the elevated concentrations of other amino acids (Tyr, Phe, Met) should point to the correct interpretation of an infant receiving hyperalimentation. Although tyrosine is elevated, succinylacetone was not elevated in this sample, excluding tyrosinemia type I. A comparison with spot #10.08EC#1 (tyrosinemia type II) highlights the differences between these two patterns. Several intravenous hyperalimentation formulations contain high concentrations of branched chain amino acids, resulting in elevated leucine/isoleucine and valine. A second tier test to detect allo-isoleucine would be indicated and useful in excluding the possibility of MSUD. Because methionine is also elevated, the measurement of total homocysteine as a second tier test could exclude homocystinuria. There are really no other conditions presenting with this pattern of multiple elevated amino acids. Very-low birth weight infants with PKU, receiving hyperalimentation, have a ratio Phe/Tyr markedly elevated, which was not the case in this sample.

Laboratories/States with a specific screening protocol for pre-term/sick babies should comply with their protocol. In absence of a specific protocol, a repeat screen would be adequate to follow-up on this case. In this case, it is important to instruct the hospital staff to collect the sample after intravenous hyperalimentation has been discontinued for at least two hours. Evaluation of plasma amino acids when the infant is discharged from the hospital and is on regular feeds for at least 48 hours may also be indicated. There is no indication for other confirmatory tests, such as urine amino acids and urine organic acids, unless results of the follow-up sample are suggestive of a metabolic disorder.

## SPOT 10.08.EC#3 - TYROSINEMIA TYPE I



**Figure 6.** MS/MS amino acid profile of a tyrosinemia type I (10.08.EC#3) case. Red lines indicate the most informative amino acids, blue lines are their respective deuterated Internal Standards (IS); m/z corresponds to the butyl esters of the amino acids.



**Figure 7.** Selective Reaction Monitoring (SRM) scan for succinylacetone in A) Tyrosinemia type I case; B) Normal Control. The red line indicates succinylacetone in the sample, the blue line is the Internal Standard.

In this sample the concentration of amino acids was within the normal range (Figures 6,2). The concentration of tyrosine was between the 90<sup>th</sup> and the 99<sup>th</sup> percentiles of the value for the normal population (Fig.2), below the target range of the cut-off for tyrosine. The concentration of tyrosine in this spot represents, according to the Region 4 MS/MS Collaborative Project data, approximately the 25<sup>th</sup> percentile of the tyrosine concentration observed in newborn screens of patients with tyrosinemia type I. The only abnormality was the elevated concentration of succinylacetone (Figure 7, Table 1). The correct assessment for this sample was possible only for laboratories monitoring succinylacetone as part of the primary screen, or, for laboratories performing analysis of succinylacetone as a second tier test, if the cut-off for tyrosine was set sufficiently low (at least at the 90<sup>th</sup>

percentile of the Normal Population) to trigger the second tier test. The likelihood of identifying an infant with tyrosinemia type I on a second screen by monitoring only tyrosine is quite low, since the majority of routine second screens are collected between 7 and 21 days of age, when tyrosine may still not be above the cut-off value.

Appropriate follow-up for this sample involves urgent confirmatory tests (plasma amino acids and urine organic acids/succinylacetone) and referral to a metabolic center.