

# Background and proposal for a pilot EQA scheme for pterins

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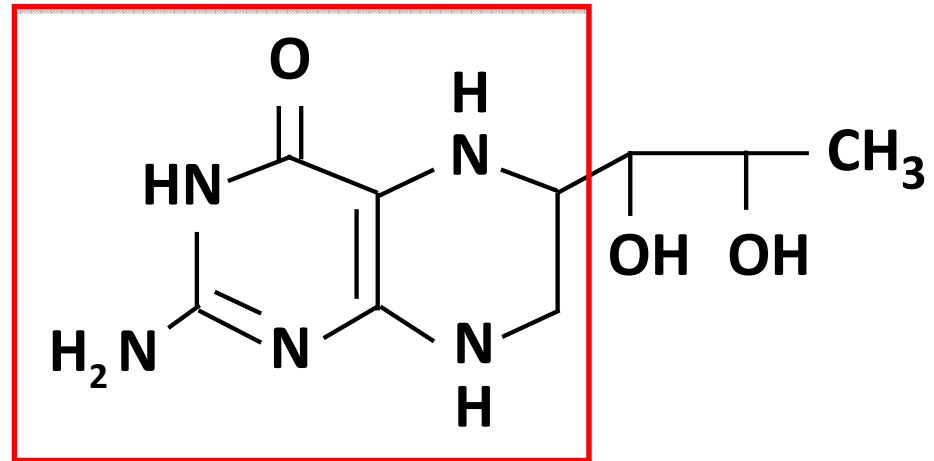
Germany

# Tetrahydrobiopterin (BH<sub>4</sub>)



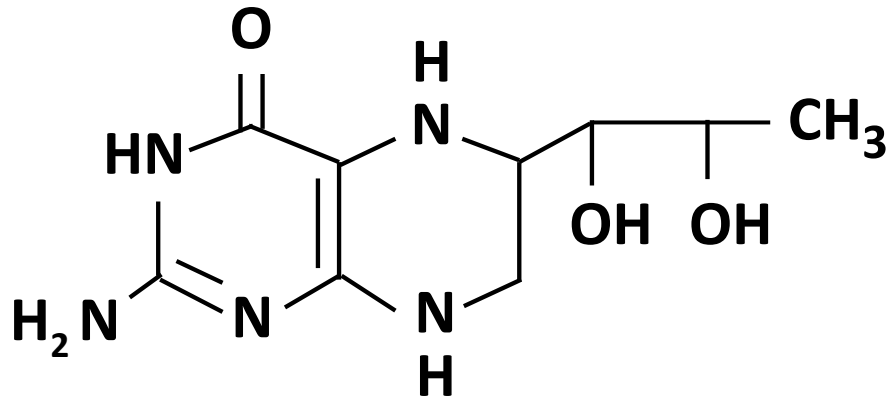
Brimstone butterfly

Gowland Hopkins (1895)

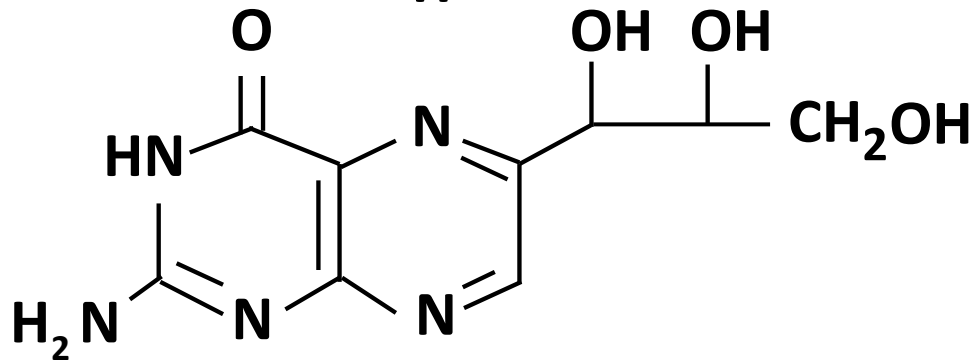


PTERON = Wing

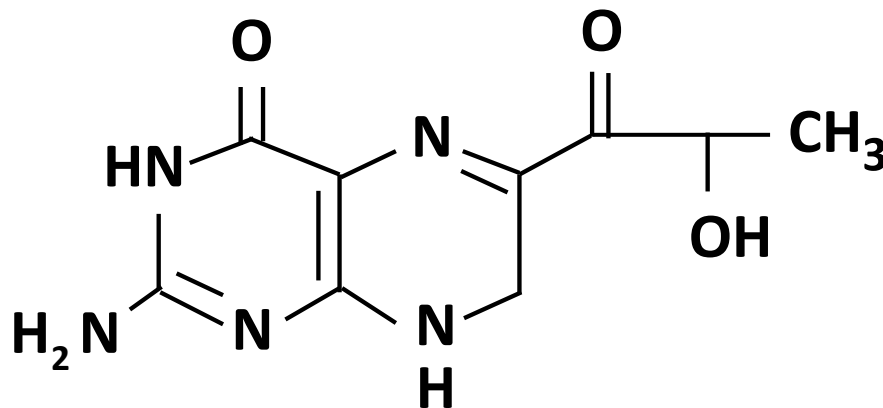
## Biologically important pterins



Tetrahydrobiopterin

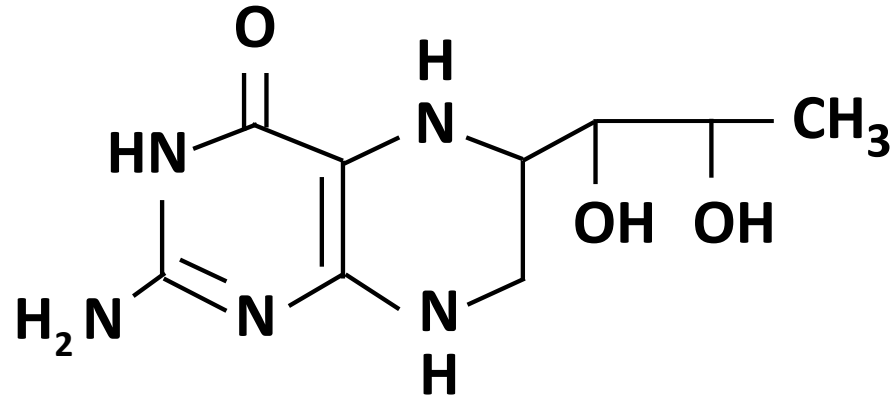


Neopterin



Sepiapterin

## Oxidation forms of BH4



**5,6,7,8-Tetrahydrobiopterin (BH4)**

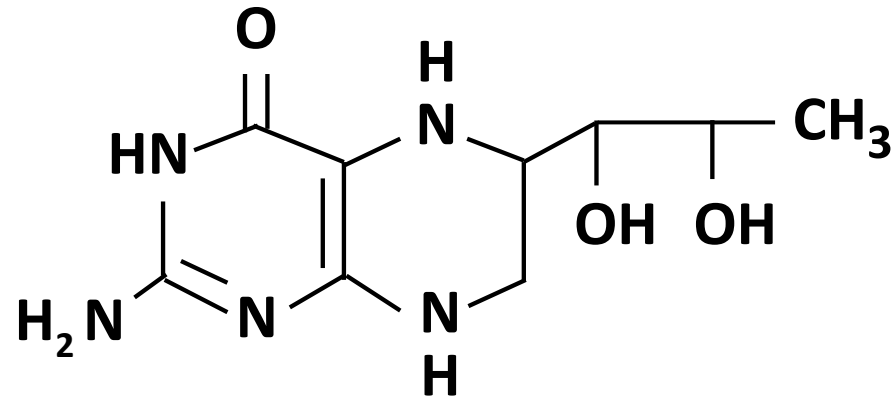


**7,8-Dihydrobiopterin (BH2)**

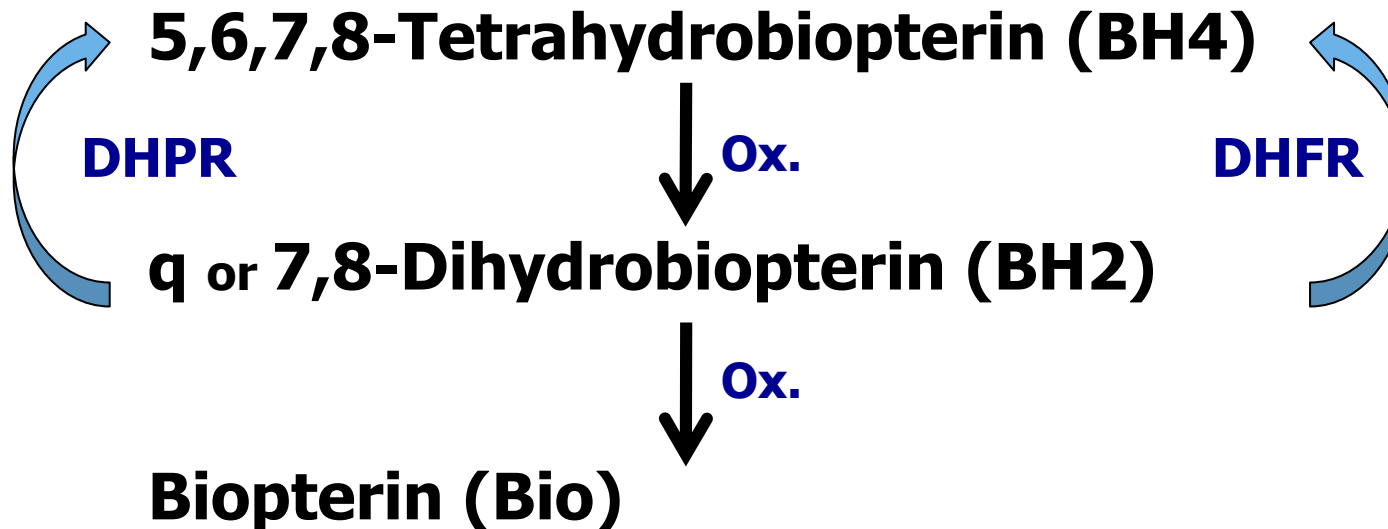


**Biopterin (Bio)**

## Regeneration of BH4



**De novo Pathway**



**Salvage Pathway**

**DHPR = Dihydropteridine reductase**  
**DHFR = Dihydrofolate reductase**

# Functions of tetrahydrobiopterin

## ■ Cofactor for:

Phenylalanine hydroxylase



Tyrosine hydroxylase



Tryptophan hydroxylase



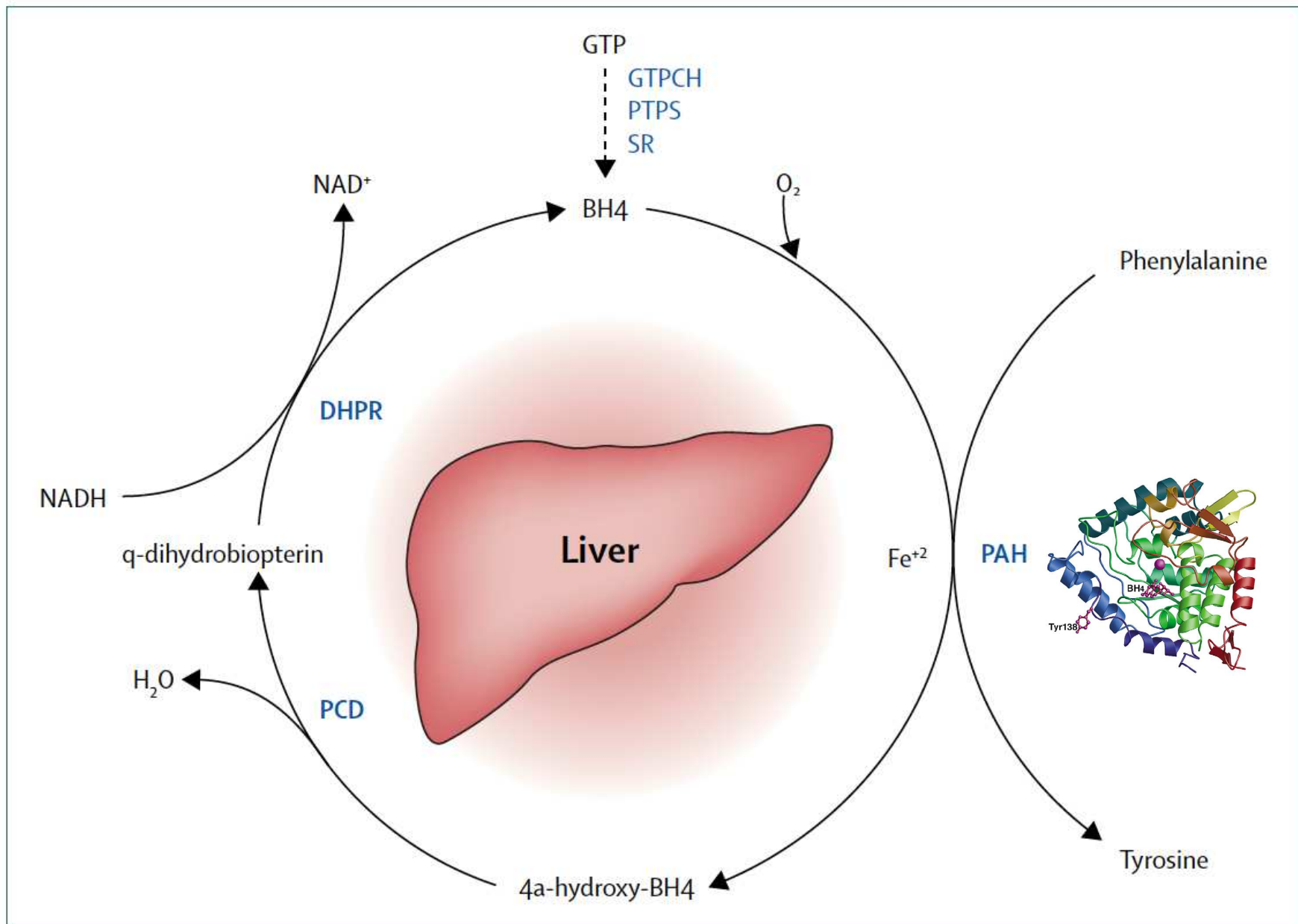
Nitric oxide synthase (NOS)



Glycerol-ether monooxygenase

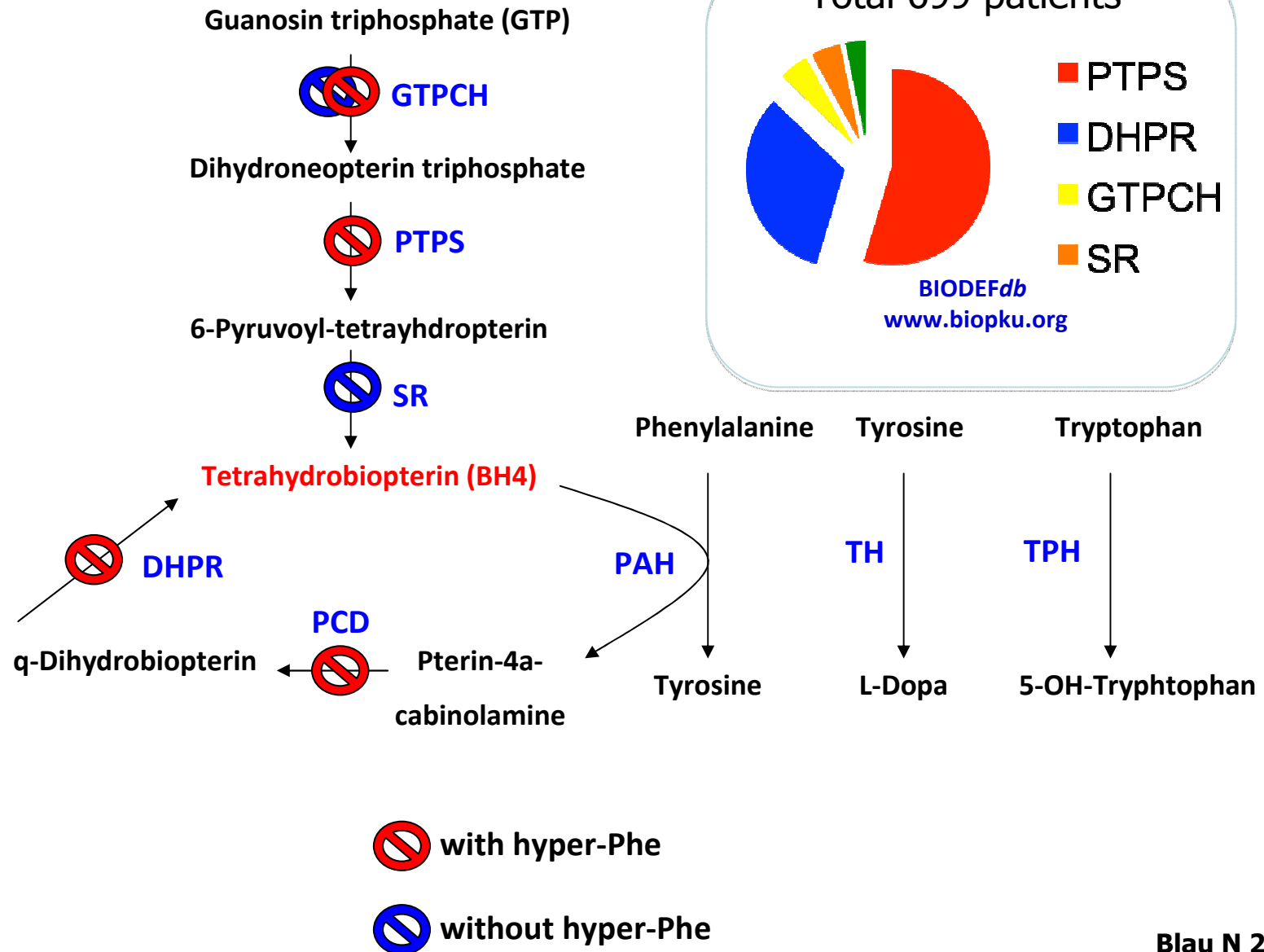


# Phenylalanine hydroxylating system



Blau, N., F. J. Van Spronsen and H. L. Levy (2010). "Phenylketonuria." *Lancet* 376: 1417-1427.

# BH4 deficiencies





# Laboratory diagnosis of PKU and BH<sub>4</sub> deficiencies

## Differential diagnosis

1. Quantitative analysis of Phe and Tyr in plasma (DBS)
2. Pterins (neopterin and biopterin) in DBS or urine
3. DHPR activity in DBS
4. BH<sub>4</sub> loading test



## Laboratory diagnosis of PKU and BH4 deficiencies

### Pterins (neopterin and biopterin) in DBS or urine

Every newborn with even slightly elevated blood Phe levels

Before introducing the diet (at high blood Phe)

**CAVE: GTPCH deficiency may present with normal blood Phe in the NBS**

# Laboratory diagnosis of PKU and BH4 deficiencies

**Neopterin, biopterin and primapterin are markers for BH4 deficiency**

- Light- and oxygen-sensitive
- In DBS pterins are stable (transport at RT)
- In urine pterins must be oxidized with  $\text{MnO}_2$
- Transport of native urine on dry ice (native urine)

# Pterins in urine or DBS?

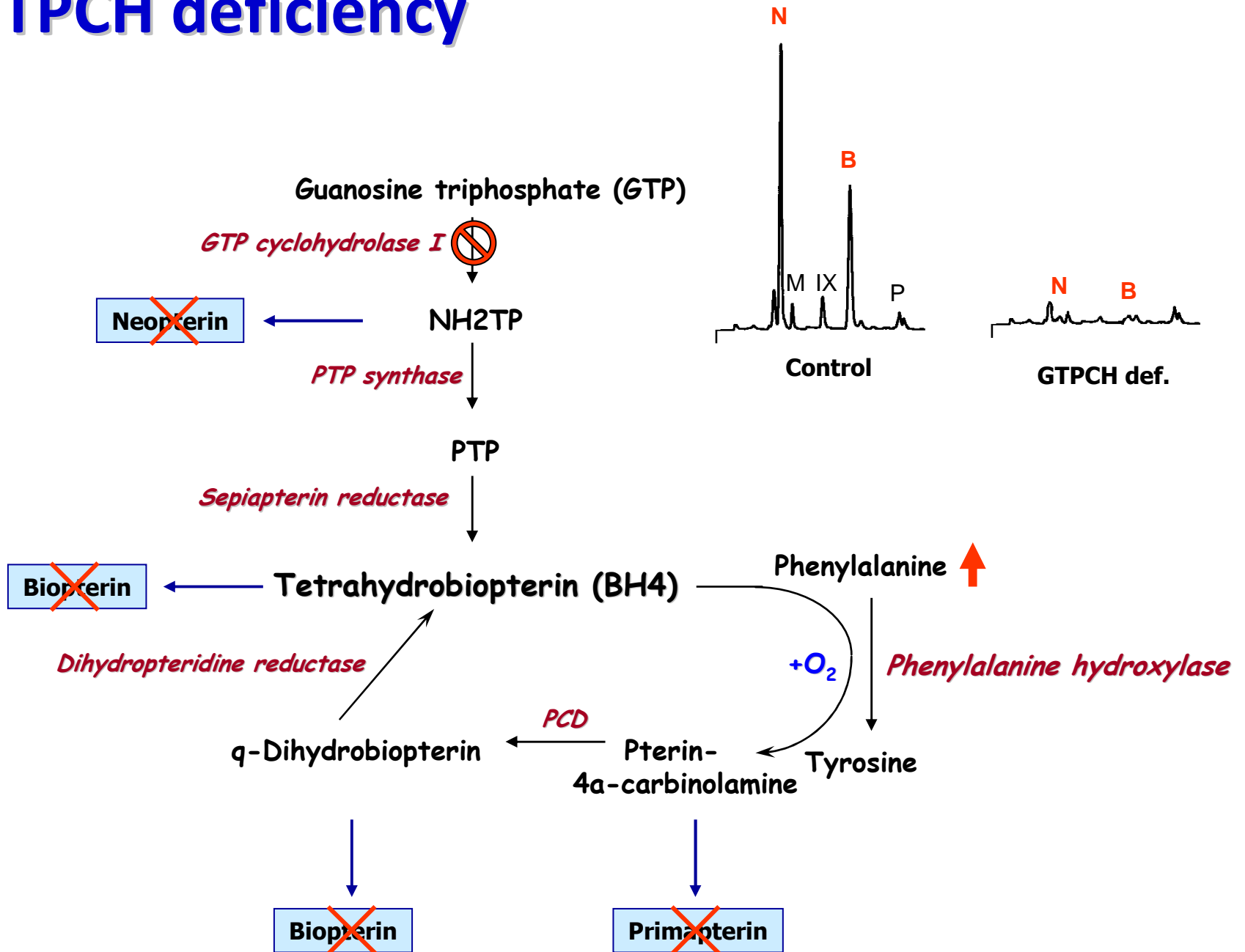
## URINE

- Higher concentrations ( $\mu\text{mol/L}$ )
- Need for oxidation or shipping on dry ice
- Light and oxygen sensitive
- Creatinine

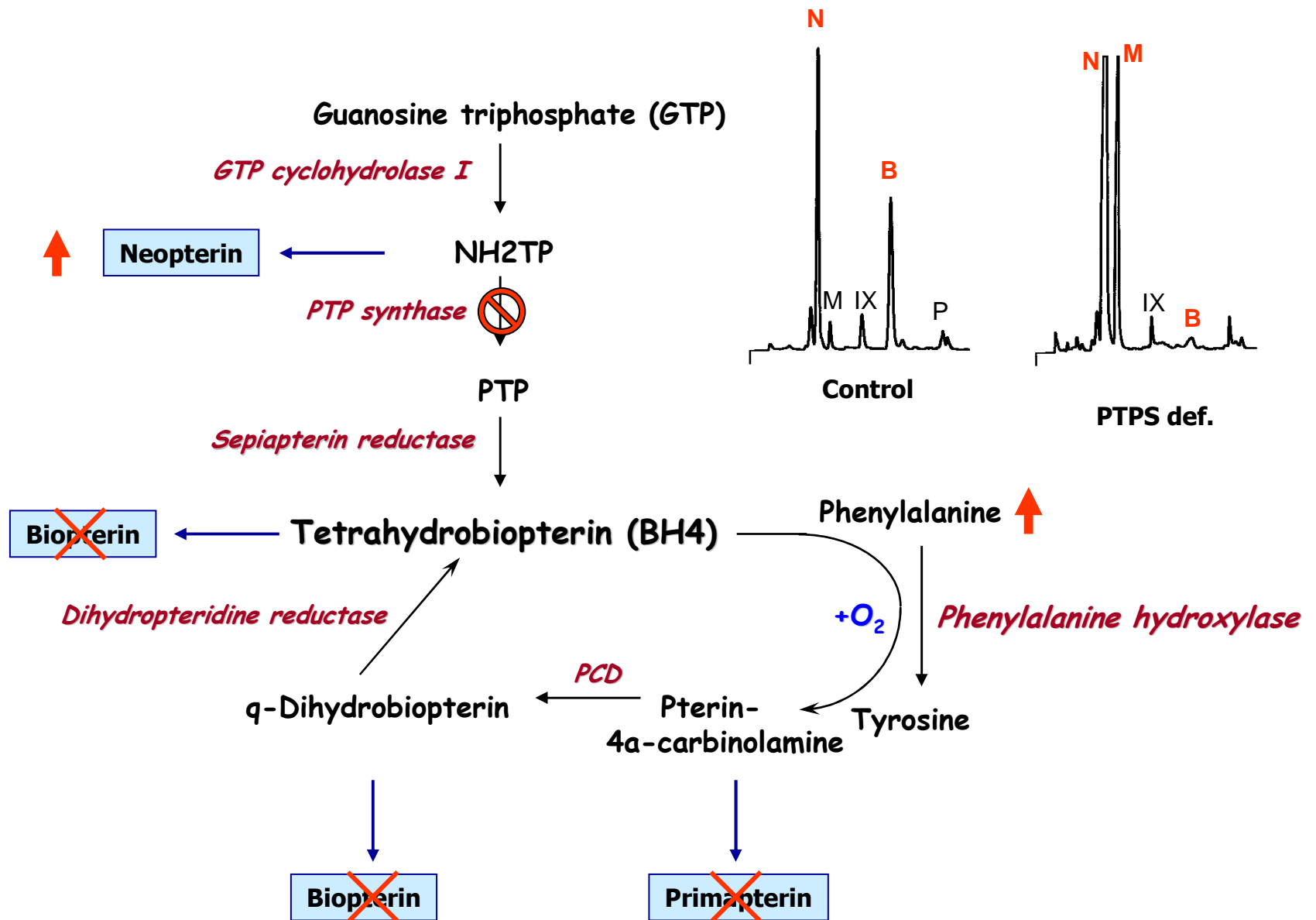
## DBS

- Lower concentrations ( $\text{nmol/L}$ )
- Oxidized during drying and stable on filter paper
- Shipping at RT in envelope
- Hemoglobine

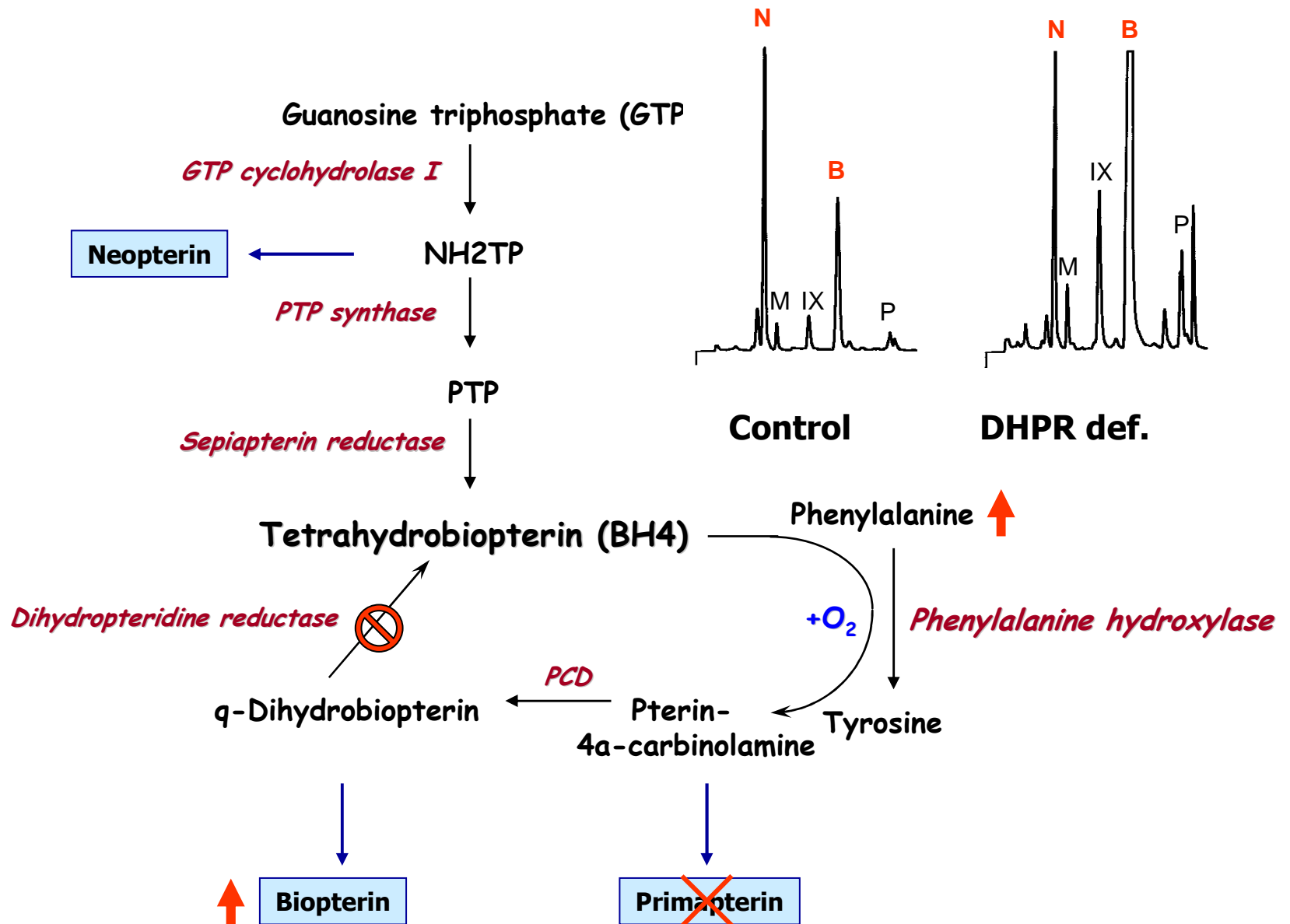
# GTPCH deficiency



# PTPS deficiency



# DHPR deficiency



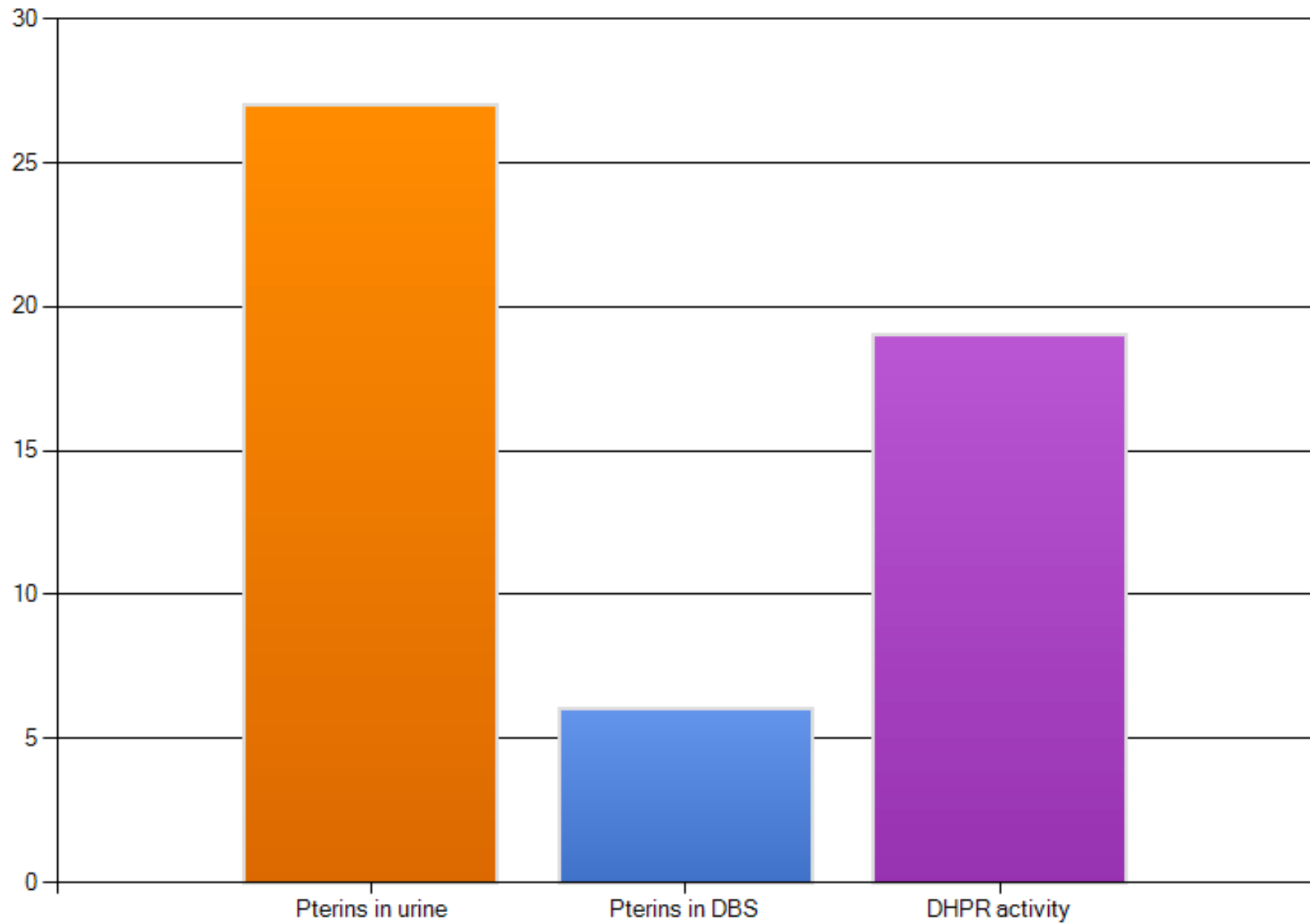
## Aim of the EQA

We plan to distribute urine and/or DBS samples twice a year. Samples will be obtained from patients with confirmed diagnosis and with known quantitative values for pterins and DHPR (tested in Heidelberg Metabolic Laboratories).

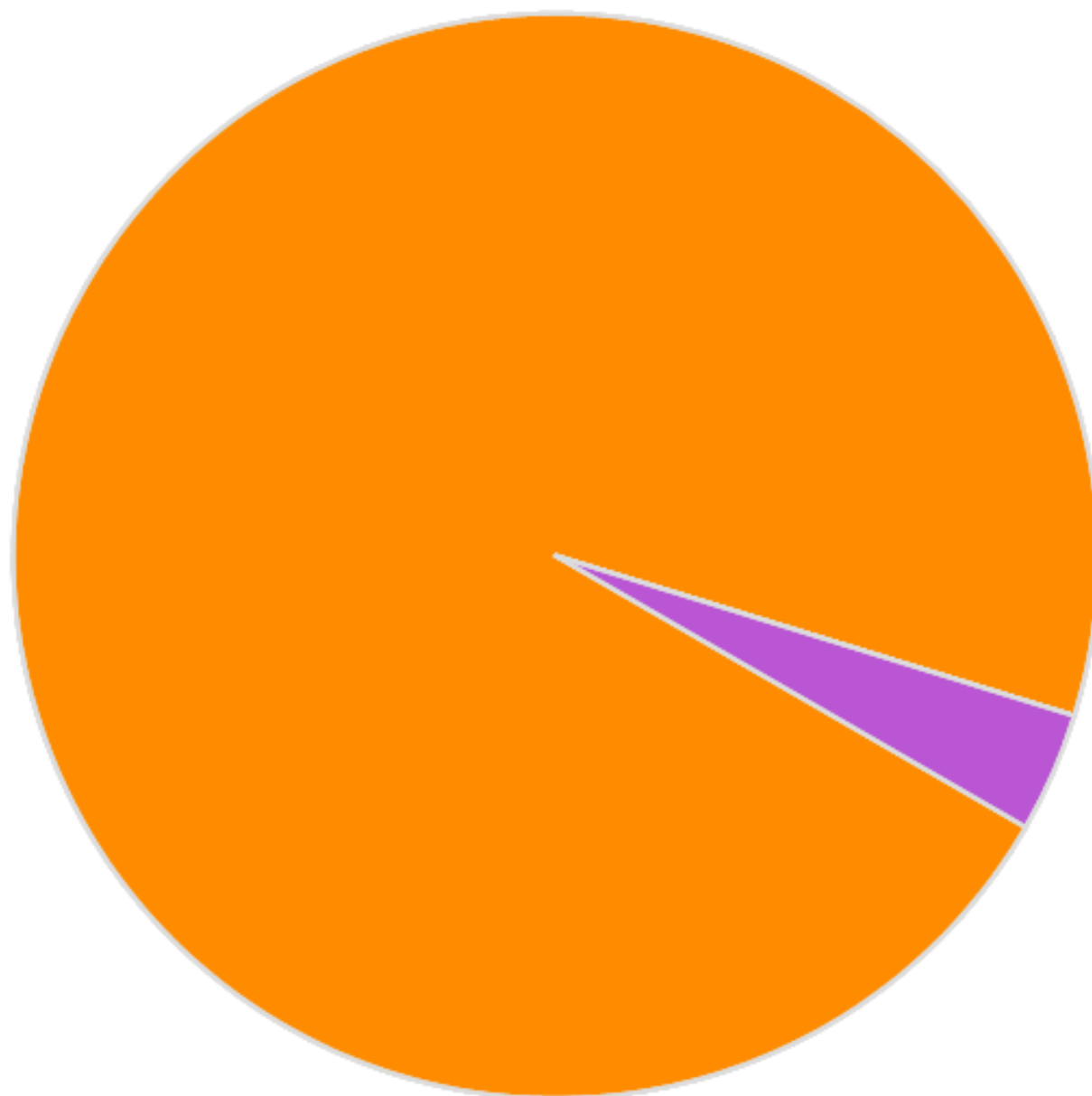
Participating laboratory should be able to separate neopterin, monapterin, biopterin, primapterin, isoxanthopterin and pterin and to quantify total neopterin and biopterin.






### Tests routinely performed in your lab:

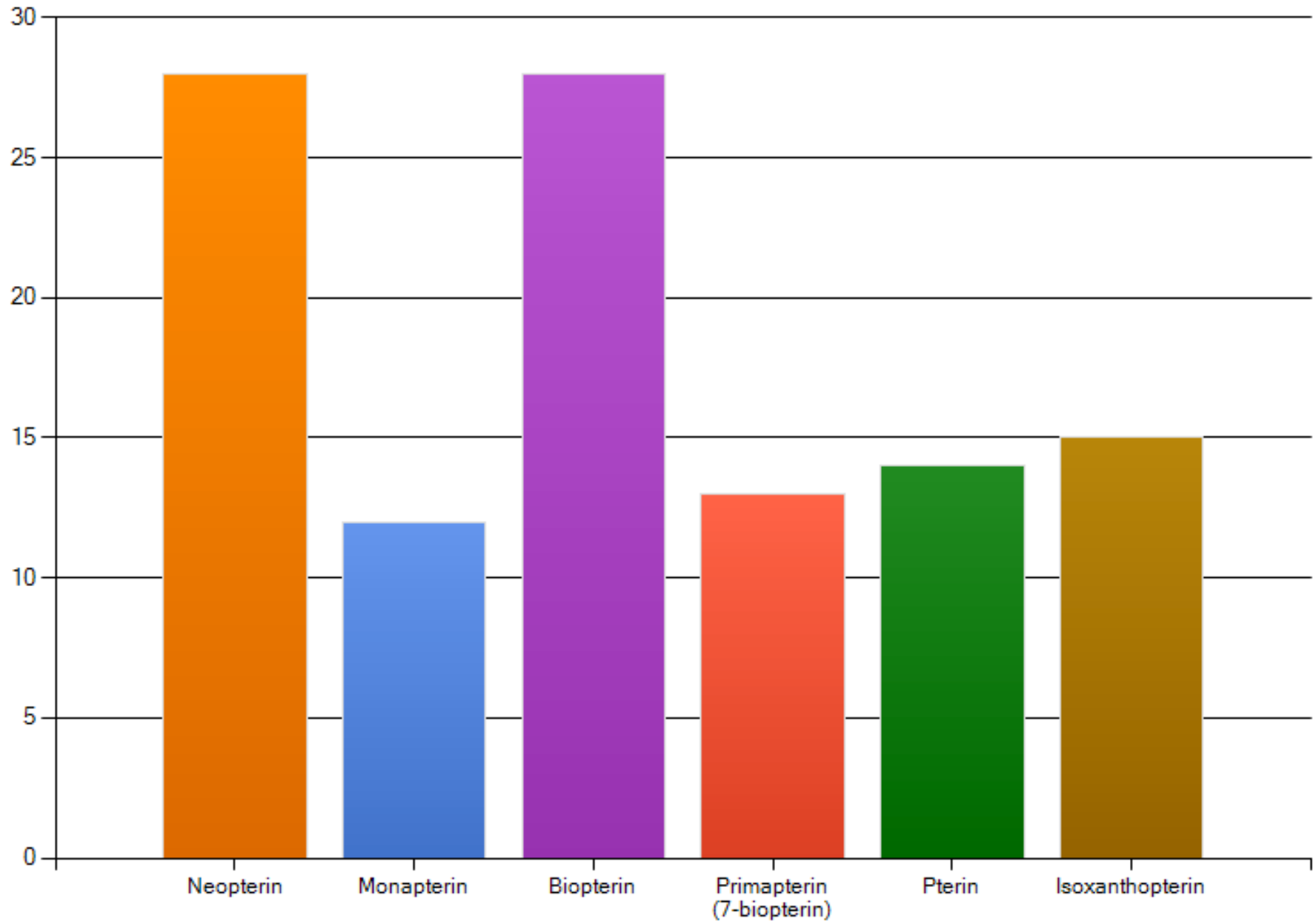


**Method you are using for pterins quantification in urine and/or DBS (not CSF):**

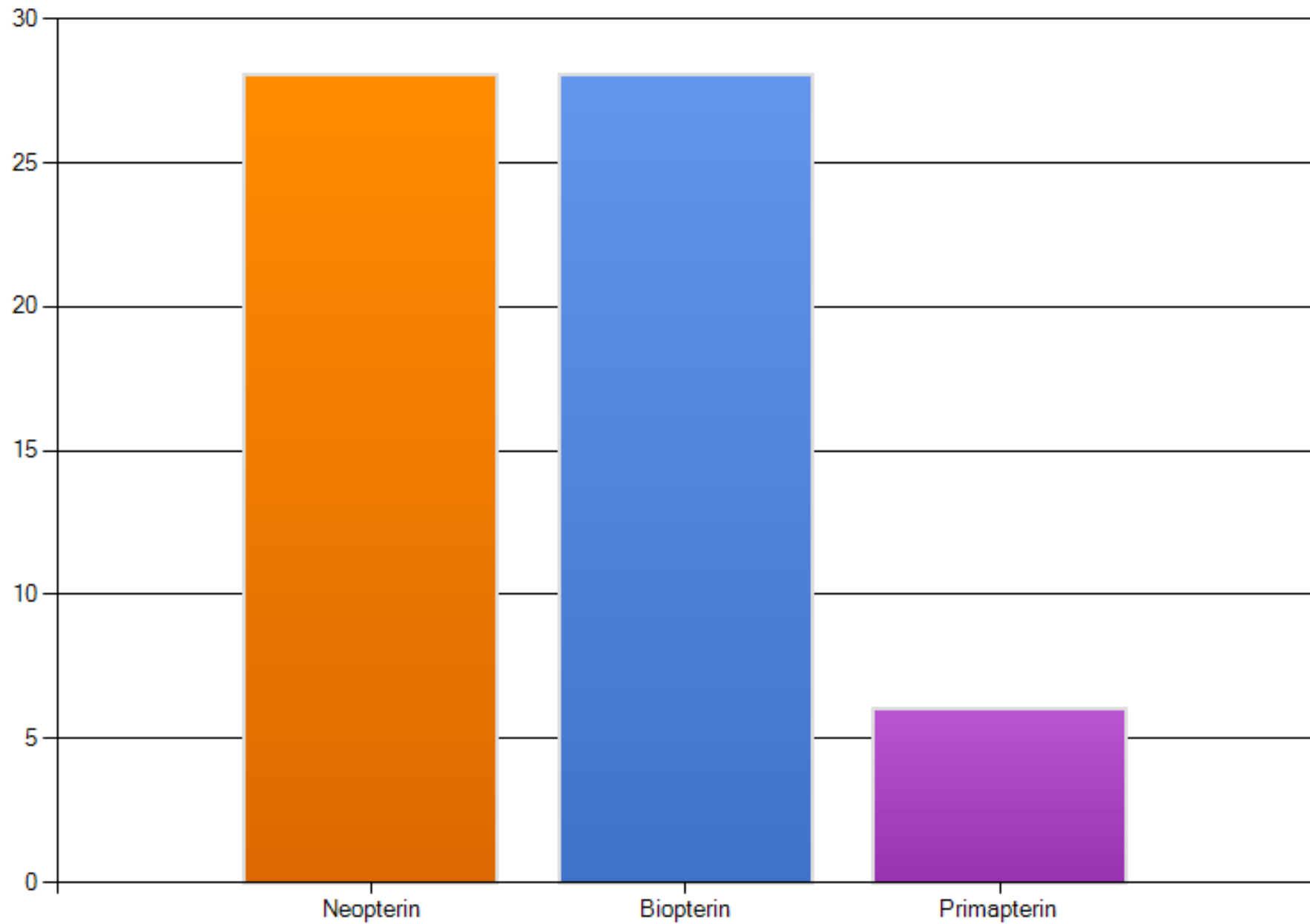


-  HPLC with fluorescence detection
-  HPLC with electrochemical detection
-  Other methods

### Pterins you can separate with your method:



### Pterins you are reporting quantitatively:



## Samples

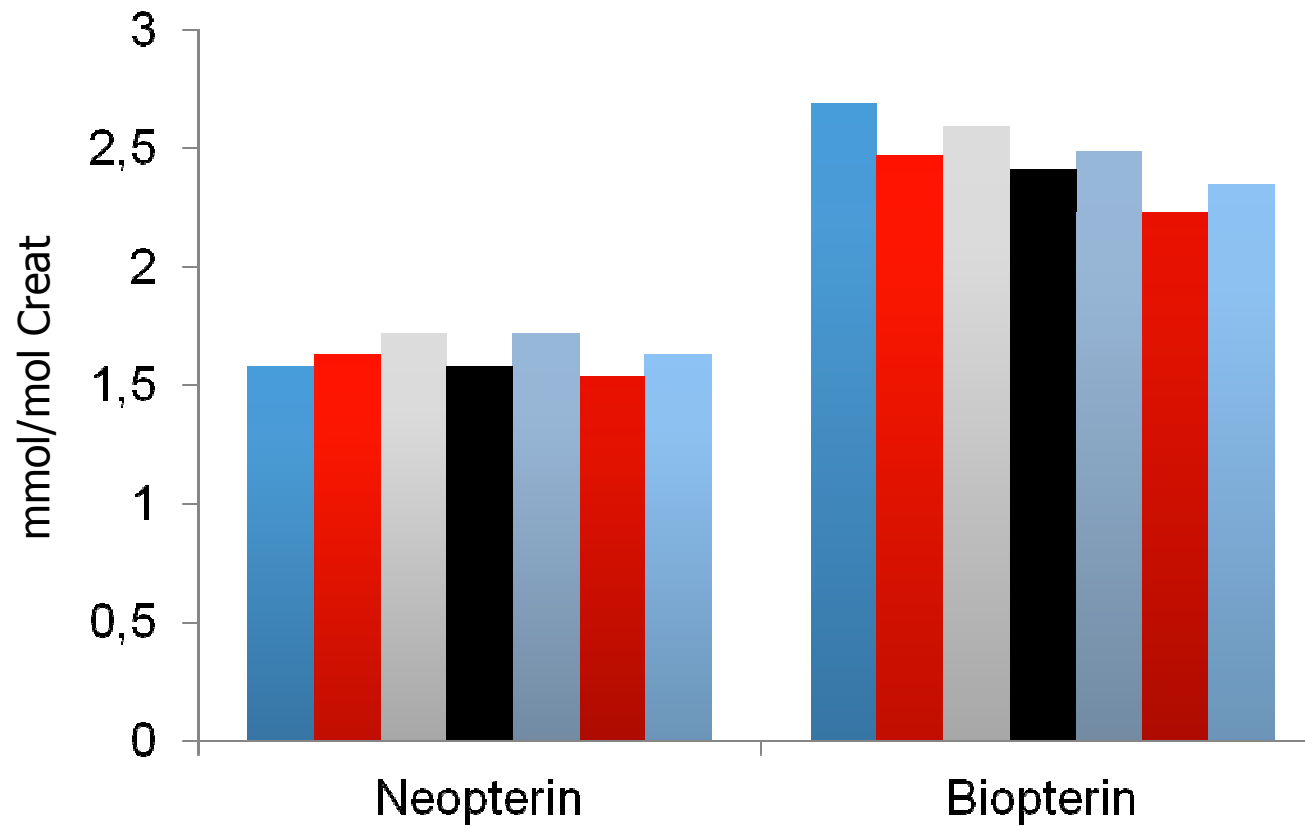
DPT-1: Two urine samples (normal or pathologic)  
Two DBS samples (normal or pathologic)

DPT-2 Two urine samples (normal or pathologic)  
Two DBS samples (normal or pathologic)

# Investigations

- Quantitative results (urine):
  - Neopterin (total)
  - Biopterin (total)
  - Creatinine
- Quantitative results (DBS):
  - Neopterin (total)
  - Biopterin (total)
  - Dihydropteridine reductase activity (Hemoglobin)
- Diagnosis

# Evaluation Test Samples Pterins\*



Frozen	+	+	-	-	+	-	-		+	+	-	-	+	-	-
Lyophilized	-	-	+	+	-	+	+		-	-	+	+	-	+	+
Cryo protect	-	-	-	-	+	+	+		-	-	-	-	+	+	+
Lyo protect	-	-	-	+	-	-	+		-	-	-	+	-	-	+

\*Cas Laboratory

## Next steps

- Registration of potential participants  
Questionnaire (method details)
- Samples collection (Heidelberg)
- Samples processing and distribution (MCA Lab)



# Acknowledgements

## Metabolic Laboratory Heidelberg

Alex Anninos  
Brigitte Schmidt-Mader  
PD Dr. Jürgen Okun

## Streekziekenhuis Koningin Beatrix Winterswijk (MCA)

Dr. Cas Weykamp

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