Measuring Phenylalanine in Dried Blood Spots:

A Comparison between An Enzymatic Assay and A Tandem Mass Spectrometry Method

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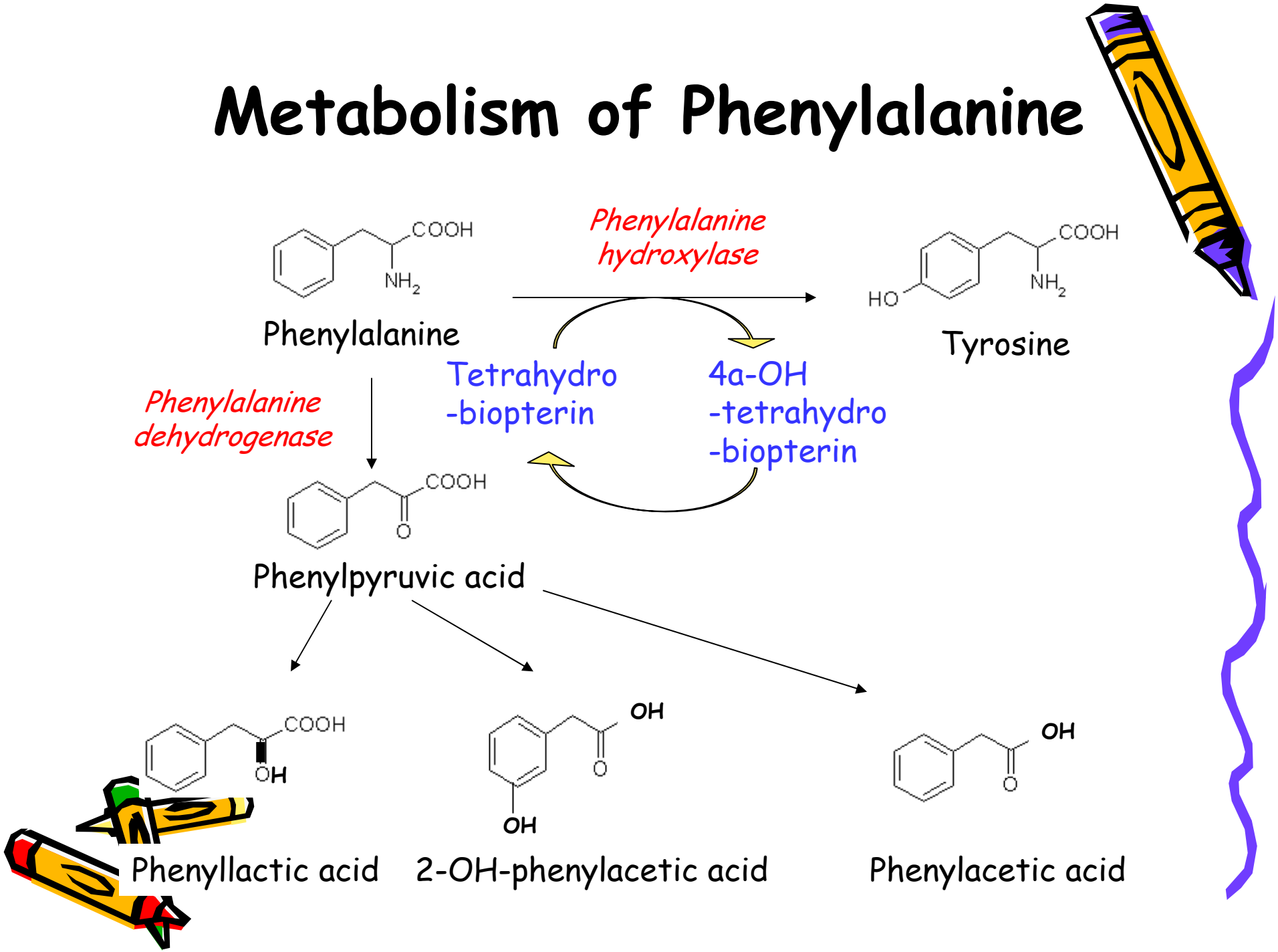
Hyperphenylalaninemias

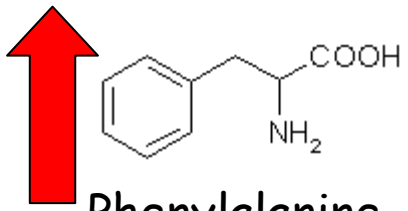
Inherited errors of metabolism caused by deficiency in the enzymes involved in phenylalanine metabolism

Classic phenylketonuria (PKU) and other types of hyperphenylalaninemias

Metabolism of Phenylalanine

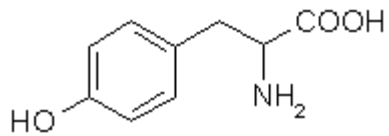


Classic Phenylketonuria (PKU)



Phenylalanine

Phenylalanine hydroxylase

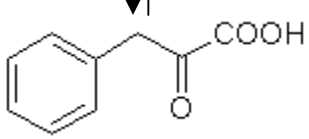


Tyrosine

Tetrahydro-biopteri

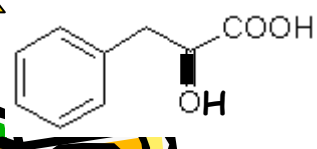
4a-OH

Transamination

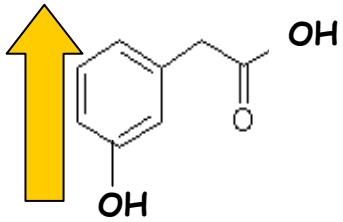


Phenylpyruvic acid

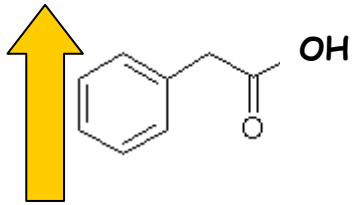
Incidence ~ 1:10,000-1:15,000
Mental retardation
Fair complexion and pigmentation
>1200umol/L
Phe/Tyr > 3
Increased urinary phe metabolites
Normal cofactor BH4



Phenyllactic acid

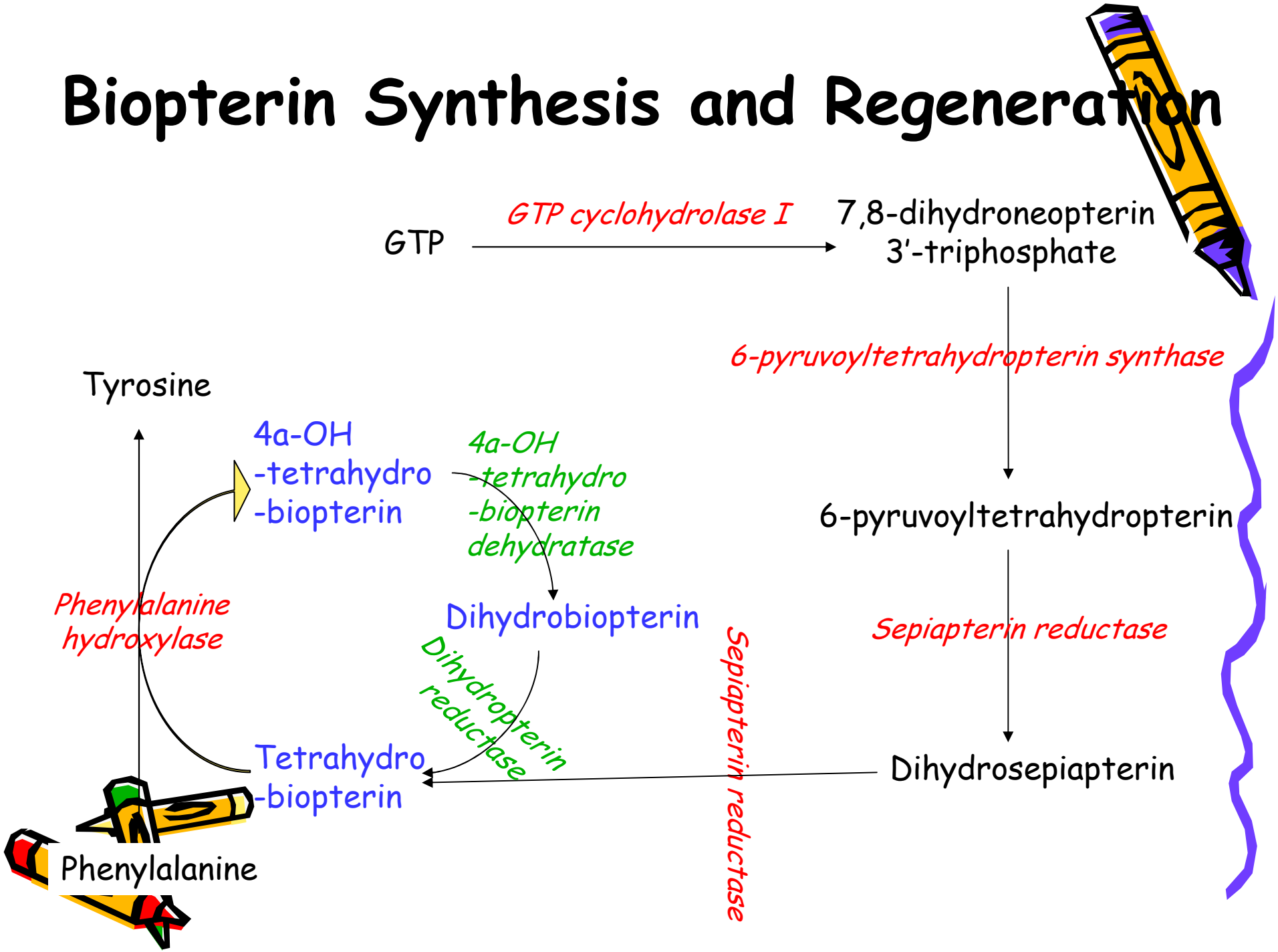


2-OH-phenylacetic acid

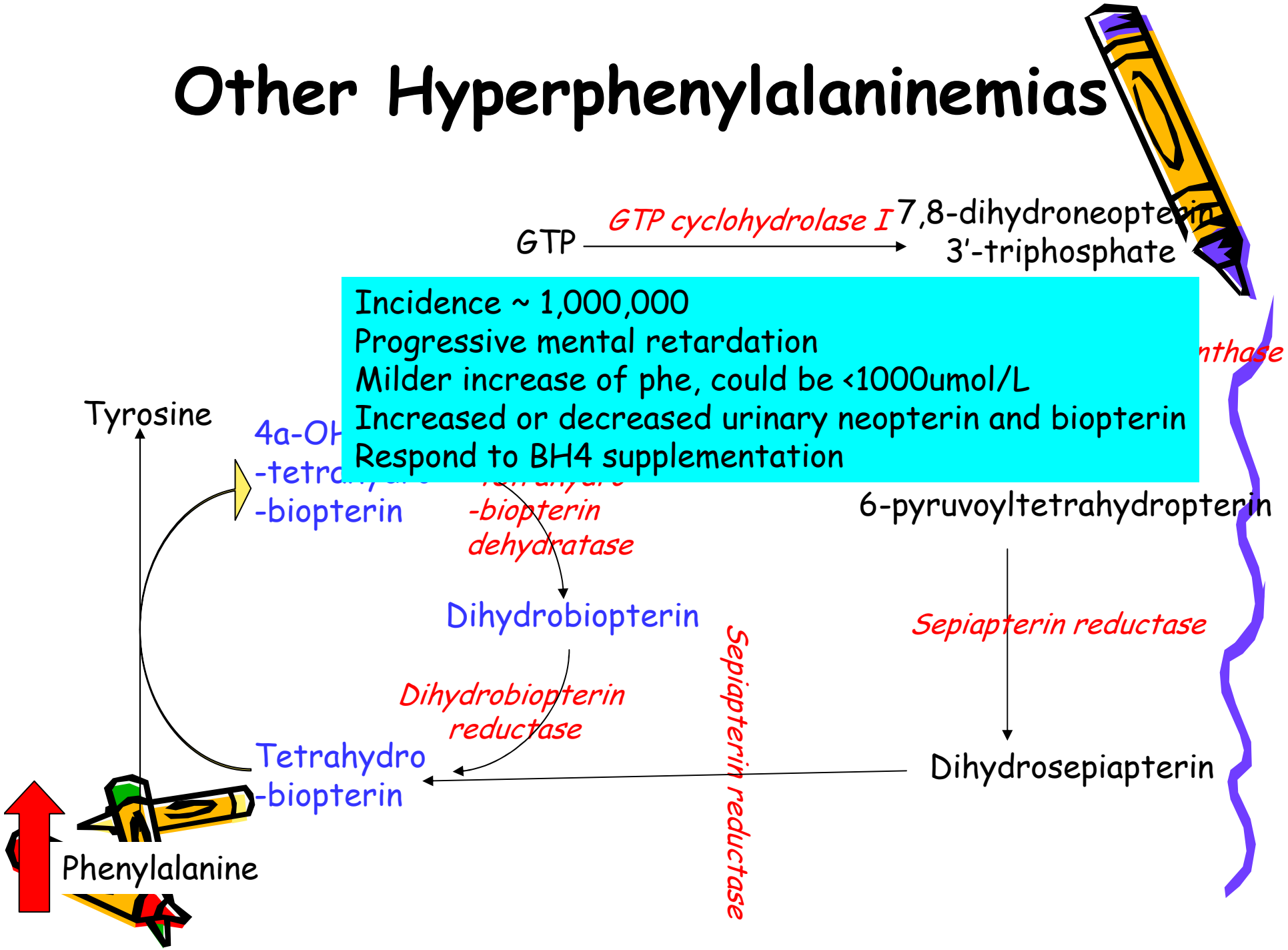


Phenylacetic acid

Biopterin Synthesis and Regeneration



Other Hyperphenylalaninemias



All Types of Hyperphenylalaninemias

Screening is done in every province of Canada and every state of USA

Prevalence 1:10,000

Autosomal recessive

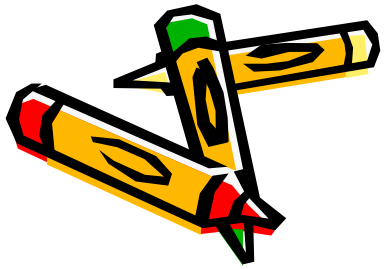
Treatment

Diet for life

BH4 treatment

Neutrotransmitter precursors supplementation

Gene therapy



What to eat


Choose the Foods Closest to the Bull's-Eye
Steak
Milk
Regular Pasta
Rice
Refried Beans
Vegetables
Corn
Pork Chops
BEST
PHENYL-FREE
FOODS
Juices
Chicken
Fruits
Peas
Bread
Eggs
Nuts
Low Protein Breads and Pastas
Potatoes
French Fries
Cheese
They are BEST for YOU
Peanut Butter
Hamburger

Collecting Blood Spots

Should not be screened until at least 24hr after birth

Measuring Methods

Guthrie Bacterial Inhibition Assay

Ninhydrin Method (Phenylalanine-ninhydrin)

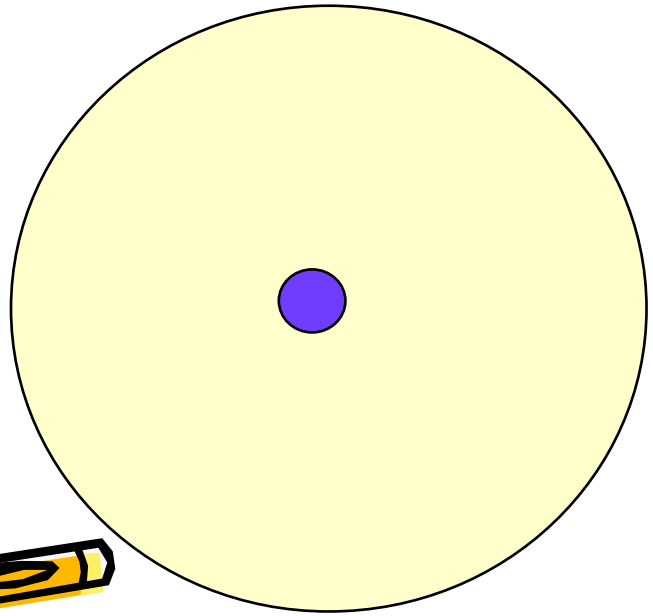
Enzymatic Method (Phenylalanine Dehydrogenase)

Tandem MS test

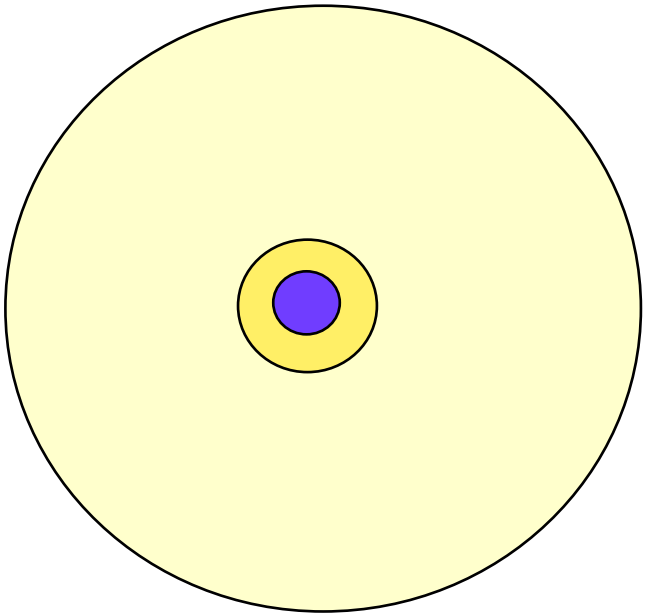
Guthrie Bacterial Inhibition Assay

Bacillus subtilis is incorporated into an agar dish along with an analogue of Phe that inhibits growth

High Phe competes with the analogue, allowing growth



Normal



PKU

Guthrie Plate for PKU screening

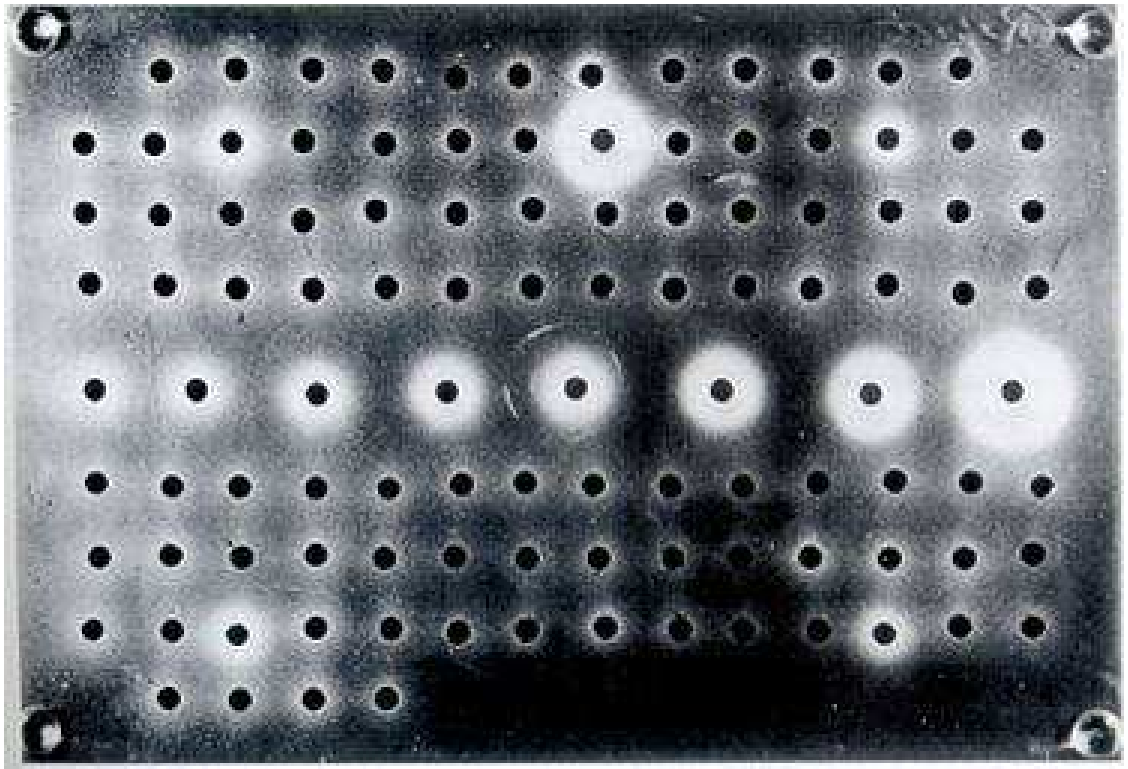
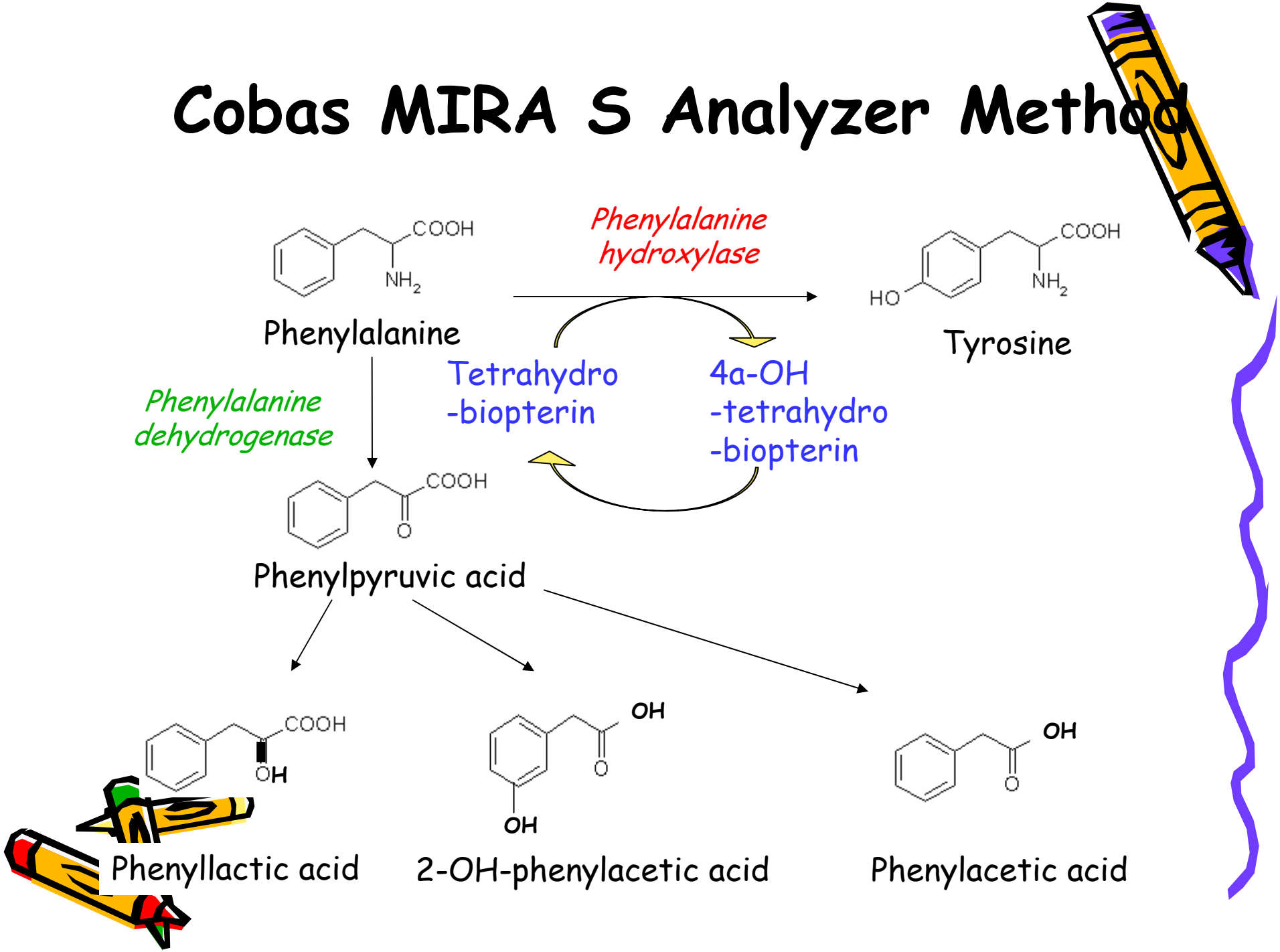
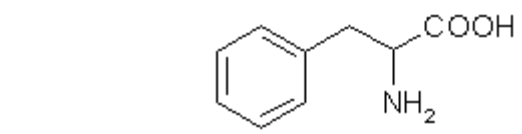


FIGURE 1-48 Guthrie plate for screening phenylketonuria. Each dark circle represents a disc of a blood spot from a newborn. The fifth row contains controls, with increasing quantities of phenylalanine from left to right. This results in a zone of bacterial growth of increasing size. The eighth sample in the second row is from a newborn with phenylketonuria, showing a large zone of bacterial growth. (Courtesy of Dr. Harvey Levy, Children's Hospital, Boston, and Massachusetts State Laboratory.)

Cobas MIRA S Analyzer Method



Cobas MIRA S Analyzer Method

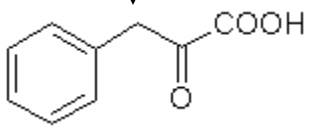


Phenylalanine

Phenylalanine dehydrogenase

NAD

NADH



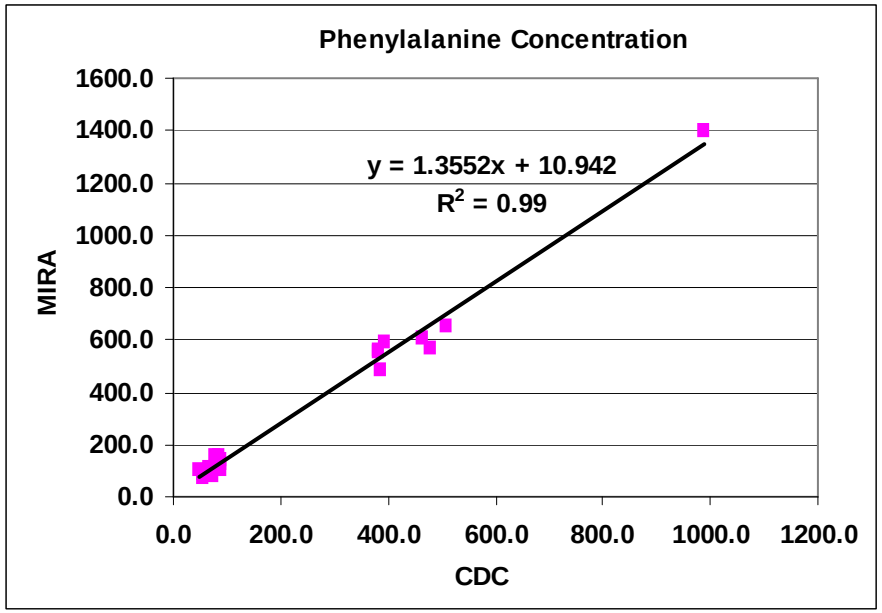
Phenylpyruvic acid

INT (idonitrotetrazolium)

Blue-purple formazan

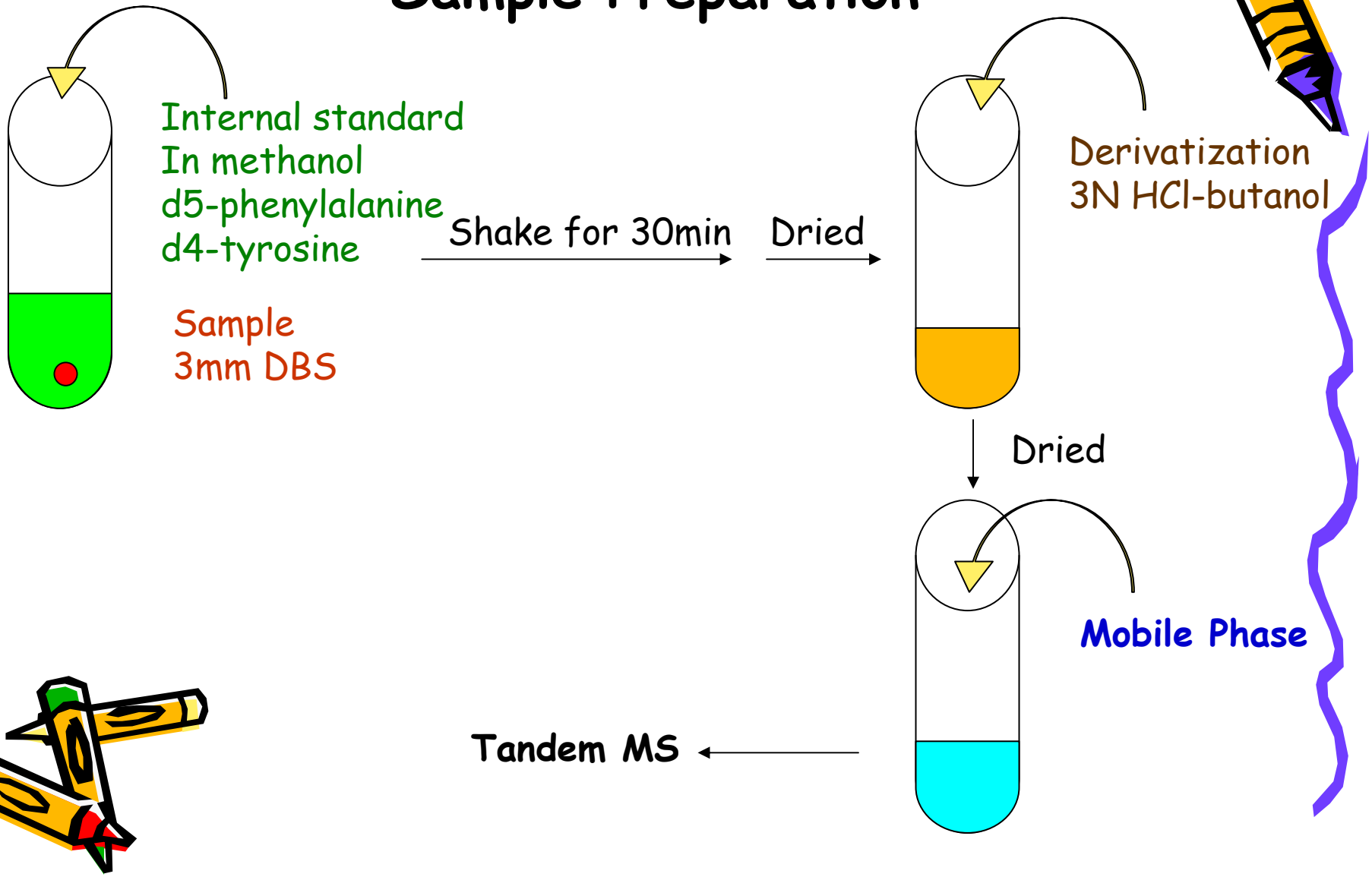
Drawbacks

- NSQ: 8mm disk to be punched out
- Positive bias in external QC program
- Interference (tyrosine and conjugate bilirubin)
- No tyrosine measured
- Out of market soon

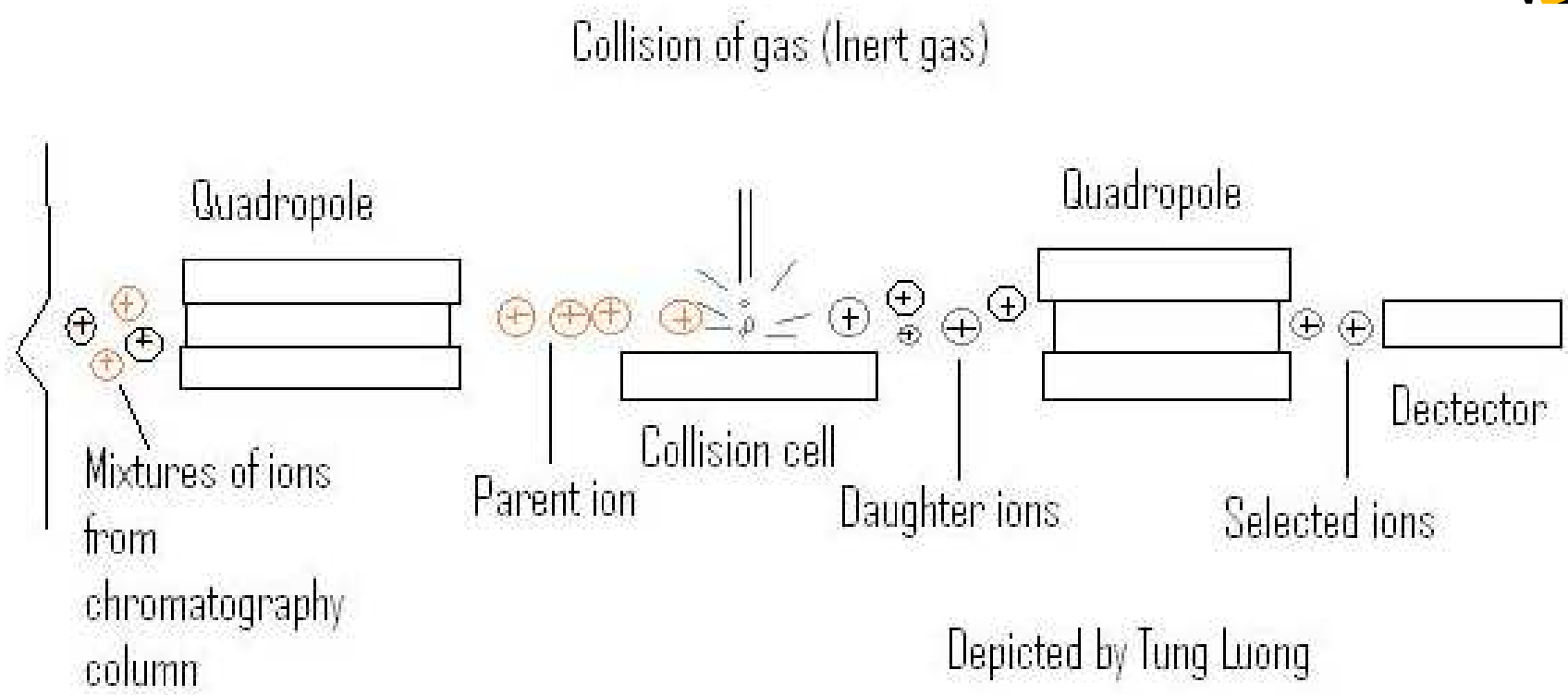


Tandem MS Method

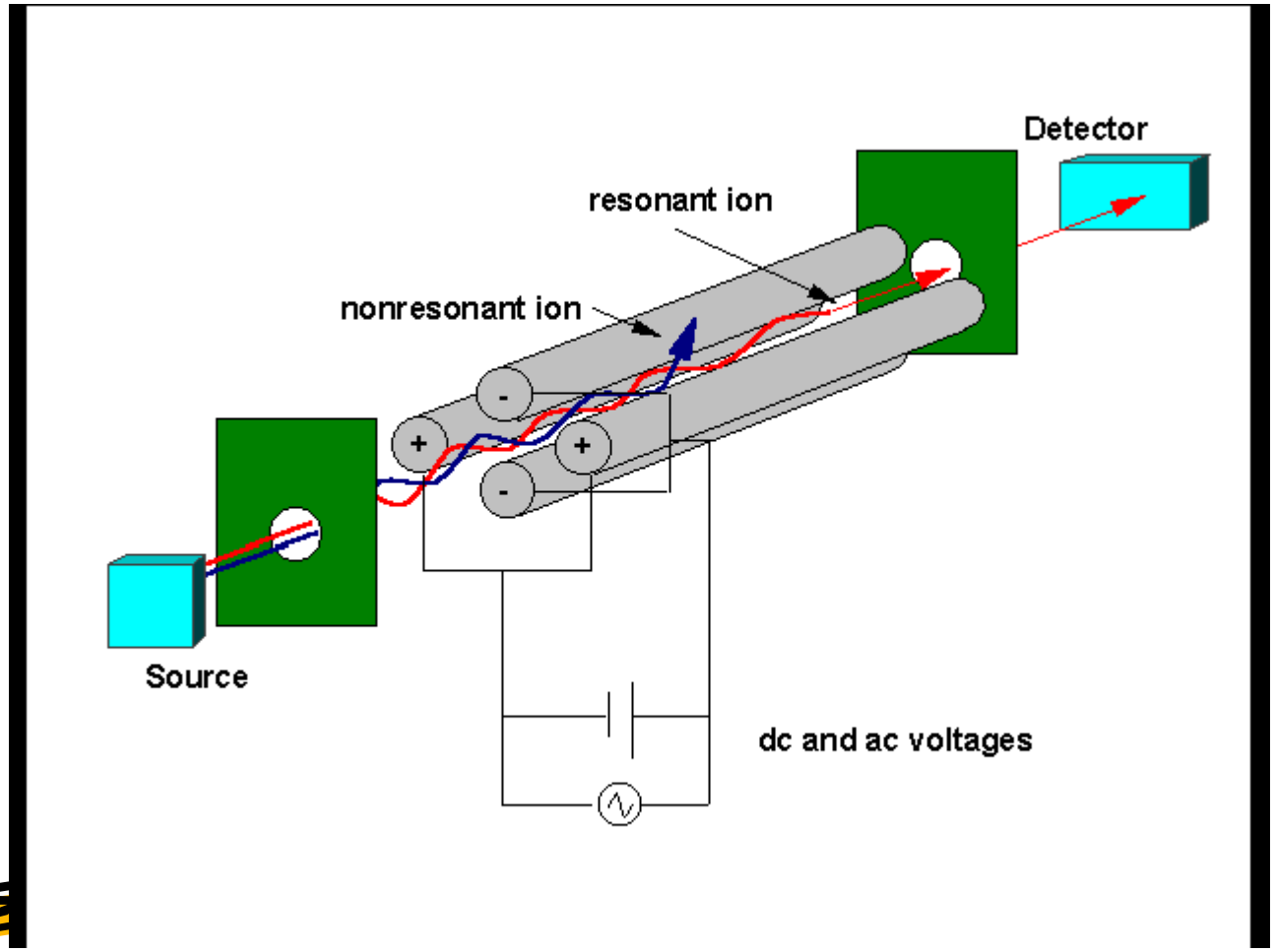
Sample Preparation



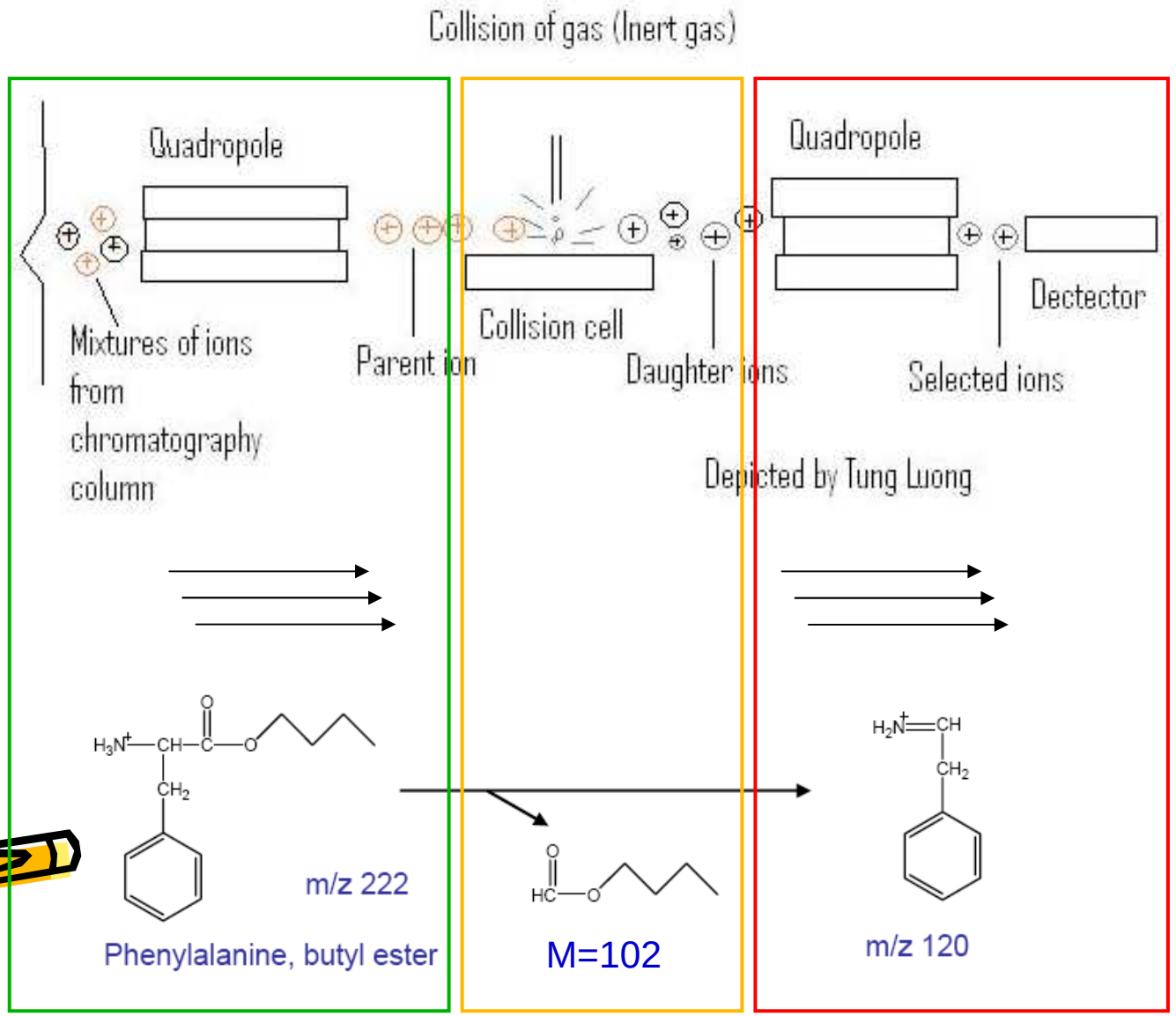
Triple Quadrupole Tandem MS



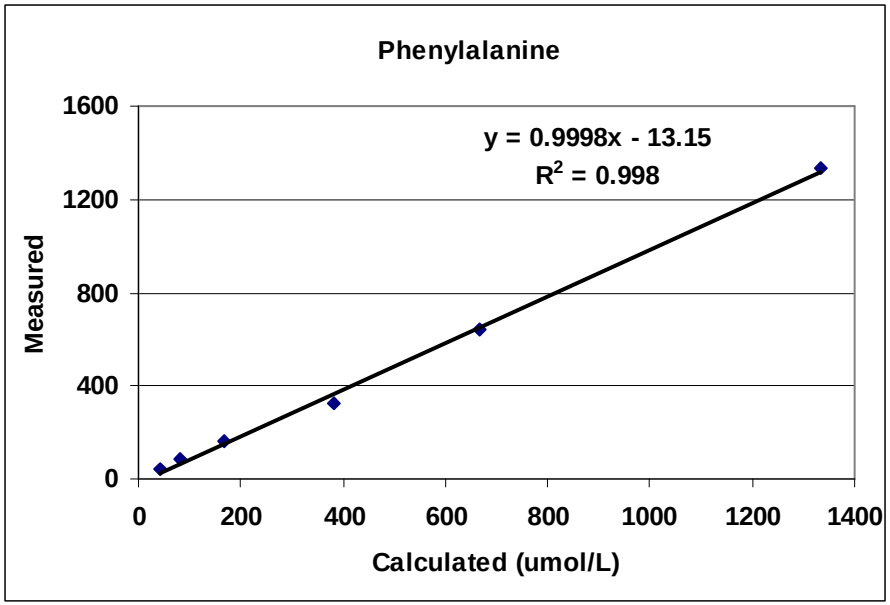
Schematic of A Quadrupole Filter

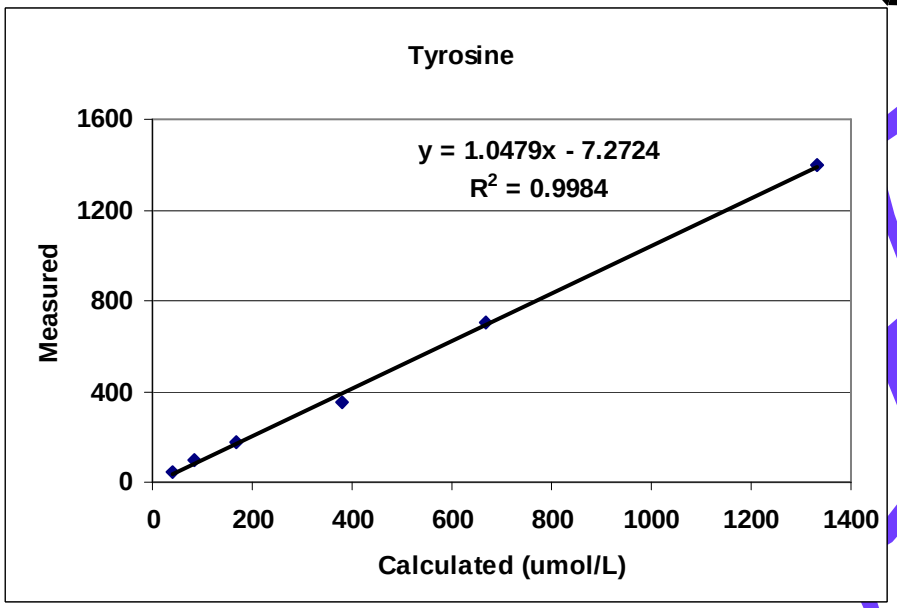


Neutral Loss Mode



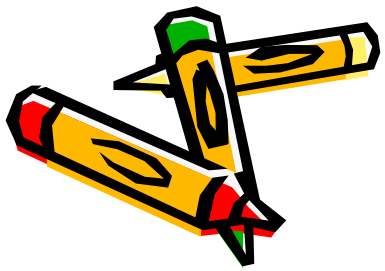
Linearity

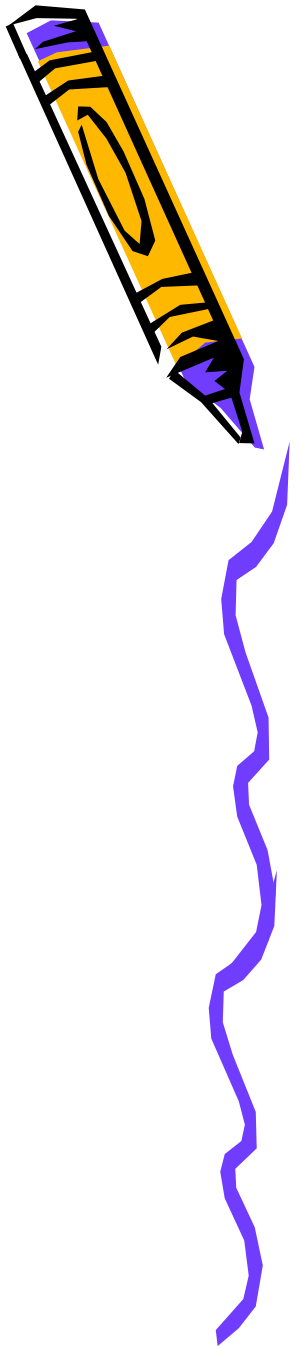

Phenylalanine
y = 0.9998x - 13.15
R2 = 0.998
Measured
Calculated (umol/L)
0
400
800
1200
1600
0
200
400
600
800
1000
1200
1400


Tyrosine
y = 1.0479x - 7.2724
R2 = 0.9984
Measured
Calculated (umol/L)
0
400
800
1200
1600
0
200
400
600
800
1000
1200
1400

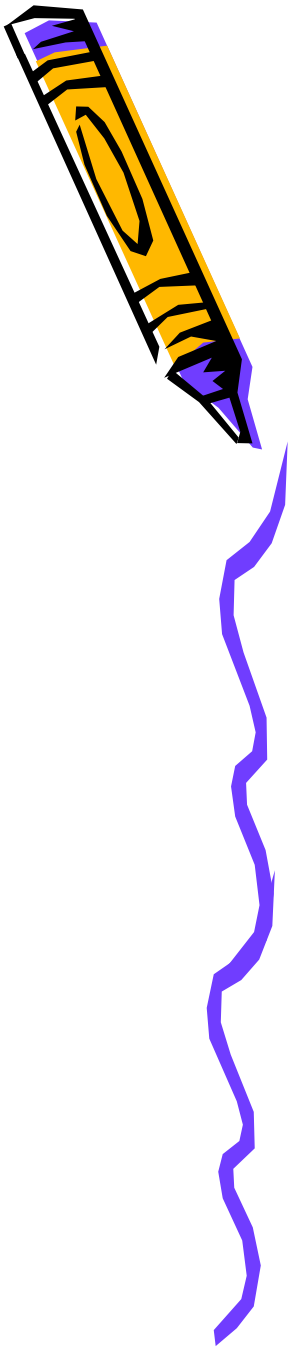
Within-run Precision

	Phe QC1	Phe QC2	Phe QC3	Tyr QC1	Tyr QC2	Tyr QC3
	83.6	220.0	487.0	76.1	195.0	463.0
	91.1	224.0	446.0	84.7	221.0	445.0
	89.0	196.0	514.0	74.7	202.0	544.0
	95.4	227.0	487.0	81.1	210.0	469.0
	85.5	211.0	517.0	72.8	201.0	501.0
	88.2	197.0	505.0	82.0	187.0	493.0
	81.3	230.0	527.0	73.4	234.0	480.0
	96.9	226.0	484.0	78.6	211.0	489.0
	88.7	229.0	459.0	75.2	207.0	432.0
	79.7	225.0	483.0	72.7	204.0	490.0
	91.0	209.0	508.0	73.2	196.0	501.0
	83.8	215.0	588.0	74.1	201.0	600.0
	83.7	208.0	450.0	76.9	204.0	468.0
	83.0	221.0	486.0	75.5	215.0	463.0
	85.9	213.0	476.0	76.6	211.0	479.0
	89.4	230.0	475.0	77.9	206.0	452.0
	94.1	205.0	470.0	84.0	203.0	464.0
	84.6	221.0	480.0	75.1	198.0	466.0
	90.1	222.0	446.0	83.7	211.0	465.0
	90.0	198.0	500.0	74.6	202.0	524.0
MEAN	87.8	216.4	489.4	77.1	206.0	484.4
SD	4.7	11.2	32.8	3.9	10.1	37.8
CV%	5.3	5.2	6.7	5.1	4.9	7.8



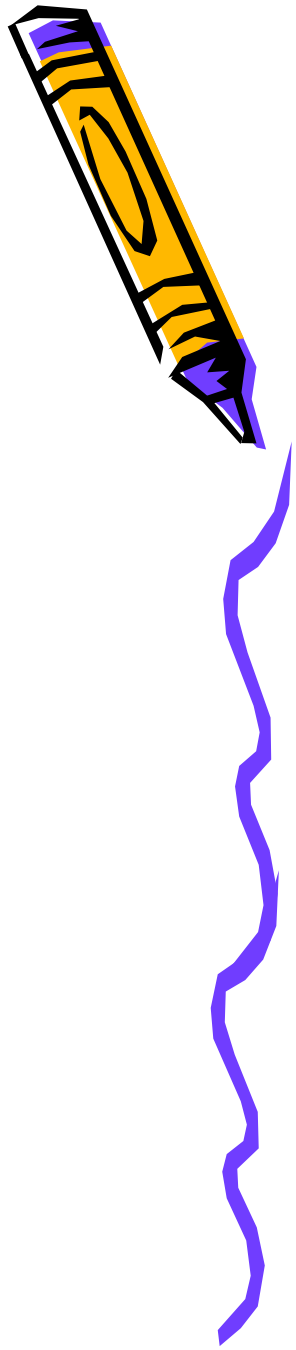


Between-run Precision



	Phe QC1	Phe QC2	Phe QC3	Tyr QC1	Tyr QC2	Tyr QC3
	93.6	234.5	508.5	78.8	215.0	509.5
	96.5	232.0	498.0	80.6	217.0	501.0
	90.6	237.0	519.0	76.9	213.0	518.0
	94.5	220.0	524.0	77.7	203.0	498.0
	92.5	208.0	541.0	74.1	191.0	534.0
	83.8	213.0	505.0	69.9	197.0	500.0
	90.4	210.0	474.0	75.7	197.0	478.0
	92.2	205.0	551.0	78.6	182.0	559.0
	91.0	217.0	507.0	70.8	195.0	490.0
	83.6	220.0	487.0	76.1	195.0	463.0
	91.1	224.0	446.0	84.7	221.0	445.0
MEAN	90.9	220.0	505.5	76.7	202.4	499.6
SD	4.0	10.9	29.7	4.2	12.4	31.7
CV%	4.4	4.9	5.9	5.5	6.1	6.3

Precision for Phenylalanine

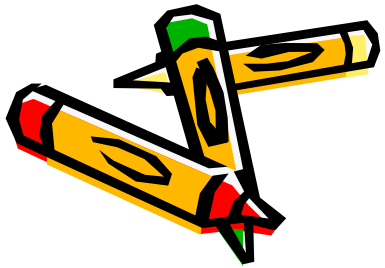


Cobas Mira S Method

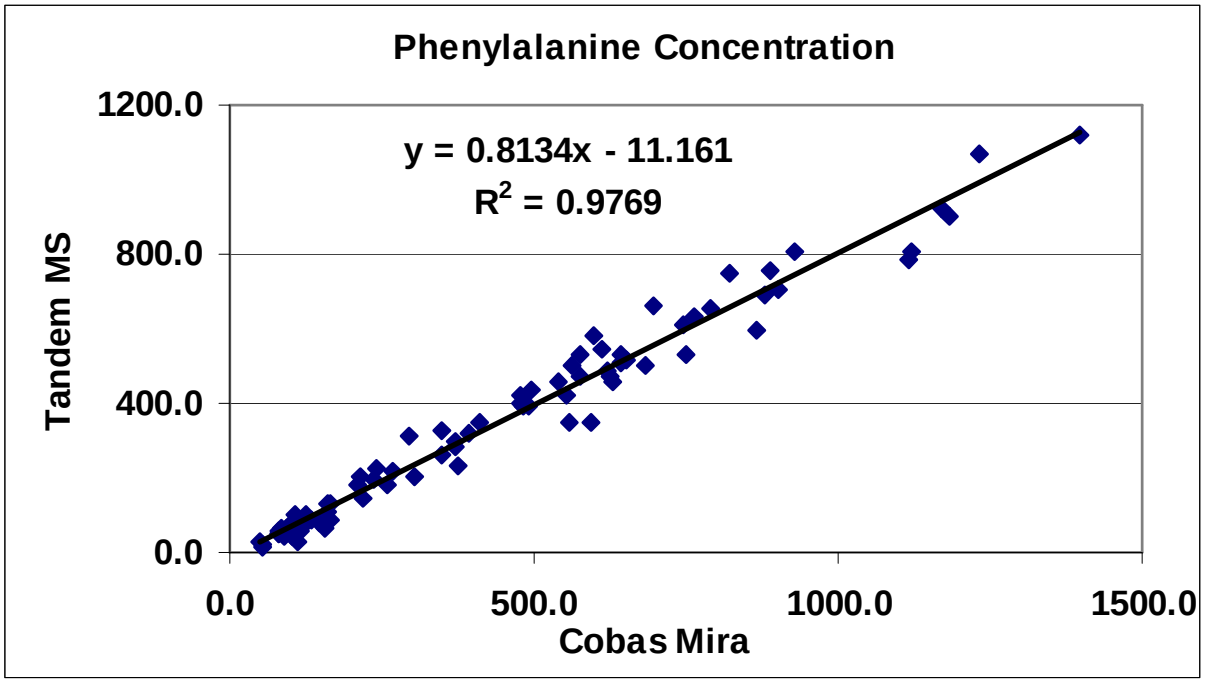
	n	Mean (μmol/L)	SD	CV%
Within-run	20	77	6.8	8.8
	20	787	42	5.3
Between-run	15	104	16	15
	17	748	42	5.6

Tandem MS Method

	n	Mean (umol/L)	SD	CV%
Within-run	20	88	5	5.3
	20	216	11	5.2
	20	489	33	6.7
Between-run	11	91	4	4.4
	11	220	11	4.9
	11	506	30	5.9

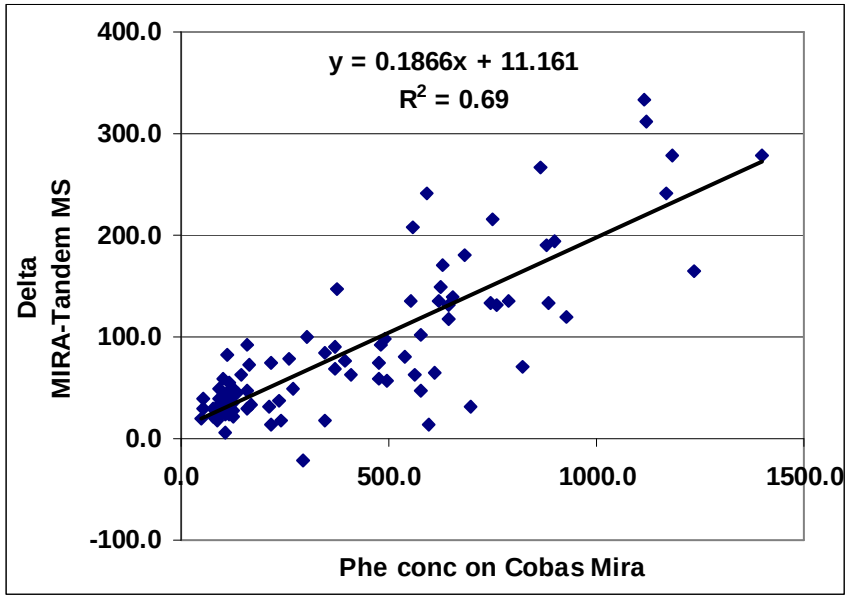


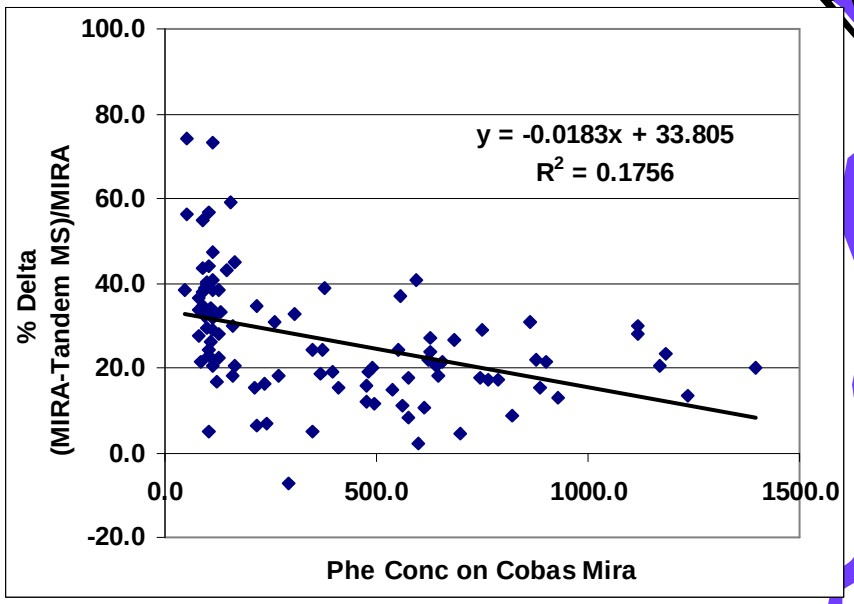
Correlation



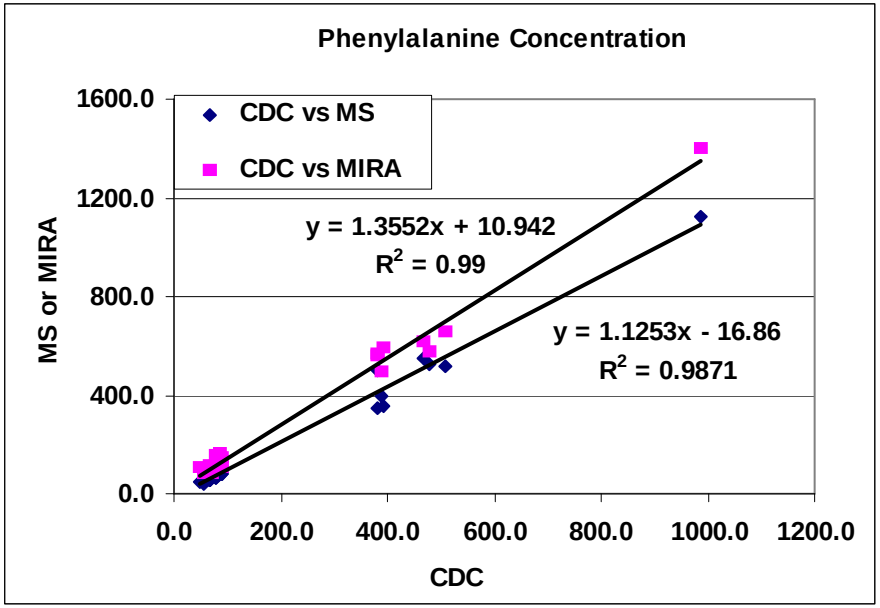
N=95

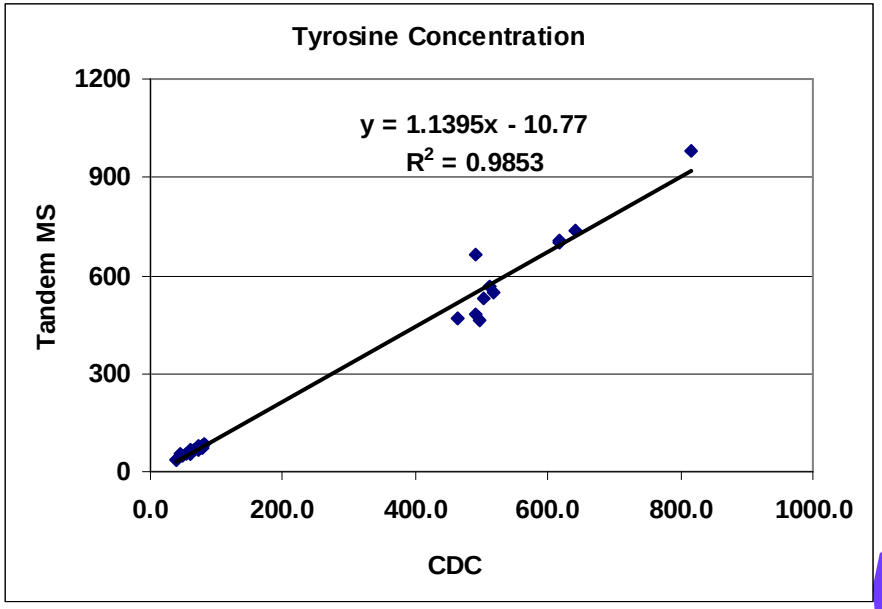
Bias Plots

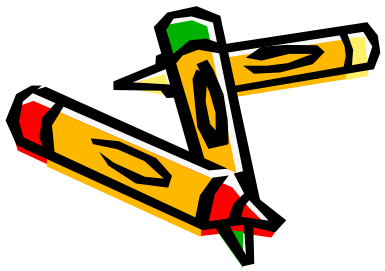

y = 0.1866x + 11.161
R2 = 0.69
Delta
MIRA-Tandem MS
400.0
300.0
200.0
100.0
0.0
-100.0
0.0
500.0
1000.0
1500.0
Phe conc on Cobas Mira


y = -0.0183x + 33.805
R2 = 0.1756
% Delta
(MIRA-Tandem MS)/MIRA
100.0
80.0
60.0
40.0
20.0
0.0
-20.0
0.0
500.0
1000.0
1500.0
Phe Conc on Cobas Mira

External Quality Control







Recovery

Tandem MS

Phenylalanine (umol/L)

	DBS	spiked (umol/L blood)	Recovered	Recovery%
QC1	94			
QC2	235	150	141	94%
QC3	509	606	415	68%

Tyrosine (umol/L)

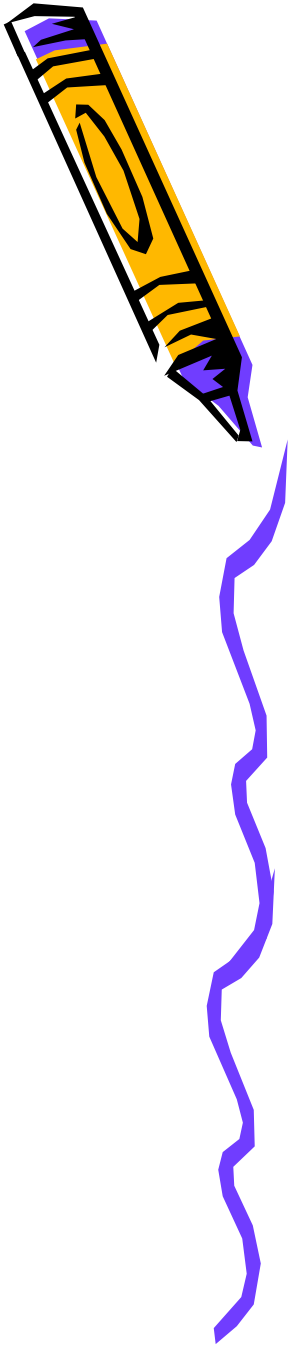
	DBS	spiked (umol/L blood)	Recovered	Recovery%
QC1	79			
QC2	215	150	136	91%
QC3	510	606	431	71%

Cobas MIRA S

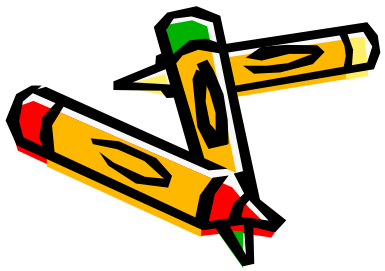
Phenylalanine (umol/L)

	DBS	spiked (umol/L blood)	Recovered	Recovery%
QC1	114			
QC2	284	150	170	113%
QC3	633	606	519	86%

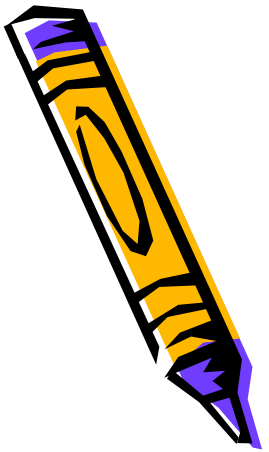
Method Detection Limit



		Phe	Tyr
		70.7	61.2
		62.4	54.5
		68.5	58
		80.9	52.4
		85.7	68.0
		65.9	56.3
		62.5	54.9
		59.1	52.5
		86.4	74.3
		69.1	55.7
		74.8	67.4
		67.9	57.0
		78.3	67.3
		71.9	66.6
		66.9	59.0
		68.9	62.0
	MEAN	71.2	60.4
	SD	8.1	6.5
	CV%	11.3	10.8
t=2.002	MDL=SD*t	**16.1**	**13.1**
	LOQ=10*SD	80.6	65.3
	1. 10*MDL>spiked	16.1*10 = 161 > 71.2	13.1*10 = 131 > 60.4
	2. MDL<spiked	16.1 < 71.2	13.1 < 60.4
	3. MDL<LOQ	16.1 < 80.6	13.1 <65.3
	4. S/N (Ave/SD)=2.5-10	71.2/8.1 = 8.8	71.2/8.1 = 9.3
	5. Recovery (80-120%)	n.a.	n.a.



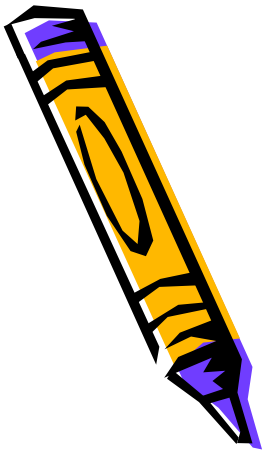
Conclusion



Enzymatic Method

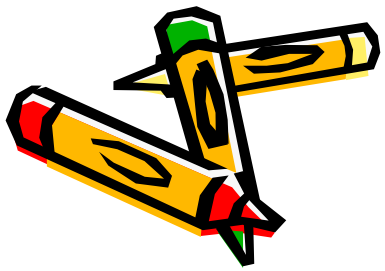
- NSQ: 8mm disk to be punched out
- Positive bias in external QC program
- Interference?
- No tyrosine measured
- Out of market soon

Conclusion



Tandem MS Method

- NSQ: 8mm disk to be punched out
- **3mm disk needed**
- Positive bias in external QC program
- **No bias in external QC program**
- Interference?
- **Robust to interference**
- No tyrosine measured
- **Tyrosine measured**
- Out of market soon
- **Not a probelm**



Thank You