Newborn Screening for Phenylketonuria: Latin American Consensus Guidelines

Gustavo J. C. Borrajo, PhD

Abstract
Newborn screening (NBS) for phenylketonuria in Latin America gave its first step in an organized way 3 decades ago when the first national NBS program was implemented in Cuba. From then onward, it experienced a slow but continuous growing, being currently possible to find from countries where no NBS activity is known to several countries with consolidated NBS programs. This complex scenario gave rise to a great diversity in the criteria used for sample collection, selection of analytical methods, and definition of cutoff values. Considering this context, a consensus meeting was held in order to unify such criteria, focusing the discussion in the following aspects—recommended blood specimens and sample collection time; influence of early discharge, fasting, parenteral nutrition, blood transfusions, extracorporeal life support, and antibiotics; main causes of transient hyperphenylalaninemas; required characteristics for methods used in phenylalanine measurement; and finally, criteria to define the more appropriate cutoff values.

Keywords
phenylketonuria, hyperphenylalaninemia, newborn screening, sample collection, methods, cutoff values

Origins of Newborn Screening
Newborn screening (NBS) for phenylketonuria (PKU) began in the United States in 1963 when Robert Guthrie developed the bacterial inhibition assay (BIA) for the measurement of phenylalanine (Phe) and introduced the filter paper device for collection of whole blood by heel stick. Both findings were the cornerstone for one of the most important advances in preventive medicine, providing an opportunity for early diagnosis of PKU. Early disease detection also made possible the prevention of the neurologic damage caused by the disease, given that Bickel had demonstrated some years before that early implementation of a treatment consisting of a Phe-restricted diet was able to prevent the clinical expression of PKU.

In subsequent years, the results of the first NBS programs encouraged the implementation and expansion of new programs in the United States and Europe, and thus, by the end of the 80s, NBS for PKU became universal in most developed countries. At present, and considering the cost/benefit arising from its implementation, NBS for PKU together with congenital hypothyroidism has achieved high acceptance and coverage around the world.

Newborn Screening for PKU in Latin America
The NBS for PKU in Latin America can be characterized as diverse, showing a practically continuous spectrum of possibilities. In some countries, NBS began 20 years ago or more, whereas in others, NBS activities are minimal or virtually nonexistent. Such heterogeneity is clearly exhibited in some characteristics like the year and modality of NBS implementation, legislation in force, and coverage reached.

There are 3 pioneering countries in terms of implementation of organized NBS programs for PKU at a national level—Cuba in 1986, Costa Rica in 1990, and Chile in 1992. Today, only 6 other countries in Latin America have implemented national or regional NBS programs—Argentina (1995, 1999, and 2000 regional; 2006 national), Brazil (2001), and more recently Uruguay (2007), Paraguay (2007), Panama (2008), and Ecuador (2011). Other countries like Mexico, Venezuela, Peru, and Guatemala have implemented NBS activities with varying degrees of success and coverage, whereas countries like Colombia, Bolivia, Nicaragua, and Dominican Republic only...

1 Detección de Errores Congénitos, Fundación Bioquímica Argentina, La Plata, Argentina

Received June 17, 2016, and in revised form September 25, 2016. Accepted for publication October 19, 2016.

Corresponding Author:
Gustavo J. C. Borrajo, PhD, Fundación Bioquímica Argentina, Calle 6 N 1344, La Plata 1900, Argentina.
Email: borrajog@net-alliance.net

This article is distributed under the terms of the Creative Commons Attribution 3.0 License (http://creativecommons.org/licenses/by/3.0/) which permits any use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).
offer screening testing for PKU in the private sector by request and without a formal program structure. The most critical situation is observed in El Salvador, Honduras, and Haiti, where NBS for PKU is virtually nonexistent.5-7

Regarding coverage reached in the region, a recent survey estimates that out of a total of around 10.5 million live births per year, approximately 46% of newborns in Latin America have access to NBS for PKU. Incidence of PKU and persistent hyperphenylalaninemias (HPAs) is estimated at 1:24 617 and 1:20 775, respectively; data obtained on a population of 37 023 392 newborns screened in 11 Latin American countries. However, it must be highlighted that a detailed analysis by country showed that the incidence of PKU and HPA in countries located above the equatorial line was significantly lower than in South American countries, a fact probably linked to ethnic composition. Finally, despite the significant growth and evolution observed in the region in the last decade, NBS for PKU in Latin America still have a lot of issues to deal with and improve.

Methods Used in NBS for PKU in Latin America
Data provided by the External Quality Assurance Program for NBS (Programa de Evaluación Externa de Calidad para Pesquisa Neonatal [PEEC-PN]) from the Fundación Bioquímica Argentina (Argentine Biochemical Foundation) corresponding to the period 2000 to 2015 show that the methods used in NBS for PKU include fluorometric and enzymatic colorimetric methods, BIA, tandem mass spectrometry (MS/MS), thin layer chromatography, and high-performance liquid chromatography. However, since 2006 onward, fluorometric and enzymatic colorimetric methods have represented more than 90% of analytical principles used, with a growing trend of MS/MS use in the last 6 years.

Cutoff Values Used in NBS for PKU in Latin America
Data corresponding to the June 2015 survey of the PEEC-PN, which counted on the participation of 65 Latin American laboratories, show that cutoff values ranged from 1.0 to 4.1 mg/dL, 38.5% of which were 3.0 mg/dL or higher. This last fact has a great diagnostic relevance as a cutoff value equal or greater than 3.0 mg/dL is above the recommended level to be used in order to assure not only an appropriate diagnostic sensitivity for PKU but also the detection of HPA variants.8

Recommendations for NBS
Blood Specimens
The NBS for PKU must be conducted using dried blood spots (DBSs) collected on filter paper (Whatman 903, GE Healthcare Life Sciences, Buckinghamshire, UK; Ahlstrom Grade 226, Ahlstrom Corporation, Helsinki, Finland; Munktell Grade TFN, Ahlstrom Germany GmbH, Bärenstein, Germany) by heel stick.9 The sample collection procedure must be performed by trained, experienced, and suitable technical staff, following the rules and technical recommendations established by each NBS program, which in turn must be in accordance with international recommendations like those published in the document “Blood collection on filter paper for Newborn Screening Programs”—Clinical and Laboratory Standards Institute.10

A summary of the main steps to be considered for sample collection is described as follows:

a. Wash hands and wear powder-free gloves.
b. Confirm identity of newborn and complete all fields on the card at the time of sampling using legible handwriting.
c. Warm the heel site to increase blood supply to the area by covering the puncture site with a soft cloth moistened with warm water at a temperature not more than 42°C for 3 to 5 minutes or using a heel-warming device containing an exothermic thermochemical composition.
d. Place the newborn’s leg lower than the heart to increase venous pressure.
e. Cleanse the puncture site with 70% isopropyl alcohol. Wipe dry with sterile gauze.
f. Select the appropriate site of puncture: for full-term and preterm infants, the external and internal limits of the heel are the recommended.
g. Obtain the sample using a sterile lancet or an automated incision device designed for use on newborns. Skin puncture must be no deeper than 2.0 mm.
h. Wipe away the first drop of blood with sterile gauze.
i. Apply gentle pressure with thumb around the heel and ease intermittently as drops of blood form.
j. Fill each circle on the filter paper card, using a single drop of blood. Do not allow the heel to make contact with the card.
k. Allow blood to soak through and completely fill the preprinted circle by natural flow with a single application to 1 side of the filter paper only. Do not layer successive drops of blood.
l. Do not squeeze the foot in an attempt to increase blood flow.
m. After collection, examine both sides of the filter paper to ensure saturation.
n. Allow blood spots to air-dry away from direct sunlight or heat, on a horizontal level, for a minimum of 3 hours, at room temperature, avoiding the contact with surfaces or liquids.
o. After the specimen has dried, place in an envelope and dispatch immediately or as much within 72 hours of taking the sample. Use of sealed plastic bags requires including desiccants.

Good quality DBS requires a sufficient quantity of blood to soak through and completely fill a preprinted circle on the filter paper in order to assure reliable results.10

Sample collection time
Specimens for NBS for PKU must be collected between 24 hours and seventh day of life. In physiological terms, 24 hours
of life is an appropriate time to obtain reliable results due to the convergence of 2 factors—on one hand, the endogenous Phe contribution coming from the increased protein degradation observed along the first hours of life as a consequence of the increased catabolism triggered by the stress of labor and by the low caloric content of colostrum and, on the other hand, the exogenous Phe contribution coming from breast or formula feeding. Thus, the sample collection at 24 hours of life or later allows for the establishment of a clear differentiation between Phe levels of nonaffected and potentially affected individuals when quantitative methods are used for Phe measurement.11-13

Nevertheless, the statement above, sample collection time must be established taking into account that NBS for PKU is not an isolated test but is part of a NBS panel of congenital diseases whose amplitude depends on the public health policies defined in each country or region. Thus, every NBS program must define its own sample collection time taking into account the diseases screened and the optimal screening windows when there is the greatest chance of diagnosing and treating the disorders in question before symptoms or permanent damage occurs. Additionally, it should be remembered that such optimal screening windows do not always overlap along the time, being very short in some cases, with illness beginning by the end of the first week of life and death in the second week if not diagnosed.14 Therefore, an equilibrium point is needed to be reached to decide on a sample collection time that allows for completing NBS for every infant in the shortest period of time, with the highest degree of reliability and using the fewest number of specimens.

Special newborn situations to be considered

Early sample collection (before 24 hours of life). In practice, sample collection before 24 hours of life usually occurs due to early hospital discharge or because of the clinical urgency to refer the newborn to a more complex health-care center. In such cases, considering the importance of admitting the newborn into program registries, it is recommended to collect the sample for testing before discharge even if the newborn is less than 24 hours old and, given the lack of diagnostic value of a normal result in this sample, to collect a second sample before the seventh day of life.

Fasting. Samples collected in fasting newborns lack of diagnostic value because babies have not received sufficient protein to be able to biochemically express the metabolic defect, which leads to false-negative results. In these cases, a second sample must be collected after the baby has fulfilled 24 hours of milk or high biological value protein intake.

Parenteral nutrition. Parenteral nutrition produces a marked increase in Phe levels, giving rise to false-positive results. However, and considering that this increase affects not only Phe but also other amino acids concentrations, mainly branched chain amino acids, it can be easily detected when screening is conducted by MS/MS. In order to obtain reliable results, a second sample must be collected 24 hours after parenteral nutrition is discontinued.14

Blood transfusion and extracorporeal life support. Sample collection must be done before the transfusion procedure or before the newborn connection to extracorporeal life support. However, since the pretransfusion collection is often forgotten or not always possible due to clinical reasons, a sample should be collected 72 hours after the transfusion or after disconnection from life support. In this way, false-negative results caused by the dilution effect of transfusion or by the dilution effect plus the volume replace in extracorporeal life support will be avoided.14

Antibiotics. Administration of antibiotics causes interferences in BIA giving rise to false-negative results. In these cases, testing should wait until the interruption of the antibiotic administration and its cleansing from the blood and then proceed to collect a second sample.1,15

Transient increase in Phe. In daily practice, there are several situations that cause a transient increase in Phe concentrations, which are detailed as follows:

a) Liver immaturity.
b) Infectious diseases affecting the liver, like hepatitis or sepsis.
c) Other inborn errors of metabolism affecting the liver, like galactosemia.
d) Intake of whole cow’s milk.

Methods

The methods used in NBS for PKU must fulfill the requirements applicable to all NBS methods with regard to analytical (accuracy, precision, specificity, sensitivity, linearity, recovery, total analytical error, robustness, limit of detection, and limit of quantitation), diagnostic (sensitivity, specificity, positive predictive value, rate of false positives, and false negatives), and performance must be guaranteed over time through the implementation of a strict daily internal quality control system and through the participation in external quality assurance schemes.18,19

Currently, there are several diagnostics companies that provide reagent kits for measurement of Phe in DBS based on different analytical principles, some of which can also be developed “in house.” Below is a list of the most internationally used methods for Phe measurement, ordered
Cutoff values provided by commercial kits should be used only as a reference. In addition to the definition of the main cutoff value, establishing a second cutoff in order to decide what newborns should be retested in a second DBS or immediately referred for clinical evaluation and confirmation testing is recommended.

In general, cutoff values used for methods like fluorometrics or enzymatic colorimetrics are defined in the range 2.0 to 2.5 mg/dL (120-150 μmol/L), whereas in the case of MS/MS, they are lower, around 1.0 to 1.5 mg/dL (60-90 μmol/L). In the case of BIA, cutoff values are usually around 3.0 mg/dL (180 μmol/L) or higher due to its limited sensitivity to concentrations below 2.0 to 2.5 mg/dL.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

References