

# Instructions for use

For *in vitro* diagnostic use only

## NEONATAL PHENYLALANINE

Fluorometric determination of phenylalanine from blood specimens dried on filter paper.

Product no. 61 99 896 (S&S 903)  
(960 wells)

Product no. 61 99 897 (S&S 903)  
(4800 wells)

CE 0537

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CONTENTS	Page
INTENDED USE .....	1
INTRODUCTION .....	1
PRINCIPLE OF THE ASSAY .....	1
KIT CONTENTS .....	2
REAGENT PREPARATION .....	2
MATERIALS REQUIRED BUT NOT PROVIDED.....	3
SPECIMEN COLLECTION AND HANDLING .....	3
PRECAUTIONS .....	3
TEST PROCEDURE (Manual punch).....	4
TEST PROCEDURE (Woodpecker system).....	4
PROCEDURAL NOTES .....	5
RESULTS .....	5
PERFORMANCE CHARACTERISTICS .....	6
CLINICAL EVALUATION.....	7
LIMITATIONS OF THE PROCEDURE .....	7
TROUBLE SHOOTING.....	8
REFERENCES .....	8

### INTENDED USE

The kit is designed for the quantitative *in vitro* determination of phenylalanine concentration in blood specimens dried on filter paper intended for newborn screening for phenylketonuria (PKU).

### INTRODUCTION

Hyperphenylalaninemia (HPA) is a general name for all clinical variants of elevated phenylalanine. Classical phenylketonuria (PKU), or phenylalanine hydroxylase

(PAH) deficiency, oligophrenia phenylpyruvica, 261600 according to McKusick (1990), or Fölling's disease is an inborn error of metabolism, that was first diagnosed in 1934 by Fölling [1], who used the ferric chloride test to detect phenylpyruvic acid in urine of a grossly retarded child.

The classical PKU is caused by the deficiency of the hepatic PAH, which is responsible for the hydroxylation of phenylalanine to tyrosine. Due to the block of tyrosine formation phenylalanine is metabolized via the alternative pathway through the formation of phenylpyruvate, phenyllactic acid, phenylacetic acid, o-hydroxyphenylacetic acid, etc [2]. The accumulation of phenylalanine and its toxic metabolites in brain tissue during the neonatal period causes a metabolic encephalopathy observed in PKU patients. The exact molecular mechanisms involved in the pathogenesis of brain tissue damage leading to irreversible mental retardation are not yet known, however disturbed myelination processes and/or disturbances in amino acid transport across the blood-brain barrier has been proposed as pathophysiological factors [3, 4].

PKU when left undiagnosed in early neonatal period and untreated by diet low in phenylalanine invalidizes the patient who will need institutional care.

Bacterial inhibition assay (BIA) of phenylalanine, the first screening method for PKU, was developed by Guthrie [5], and has been successfully used for nearly three decades. Recently, more attention has been drawn to the drawbacks of BIA: being a semiquantitative method it does not allow to set the age-adjusted cut-off values which is necessary in cases of early discharge from the nursery [6-8]. Besides being a threshold method BIA is also prone to variations especially in cases receiving antibiotic therapy. It has been also reported that BIA causes more false negative results than the quantitative chemical methods [9].

The present procedure of determination of phenylalanine from blood specimens dried on filter paper is based on the modification of the McCaman and Robins quantitative fluorometric method [10] modified for dried blood spots [11] and adapted to a microplate fluorometer with high throughput [12 - 14].

### PRINCIPLE OF THE ASSAY

Neonatal phenylalanine assay is a chemical method intended for the quantitative determination of phenylalanine from dried blood spots. Phenylalanine eluted from dried blood spots forms a fluorescent compound with ninhydrin. The fluorometric response is greatly enhanced by the presence of a dipeptide L-leucyl-L-alanine. The pH during the reaction is strictly controlled by succinate buffer at  $5.8 \pm 0.1$  in order to optimize fluorescence and maximize specificity. Copper reagent is added to stabilize the fluorescent complex and enhance the signal. Fluorescence is measured with e.g. Fluoroskan Ascent at 485 nm (excitation wavelength being 390 nm).

## KIT CONTENTS

- For *in vitro* diagnostic use.
- Reagents are sufficient for 960 wells or 4800 wells. The reagent volumes for 4800 wells are indicated below in parenthesis.
- Reagents are stored between +2°C and +8°C.
- The expiry date is printed on each component and package.
- Avoid exposure of calibrators and controls to moisture and exposure of succinate buffer to excessive light.
- **Once opened, the components must be sealed tightly e.g. with parafilm or tape!**

**1** SUCCINATE BUFFER 40 ml (200ml)  
Succinic acid, pH 5.8 ± 0.1 with 0.05 % Bronidox® as preservative.

**2** L-LEUCYL-L-ALANINE 9 ml (5x9ml)  
L-leucyl-L-alanine solution with 0.05 % Bronidox® as preservative.

**3** NINHYDRIN 2x10 ml (10x10ml)  
Ninhydrin. Xn (Harmful)  
R 20/21/22, R36/37/38  
S 26/28/36

**4** COPPER REAGENT 2x125 ml (2x625ml)  
Copper sulphate pentahydrate, sodium potassium tartrate and sodium carbonate.

**5** CALIBRATORS, 1 sheet with 5 sets of 6 calibrators, in a foil package with desiccant (5 sheets with 5 sets of 6 calibrators)

Phenylalanine in dried blood spots on filter paper S&S 903.

Approximate values:  
(mg/100 ml)

A	=	0.2
B	=	1.5
C	=	3.0
D	=	5.0
E	=	10.0
F	=	15.0

**The values of calibrators are lot specific. For exact values refer to the calibrator sheet included in each kit.**

The calibrators are prepared from human blood with a hematocrit value of 50 % ... 54 % and calibrated against the 1<sup>st</sup> ISNS Reference Preparation for Neonatal Screening for thyrotropin, phenylalanine and 17-alpha-hydroxyprogesterone in blood spots [20].

**6** CONTROLS, 1 sheet with 5 sets of 2 controls in a foil package with a desiccant (5 sheets with 5 sets of 2 controls)

Phenylalanine in dried blood spots on filter paper S&S 903 .

Approximate values:  
(mg/100 ml)

C1	=	3
C2	=	7

**The values of controls are lot specific. For exact values refer to the control sheet included in each kit.**

The controls are prepared from human blood with a hematocrit value of 50 % ... 54 %.

Phenylalanine values are in gravimetric units (mg/dl = mg/100 ml = mg %) [15]. Conversion to the SI units may be accomplished by using the following equation:

Phenylalanine (mg/100 ml) x 60 = Phenylalanine (µmol/l)

REACTION PLATES 10 pcs (5x10 pcs)  
Non coated solid microplates.

PLASTIC COVER SHEETS 20 pcs (100 pcs)  
Plastic sheets to cover the plates during incubation and elution.

SHEET WITH VALUES FOR CONTROLS AND CALIBRATORS, 1 pc each

## REAGENT PREPARATION

**Table 1** Reagent preparation

Reagent	Preparation	Stability of opened and diluted reagents (+2°C to +8°C)
1 Succinate buffer	Ready for use	6 months. Discard if turbidity develops.
2 L-Leucyl-L-Alanine	Ready for use	6 months.
3 Ninhydrin Incubation mixture	Ready for use Mix reagents 1, 2 and 3 in proportion 5+1+2. <b>See table 2.</b>	6 months. Discard unused incubation mixture.
4 Copper reagent	Ready for use	6 months.
5 Calibrators	Ready for use	When opened stable at least 1 month
6 Controls	Ready for use	When opened stable at least 1 month.
Reaction plates	Ready for use	Can be stored at RT.

**Table 2** Preparation of incubation mixture

No of plates	Vol of Succinate buffer (reagent 1) (ml)	Vol of L-leucyl-L-alanine (reagent 2) (ml)	Vol of Ninhydrin (* (reagent 3) (ml)
1	3.5	0.7	1.4
2	7.0	1.4	2.8
3	10.5	2.1	4.2
4	14.0	2.8	5.6
5	17.5	3.5	7.0
6	21.0	4.2	8.4
7	24.5	4.9	9.8
8	28.0	5.6	11.2
9	31.5	6.3	12.6
10	35.0	7.0	14.0

(\* Ninhydrin may form crystals in low temperatures. Warm it up in room temperature so that the crystals will disappear.

## MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate fluorometer eg. Fluoroskan Ascent, cat. no. 5210 470
- Microplate shaker eg. iEMS Incubator/Shaker with 9 thermal microplate holders, temp. range: RT – +40°C (cat.no. 5112 200) or iEMS Incubator/Shaker HT, with 3 thermal microplate holders, temperature range: +14°C – +69°C (cat. no. 5112250)
- Microplate washer eg. Wellwash 4 Mk 2 (cat. no. 516 0770) or 8-channel pipette with 300 µl volume
- Disk puncher with a diameter of 3 mm to cut off paper disks of dried blood controls, calibrators and samples, and water suction with eg. Pasteur pipette to remove paper disks from microtitration wells or Woodpecker disk processor to punch disks (cat. no. 5600 200 )
- Disk holders for Woodpecker, disposable (100 pcs, cat. no. 9700300X)
- 8-channel pipette to pipet 100 µl and 200 µl volumes
- Liquid dispenser eg. Multidrop 384 (cat. no. 584 0150)
- 80 % ethyl alcohol
- Microplate for disk elution in manual punch procedure

## SPECIMEN COLLECTION AND HANDLING

A blood spot on the filter paper is obtained by one application of the filter paper onto a drop of blood from the pricked heel of the baby 3-5 days after birth. Schleicher & Schuell 903 filter paper is suitable for collection of blood spots. Make sure that the filter paper sample is fully covered and soaked through. The blood spot is dried for at least 3 hours.

The specimen collection technique is described in detail in NCCLS document LA4-A3 [16].

It is essential that the blood spots are collected by application of a single drop of blood. Layering of successive drops, often recognizable by a visible caking of blood on the filter paper support, will produce falsely elevated results. It is likewise important that the drop of blood is large enough to spread out over the required area, penetrating the filter paper from one side to the other; incomplete saturation of the support medium may well result in underestimation of phenylalanine content.

To enhance blood flow at the puncture site, the infant's heel can be covered with a warm, moist towel at a temperature of 42°C or less for three minutes. Clean the skin with 70 % alcohol and wipe with dry sterile gauze. To minimize the risk of injury to the bone, use a disposable lancet. Puncture the skin on the lateral side of the flat walking surface of the heel. (Avoid the arch and the posterior curvature of the heel.)

According to NCCLS standard LA4-A3 the infant's heel should be punctured to a depth of approximately 2,0 mm. It has been suggested that blood may be obtained when depths of the lancet or puncturing device are as little as 1 mm - e.g., Simplate® (Organon Teknika Corp., Durham, NC), and Tenderfoot™ Surgicut® (International Technidyne Corp., Edison, NJ) [17]. Wipe off the first drop of blood and encourage the formation of subsequent drops, while holding the foot in a dependent position, by applying gentle pressure. Excessive squeezing may result in hemolysis or dilution of the sample with tissue fluid.

Once a drop of blood of adequate volume has formed, touch the filter paper gently to it - do not press it against the heel - and watch from the opposite side as the blood saturates the paper. Ordinarily at least three blood spots are collected from each infant. Be careful not to handle the filter paper in the region of the preprinted circles prior to collection and not to touch or smear the blood spots. Avoid contamination with water or alcohol.

Allow the blood spots to air dry in a horizontal position for 3 to 6 hours at ambient temperature. During the drying process, do not subject the spots to heat and neither stack them nor let them touch other surfaces.

Once dry, place each specimen in a separate paper envelope and mail it to the laboratory. Blood spot specimens received in the laboratory should be stored at +2°C ... +8°C protected against moisture.

## PRECAUTIONS

For *in vitro* diagnostic use.

### WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Each donor unit used in the preparation of the kit calibrators and controls has been tested for the presence of the antibodies to HIV (human immunodeficiency virus) and HCV as well as for hepatitis B markers and found to be negative. Because no test method can offer complete assurance that HIV, HCV, hepatitis B virus, or other infectious agents are absent, these calibrators, controls as well as specimens should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", 1999. In addition, handle and dispose of the Microstrips® as well as all material coming into contact with them or with the specimens, calibrators and controls as if capable of transmitting infection.

Discard solutions into a waste drainage network and flush with large volumes of water.

Note that kit component 3 (Ninhydrin) contains ninhydrin, which is harmful by inhalation, in contact with skin and if swallowed (R20/21/22) and irritating to eyes, respiratory system and skin (R36/37/38). In case of contact with eyes, rinse immediately with plenty of water and seek medical advice (S26). After contact with skin, wash immediately with plenty of water (S28). Wear suitable protective clothing (S36). Material Safety Data Sheet (MSDS) will be delivered to professional user on request.

### TEST PROCEDURE (Manual punch)

1. Punch out 3 mm disks containing blood calibrators (reagent 5), controls (reagent 6) and patient specimens into microplate wells (not included in the kit). Use calibrators as duplicates, and controls and specimens as single.
2. Add 80  $\mu$ l of 80 % ethyl alcohol to each well, cover the plate and elute phenylalanine for 30 min at room temperature on a shaker.  
**NOTE:** With iEMS Incubator/Shaker use speed 900 rpm (shaking value 3).
3. Transfer 50  $\mu$ l eluates with a multichannel pipette into a reaction plate.
4. Add 50  $\mu$ l of reaction mixture (see table 2), seal tightly with a new incubation cover, shake for 1 min at room temperature and incubate according to the chosen procedure:

Procedure A: 60 min at +60 °C  
Procedure B: 120 min at +37 °C

5. Add 200  $\mu$ l of cold (directly from refrigerator) copper reagent (reagent 4) to each well. Cold reagent is used to cool the reaction mixture down to room temperature.
6. Incubate the plate for 15 min at room temperature.
7. Read the fluorescence within 15-30 min after addition of the copper reagent using e.g. Fluoroskan Ascent with filters excitation 390 nm/emission 485 nm.

**NOTE:** The fluorescence readings of the plate may change slightly during the 15 min period, however with no effect on the imprecision or the calculated concentrations.

### TEST PROCEDURE (Woodpecker system)

1. Add 120  $\mu$ l of 80 % ethyl alcohol to each well of the reaction plate.
2. Punch out 3 mm disks containing blood calibrators (reagent 5), controls (reagent 6) and patient specimens.
3. Insert disk holders with disks into reaction plate wells. Use calibrators as duplicates, and controls and specimens as single. Elute phenylalanine for 30 min at room temperature on a shaker.

**NOTE:** With iEMS Incubator/Shaker use speed 900 rpm (shaking value 3).

4. Remove the disk holders.
5. Add 50  $\mu$ l of reaction mixture (see table 2), seal tightly with a incubation cover, shake for 1 min at room temperature and incubate:  
Procedure A: 60 min at +60 °C
6. Add 150  $\mu$ l of cold (directly from refrigerator) copper reagent (reagent 4) to each well. Cold reagent is used to cool the reaction mixture down to room temperature.
7. Incubate the plate for 15 min at room temperature.
8. Read the fluorescence within 15-30 min after addition of the copper reagent using e.g. Fluoroskan Ascent with filters excitation 390 nm/emission 485 nm.

**NOTE:** The fluorescence readings of the plate may change slightly during the 15 min period, however with no effect on the imprecision or the calculated concentrations.

## PROCEDURAL NOTES

1. Bring the calibrator and control foil packages to room temperature before starting the assay. Once the calibrator and control foil packages have been opened they have to be resealed tightly and stored at +2°C to +8°C with a desiccant.
2. Because the fluorescence values of calibrators, controls and unknowns may vary between assays, **the use of calibrators with each plate** for correct calculation of concentrations of unknowns is obligatory!
3. Prolongation of the elution time will not affect the results, however the possible evaporation of the 80 % ethanol should be considered.
4. It is recommended to use Procedure A because of the deeper slope obtained. Procedure B is an alternative for those laboratories where +60°C incubators are not available. When incubating the plate at +60°C the reaction achieves equilibrium after 60 min, whereas at +37°C some increase of fluorescence is observed up to 180 min. However, for practical reasons (i.e. shorter assay) the reaction of Procedure B is stopped after 120 min of incubation. **Procedure B is not recommended with Woodpecker application.**
5. Slight variations in incubation time and temperature are allowed: time tolerances for Procedures A and B are 55-65 min and 110-120 min, respectively and temperature tolerances for Procedures A and B are +55-65°C and +35-39°C, respectively. However, lower temperatures and shorter incubation time result in somewhat decreased fluorescence signal.
6. Do not use vigorous shaking when copper reagent (reagent 4) is added to avoid splashing.

## RESULTS

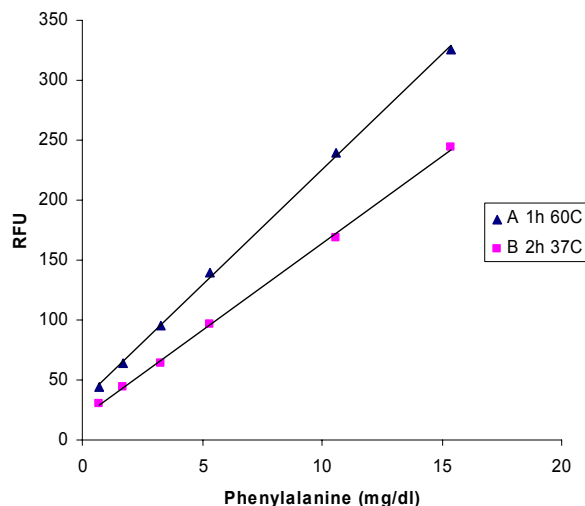
### Calculation of the Results

Measuring in clear reaction plates will produce lower signal than measuring in white cliniplates used previously. However, signal/background ratio is not changed and sensitivity of the test remains the same as before. If similar signal level as measuring in white cliniplates is needed, apply a suitable scaling factor.

If automatic data processing can be used, linear or cubic spline curve fitting with lin-lin axis scaling is recommended.

Manual calculation:

1. Draw a standard curve on graph paper with fluorescence values on the ordinate and phenylalanine concentrations of the calibrators on the abscissa.
2. Read the phenylalanine concentrations of controls and unknowns from the calibration curve.



**Figure 1:** Comparison of calibration curves using two procedures and with linear regression curve fitting

### Quality Control Values

The expected values for the controls are given for each lot on a separate sheet included in the kit. Test results can be accepted only if the expected values of controls are reached.

When using Fluoroskan Ascent for processing the results it calculates the slope and intercept for the calculation curve.

As an example in the Procedure A with manual punch the value ranges are as follows:

slope	14 - 30
intercept	< 80

### Expected Values and Interpretation of the Results

The discrimination between normal subjects and presumptive positive for PKU is based on the predetermined cut-off point. It is recommended that each laboratory sets its own cut-off value based on the reference range of the given normal population. The reference range depends mainly on the age of infants when the blood sample is withdrawn [6].

## PERFORMANCE CHARACTERISTICS

### Reproducibility

**Table 3** Procedure A

Sample no.	Mean concentration (mg/100 ml)	Standard deviation (mg/100 ml)	CV%
Within-run imprecision (10 replicates)			
1	1,5	0,2	12,1
2	5,2	0,3	5,9
3	9,3	0,6	6,3
Between-run imprecision (10 successive runs, averages of 4 replicates)			
4	1,8	0,2	11,4
5	5,2	0,5	9,4
6	10,6	0,8	7,9

**Table 4** Procedure B

Sample no.	Mean concentration (mg/100 ml)	Standard deviation (mg/100 ml)	CV%
Within-run imprecision (10 replicates)			
1	1,7	0,1	7,7
2	5,3	0,2	3,8
3	9,9	0,6	6,1
Between-run imprecision (10 successive runs, averages of 4 replicates)			
4	1,8	0,2	11,4
5	5,1	0,3	5,8
6	11,3	0,7	5,8

### Detection Limit

The detection limit of phenylalanine calculated as a mean + 3SD of the near-to-zero blank using Procedures A and B was 0.5 mg/100 ml (= 30 µmol/l).

### Analytical Recovery

Blood from an adult volunteer was adjusted to hematocrit 50 % and spiked with phenylalanine.

**Table 5** Procedure A

Endogenous (mg/100 ml)	Added (mg/100 ml)	Measured (mg/100 ml)	Expected (mg/100 ml)	% Recovery
1.0	2.0	3.6	3.0	120
	4.0	5.4	5.0	108
	10.0	12.2	11.0	110

**Table 6** Procedure B

Endogenous (mg/100 ml)	Added (mg/100 ml)	Measured (mg/100 ml)	Expected (mg/100 ml)	% Recovery
0.7	2.0	3.0	2.7	111
	4.0	5.1	4.7	108
	10.0	11.5	10.7	107

(The same samples were used in both Procedures.)

### Interference by Other Amino Acids

For this study amino acids showing the highest interferences were chosen [18]. Blood from an adult volunteer was adjusted to hematocrit 50 % and spiked with

- A) L-phenylalanine 2 mg/100 ml
- B) L-methionine 2 mg/100 ml
- L-citrulline 2 mg/100 ml
- L-glutamic acid 2 mg/100 ml
- L-histidine 2 mg/100 ml
- L-leucine 4 mg/100 ml
- L-glycine 4 mg/100 ml
- C) L-phenylalanine 2 mg/100 ml and amino acids as in sample B)

The samples were analyzed in 4 replicates with the reaction mixture, in which pH of the 1.2 M succinate buffer varied from 5.0 to 6.5.

#### Results:

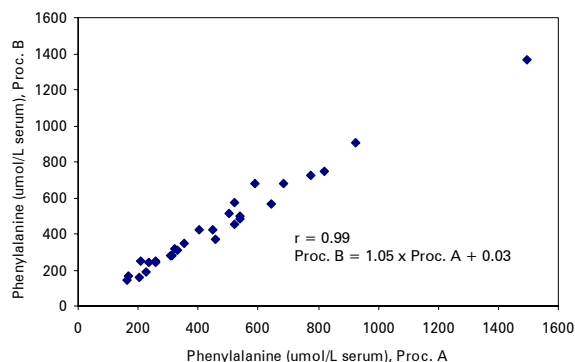
- 1) at the incubation condition of the kit (pH 5.8 ± 0.1) there was no interference by the tested substances.
- 2) at high pH (pH 6.5) the impact of other amino acids yielded in average 17 % of the false increase of phenylalanine concentration.
- 3) at low pH (pH 5.0) the sensitivity of the assay was compromised.

### Interference by Bilirubin

No interference by bilirubin was found at concentrations 50, 100 and 200 µmol/l blood, the concentration of phenylalanine being 3 mg/100 ml.

### Comparison of Procedures

Figure 2 represents the correlation between the calculated concentrations of unknowns and controls assayed using either Procedure A or Procedure B.



**Figure 2:** Correlation of calculated phenylalanine concentrations from 28 samples using procedures A and B

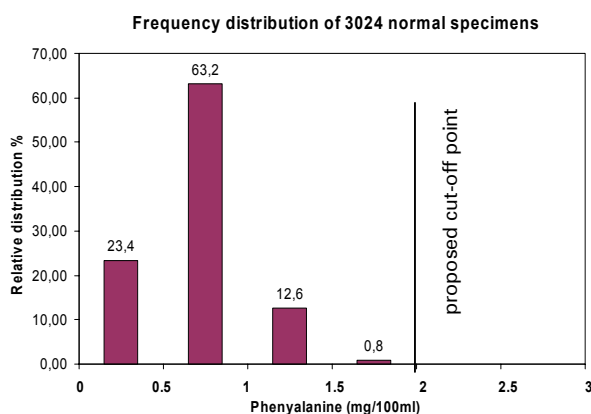
## CLINICAL EVALUATIONS

### Newborns classified as normals

Limited clinical evaluation of the Neonatal phenylalanine was performed as a retrospective study at the Belarussian Institute for Hereditary Diseases (Centre for Medical Genetics Service), Republic of Belarus. The material studied comprised randomly selected 3024 specimens from newborns classified as normals by thin-layer chromatography and in-house fluorometric micromethod. All normal samples were taken on the 4-5th day of life.

The reliability of the in-house method was proved by participation in the External Quality Assurance programs and detection of all hyperphenylalaninemias in the Republic.

The distribution pattern of phenylalanine concentrations of apparently healthy newborns is presented in the Fig. 3.



**Figure 3:** Frequency distribution (in %) of blood phenylalanine values given by the Neonatal Phenylalanine for apparently normal 3024 blood spot specimens.

Measures of the frequency distribution.

Max	=	1.97 mg/100 ml
Mean	=	0.70 mg/100 ml
Standard deviation	=	0.280 mg/100 ml
Skewness	=	0.447 mg/100 ml
Kurtosis	=	0.628 mg/100 ml
Median	=	0.680 mg/100 ml
95th percentile	=	1.20 mg/100 ml
99th percentile	=	1.47 mg/100 ml
99.9th percentile	=	1.85 mg/100 ml

Thus the proposed cut-off value was set at 2 mg/100 ml, however it is recommended that each laboratory establishes its own cut-off value. To ensure the detection of mild hyperphenylalaninemia cases it is advisable to set low cut-off value.

### PKU patients

Samples from PKU patients (n=22) being at the time of evaluation on a phenylalanine restricted diet were analysed by the present method in comparison with the in-house fluorometric micromethod. The phenylalanine values at the time the diagnosis was verified using patients sera varied from 16.4 mg/100 ml (mild PKU, n=1) to 51.4 mg/100 ml.

The correlation of phenylalanine values obtained by the Neonatal Phenylalanine and in-house fluorometric micromethod gave linear regression  $y = 1.3x - 0.55$ , where  $y$  = in-house fluorometric micromethod and  $x$  = Neonatal Phenylalanine and correlation coefficient  $r = 0.93$ . The range of phenylalanine in patients taken for this study varied from 2.2 to 10.2 mg/100 ml by Neonatal Phenylalanine.

### PKU screening

Clinical evaluation has been performed at Neonatal Screening Laboratory of Shanghai Children Hospital, China from the beginning of 1999 to June 2001 (19). Samples were taken from the routine screening program and total number of screened cases was 78,151.

Among all screened cases, 8 cases were identified and clinically confirmed as PKU patients, incidence of PKU was calculated as 1/9769. Two of the cases were low positives which have phenylalanine level of 3.0mg/dl and 3.5mg/dl (the cut-off value used was 2.0mg/dl). No missing positive cases were observed.

## LIMITATIONS OF THE PROCEDURE

The Neonatal Phenylalanine is designed for screening of newborns for phenylketonuria and in some instances for the monitoring of dietary therapy. For exact phenylalanine values use serum as a specimen.

A definite clinical diagnosis should not be based on the results of any single test. If the infant with PKU has not had a sufficient intake of protein prior to the test a false negative result may occur.

It is recommended that the assay is performed by qualified and trained laboratory technician.

## TROUBLE SHOOTING

DECREASED SLOPE OF THE CALIBRATION CURVE AND LOW FLUORESCENCES	
Cause/Error	Remedy
1. Reagents are deteriorated * due to contamination of all reagents * due to improper storage (especially of L-leucyl-L-alanine) * pH of the buffer is too low	1. To prevent deterioration use aseptic technique when pipetting reagents repeatedly from the same vial. 2. To avoid deterioration, see instructions for reagent storage. 3. pH of the buffer should be 5.7-5.9
2. Insufficient elution time	Elute phenylalanine for 30 min.
3. Incubation temperature is too low	Procedure A: 1 hour at +60°C Procedure B: 2 hours at +37°C
4. Incubation time is too short	See above
5. Fluoroskan Ascent wavelength settings are not correct	Fluoroskan Ascent excitation/emission wavelengths are 390/485
6. Fluoroskan is programmed for blanking	Reprogram Fluoroskan
7. The scaling factor is not suitable.	Use a suitable scaling factor

VIOLET COLORATION OF THE PLATE	
Cause/Error	Remedy
1. Too high temperature in the Procedure A	Check the temperature of iEMS Incubator/Shaker $t = 60^{\circ}\text{C} \pm 5^{\circ}\text{C}$
2. Improper fixation of hemoglobin and peptides on a dried blood disk	1. Avoid contamination of disks with water 2. Check the concentration of ethanol used for elution.

POOR PRECISION	
Cause/Error	Remedy
<b>Only calibration curve</b>	
1. Calibrators and controls are deteriorated due to improper storage	Protect calibrators and controls from excessive light and moisture by resealing the foil package tightly
<b>Whole plate (including calibration curve)</b>	
1. Liquid handling devices are not properly calibrated	Check calibration of your pipetting device
2. Uneven heating	Use preferably iEMS Incubator/Shaker or HT
3. Air bubbles when pipetting	Pipette carefully
4. Air contamination of wells by particles	Perform assay in clean environment
<b>Only patient samples</b>	
1. Uneven distribution of blood in the sample	When possible, use samples fully impregnated with blood

CHANGE OF THE SLOPE OF THE CALIBRATION CURVE AFTER SUBSEQUENT MEASUREMENT	
Cause/Error	Remedy
1. The measurement of the plate after addition of copper reagent is not within the recommended time interval	The plate should be measured within 15 - 30 minutes after the addition of copper reagent

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**RELATED PRODUCTS AND OTHER INFORMATION**

Product no	Product description	Kit size
61 99 850	Neonatal Galactose	960 wells
61 99 860	Neonatal G6PD	960 wells
61 99 870	Neonatal 17-OH-Progesterone FEIA	480 wells
61 99 875	Neonatal 17-OH-Progesterone EIA	480 wells
61 99 896	Neonatal Phenylalanine	960 wells
61 99 897	Neonatal Phenylalanine	4800 wells
61 90 930	Neonatal Phenylalanine Controls	5 sets of 3 levels -
61 90 940	Neonatal Phenylalanine Calibrators	5 sets of 6 levels -
61 99 880	Neonatal hTSH FEIA Plus	960 wells
61 99 881	Neonatal hTSH FEIA Plus	4800 wells
61 99 892	Neonatal hTSH EIA	960 wells
61 99 8923	Neonatal hTSH EIA	4800 wells
61 99 802	Neonatal Toxoplasma gondii IgM FEIA	480 wells

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