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Article

Disparities or Inequities in Newborn Screening (NBS) Programs

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Abstract

The subject of the title is discussed from personal experience in Galicia (Spain), with a brief allusion to the beginning of NBS, indicating that without diagnosis, there can be no treatment, including the outstanding role played by **LI Woolf,** who always advocated not limiting it to PKU, and ignorance of their work, even in the hospital itself, with a footnote referring to current situations. The beginnings of NBS programs in Spain are referred to, which were born expanded, with a methodology that I call open, chromatographic techniques, and nonspecific reagents that originate open procedures, applicable, for example, in the detection of lysosomal storage diseases, demonstrating the importance of urine in our program. The methodology used determines the pathologies to be detected, and the professionals who choose one or the other according to the criteria that are maintained today are largely responsible for the current disparities. The administrations acted on the matter sometime later when it had been rolling and running in different ways, depending on the territory. Like others, Galicia always had an expanded program and was almost always ahead. At one point, a "Dr. BESSMAN" appeared in our administration who discovered the systematic reviews and scientific evidence, ignoring our evidence; after maneuvering in different spaces, he tried to be coercive, reducing the program without knowing the i tended purpose; a petition on the *Change.org* platform stopped that claim. The approach is based on a universal public social and healthcare system with competent professionals to successfully address rare diseases and, in the event of unexpected findings, to seek the best option for the patient. What has been published on the subject and the peculiarities of Spain are discussed. It is suggested that epidemiologists who are dedicated to this matter mutate into rareologs, and the situation at the beginning of the NBS is recalled when organized opposition from doctors arose, highlighting Samuel P. **Bessman.** It was the parents, as happened with Føling and Bickel, who got them interested in looking for the cause and treatment; now, they (parents and relatives) are changing to continue expanding the NBS programs. In Galicia, it appears that the criteria set by political leaders have changed, which has resulted in the exclusion of potential candidates such as Bessman from this role. In Spain, there are still disparities, as in Europe and many other places; the approach will have to be different depending on the social-health system of origin. Prof. F. Mayor Zaragoza took steps to address the issue within the framework of the Universal Declaration of Human Rights. If the precursors of the NBS had waited for scientific evidence at its inception in 1956 and 1957, it would not exist today. It is not possible to look for differences in the health of populations with broad and narrow NBS programs because these diseases are rare and do not significantly influence global health. Seeking equity cannot curtail programmers where no harm caused by them is appreciated. The differences between programs allow them to be compared and to progress. The emergence of biomarkers, treatments, analytical technology, etc., requires frequent changes in programs; and the training, judgment, criteria, mentality and mindset of those responsible for them will determine whether these changes are adopted.

Keywords: newborn screening; disparities or inequities; Galicia; Spain

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Open Analytical Methods and Procedures for Expanded NBS and Program Disparities

Introduction

The following comments are the result of personal experience in Galicia, a historical nationality in the Kingdom of Spain, northwestern corner of the Iberian Peninsula, the cradle of the Portuguese language, and the Finisterre of the Roman Empire.

The surface of the current Galicia is 29,574 km2, with 2,555 km of indented coastline. There are 2.7 million inhabitants. In 2022, 14,495 births were registered, of which 100% were analyzed; in 1978, 43,721; and in 1993, 21,248, with 21,148 analyzed (99%), the year with the highest number of analyzed newborns; and in 2018, 16,848 births were registered, with 100% of newborns analyzed.

I remind you that Sheila Jones was of Irish origin; when Bickel detected PKU in her and, together with the pediatrician John Gerard and the head chemist of the laboratory, Evelyn Hickman, made the diagnosis, they were very happy to identify PKU, something new and rare. The mother did not understand this satisfaction if they could not cure it; the book and the article by A. Green [1,2] supply detailed information on what happened. It is evident that without diagnosis, there would be no treatment.

The dietary treatment and preparation methods were proposed by LI Woolf [3,4] when he was working at the Organic Chemistry Laboratory of the Hospital for Sick Children, Great Ormond Street, London (GOSH), arriving there in 1947 (ICI fellowship). He was the first in 1956 to treat a newborn with PKU (17 days), considering its need for lipids and the essential amino acid phenylalanine in the necessary quantity to build proteins*; this nutrient shortage causes catabolism and paradoxically increases its concentration; the latter was ignored by some, and the results were disastrous. Even at GOSH, Moncrieff and Wilkinson [5] reported in 1961 a newborn with PKU (1958 Woolf's last year at GOSH), Moncrieff wrote the 1955 paper [6] formulating the diet, but he has not discussed the newborn's diet with Woolf the following year, and he omitted the addition of lipids when he added them any problems that had arisen disappeared. This highlights the lack of attention that some pay to their work**, in this case, with the collaboration of a nutritionist. Woolf tried to clarify the reason for the high prevalence of PKU at birth in Ireland and western Scotland, suggesting that genetically determined phenylalanine levels might have a protective effect against some mycotoxin [7].

*She treated her non-PKU twin sister with the same formula to confirm that her diet was sufficient for normal development. The PKU patient reached an IQ of 90, and the non-PKU patient reached 110; the older sister (the index patient, PKU), who was not treated, did not exceed 20 [8]•pg 71

**The following three articles are examples of today's lack of knowledge about what was done in the laboratory in Galicia.

In the first article, the procedure in citation 19 from 1981 was discontinued in 1982, and the procedure in citation [19] of this paper (**without PYRIDINE**) has been used since 1997; [21] describes the evolution of the procedure in the laboratory on pages 170-194. In that paragraph, the references are incorrectly assigned.

----Couce ML, Bóveda MD, Castiñeiras DE, Vazquez-Mosquera ME, Barbosa-Gouveia S, De Castro MJ, Iglesias Rodriguez AJ, Colón C, Cocho JA, Sanchez-Pintos P. A Newborn Screening Programme for Inborn Errors of Metabolism in Galicia: 22 years of evaluation and follow-up. Orphanet J Rare Dis. 2024 May 17;19(1):202 https://doi.org/10.21203/rs.3.rs-3328532/v1

In the second article, of which I am coauthor and of which I was not informed, the same error was made in reference 27.----Sánchez Pintos P, Cocho de Juan JA, Bóveda Fontán MD, Castiñeiras Ramos DE, Colón C, Iglesias Rodriguez AJ, de Castro López MJ, Alonso-Fernández JR, Fraga JM, Couce ML. Evaluation and perspective of 20 years of neonatal screening in Galicia. Program results. *Rev Esp Salud Pública.* 2020; 94: 16 de diciembre e202012161. PMID: 33323918 https://pubmed.ncbi.nlm.nih.gov/33323918

In the third article, from a year ago, the same error was made in reference 8. Furthermore, the use of a urine reducer was not mentioned. Tests were performed with the MANDĒLIN reagent [4].-

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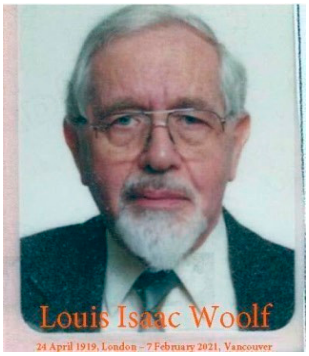
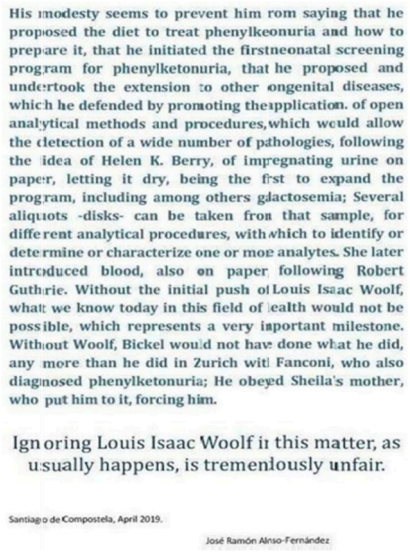
--Sánchez-Pintos P, Camba-Garea MJ, Martin López-Pardo B, Cocho de Juan JA, Bóveda MD, Barbosa-Gouveia S, Vázquez-Mosquera ME, Barros-Angueira F, Fernández Patiño R and Couce ML Clinical and biochemical evolution after partial dietary liberalization of two cases of galactosemia due to galactose mutarotase deficiency. BMC Pediatrics (2024) 24:620 https://doi.org/10.1186/s12887-024-05074-6



I have been interested for time in the contributions and figure of **LI Woolf**, a chemist like me, who is very ignored, who opened the path we have made others pilgrimages, including Bickel and Guthrie, perhaps because of their modesty, professors Mayor and Ugarte, who knew him at Oxford, were unaware of his time at GOSH in London, where everything that has brought us here was forged (they learned about it from me, consequence the article [3] prepared for his centenary; which means he did not tell them about). I inserted the translated page, which was included in the edition on his 100th birthday [8], with which to end the prologue. (Figure 1)


His modesty seems to prevent him rom saying that he proposed the diet to treat phenylkeonuria and how to prepare it, that he initiated the firstneonatal screening program for phenylketonuria, that he proposed and undertook the extension to other congenital diseases, which he defended by promoting the application. of open analytical methods and procedures, which would allow the detection of a wide number of pathologies, following the idea of Helen K. Berry, of impregnating urine on paper, letting it dry, being the first to expand the program, including among others galactosemia; Several aliquots -disks- can be taken from that sample, for different analytical procedures, with which to identify or determine or characterize one or more analytes. She later introduced blood, also on paper following Robert Guthrie. Without the initial push of Louis Isaac Woolf, what we know today in this field of health would not be possible, which represents a very important milestone. Without Woolf, Bickel would not have done what he did, any more than he did in Zurich with Fanconi, who also diagnosed phenylketonuria; He obeyed Sheila's mother, who put him to it, forcing him.

Ignoring Louis Isaac Woolf in this matter, as usually happens, is tremendously unfair.

Santiago de Compostela, April 2019.

José Ramón Alonso-Fernández

Figure 1. Insertion of the translated page, included in the edition of the monograph on the occasion of the centenary of the birth of L I. Woolf [8] with which the prologue ends.

Louis Isaac Woolf advocates an expanded Newborn Screening, not limited to PKU, as he wrote in 1968 to Prof. Federico Mayor Zaragoza.* (Figure 2)

F Mayor, who met Woolf at Oxford during the 1966-67 sabbatical with Krebs (with whom he had already been in 1959, Woolf was there, but they did not meet then), set up an NBS Laboratory financed by the Spanish Government in 1968 at the facilities of the Directorate General of Health in Granada. The inauguration in January 1969 was carried out by Dr. García Orcoyen, Director General of Health, and the highest health authority. (Figure 3)

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MRC
Medical Research Council
External Staff

reference LIW/ js

Department of the Regius
Professor of Medicine
The Radcliffe Infirmary, Oxford

telephone Oxford 48891 ext 225

January 10th, 1968

TF

Professor F. Mayor,
Universidad de Granada,
Facultad de Farmacia,
Catedra de Bioquimica,
GRANADA, Spain.

Dear Professor Mayor,

Many thanks for your letter of January 2nd, I agree with you that it would be well worth-while to set up a screening service for the newborn in Spain, particularly if this screen were not confined to phenylketonuria but also covered other treatable inborn errors of metabolism. I think it is an excellent plan that you, with the Heads of the Analytical Centre and Preventive Medicine visit me in Oxford to study the actual working of the scheme. I plan to stop laboratory work here on March 1st; I hope, therefore, it will be possible for the three of you to visit Oxford either this month or next month. If it is to be February, it is preferable early in February. I would welcome the chance of taking part in the course of formation of the Centre in Madrid and of giving the opening lecture. Will it, however, be possible to organize this in the time left before I go to Vancouver?

With best wishes and the hope of a fruitful outcome.

Yours sincerely,

L. I. Woolf

Figure 2. Louis Isaac Woolf advocates an expanded NBS not confined to PKU, as he wrote in 1968 to Prof F Mayor.

Independently and unaware of F Mayor's initiative, Dr. Juan Sabater Tobella became interested in the same subject. One year later, in Barcelona, he started an NBS Laboratory funded by the provincial government and the Juan March Foundation.

Inaugurated on 2/7/1970, chaired by HRH the then Princess Doña Sofia, later Queen, she went on in the subject, presiding over a Royal Board of Trustees on Disability that promoted a National Plan, which, in 1977, was driven by Prof. F. Mayor, during his tenure as Minister of Education, enabled the implementation of the NBS Program throughout Spain.

JEFATURA PROVINCIAL DE SANIDAD
GRANADA

Secretaría

Fecha 8 de Junio de 1968
Núm. 5147

Asunto. Comunicando aprobación Pla
para investigación alteraciones
congénitas metabolicas y cromoso

El Excmo. Sr. Director General de Sanidad por oficio nº 257 de 7 del corriente, me dice lo que sigue:

"Tengo el honor de comunicar a V.I. que estudiado por este Centro Directivo el "PLAN PARA LA INVESTIGACION Y CONTROL DE LAS ALTERACIONES CONGENITAS METABOLICAS Y CROMOSOMICAS" presenta por el Profesor F. Mayor; esta Dirección ha resuelto aprobar en principio el mismo y que se proceda a su ejecución inmediata en la provincia de Granada como experiencia en la que se perfeccionará la organización y método para en su caso extender su acción progresivamente al resto del país.

Deberá comunicar esta resolución a las personas interesadas en el mismo y prestar la colaboración precisa."

Lo que traslado a Vd. para su conocimiento y efectos oportunos.

Dios guarde a Vd. muchos años.
EL JEFE PROVINCIAL DE SANIDAD

Prof.Dr. Don Federico Mayor.- Catedrático de Bioquimica de la Facultad de Farmacia.-GRANADA

Figure 3. F Mayor set up an NBS Laboratory finance by the Spanish Government in 1968 in Granada.

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*Documentation provided by Prof. M Ugarte.

Open Methodology

Chromatography

The use of separation techniques such as planar chromatography allows the identification of multiple biomarkers using a single sample aliquot, which constitutes a window. We open the window and look at who is passing. If it is the usual one, the result is negative. If we see someone who is not but has already presented it to us, we must recognize him. If it is not known, we must determine who he is.

Chromatographic procedures are what are currently called "multiplex", although they are often ignored in NBS. All of them have been used in Galicia since 1978, for which blood and urine is available, allowing for various detections, such as methylmalonic and ethylmalonic acidemia [9]*, leucinosis, alkaptonuria, tyrosinemia, cystinuria, etc., and can reveal any unsuspected stain.

Unfortunately, **Dr. A. Maya** has not published his amino acid PC procedure, which is part of his doctoral thesis [10] and was described and commented upon in the cited monograph [8]•pp 111-121. This text [8] discusses how the introduction of NBS programs in Spain was exceptional since Guthrie's BIA was not initially used; however, expanding the use of planar chromatography resulted in the expansion of these programs, as Woolf advocated.

Paper chromatography (PC) under pressure on paper *and pressure and/or vacuum to displace the mobile phase (**MP**), about which I have already published communications [11] and described the instruments, has the advantage that when using a liquid MP and applying pressure to the paper, capillary forces increase and, therefore, increase the migration rate of the MP and the solutes (components of the sample to be separated).*

This reduces the pressure or vacuum required to displace the MP and compact the spots, in addition to considerably increasing the migration rate and shortening the process time.

*It also allows the use of gaseous MPs, supercritical fluids, or fluids close to phase equilibrium between the triple point and the critical point. It also allows the temperature to be set and programmed. In addition, it is possible to use stationary phases (**SPs**), such as asbestos, silk or wool fabrics (protein), cotton,* etc., *in addition to paper {partitioning with water (SP) and cellulosic (SP)}. In this technique, it is also possible to stack layers of the stationary phase separated by inert films, which allows the simultaneous analysis of many hundred samples with solvent fronts (paper width) of up to 46 cm -experimental- and large, and displacements as long as needed, depending on the number of components to be separated and their mobility, with the SP and MP used. This approach promises a great future.*

*The ability to perform **sequential staining** and the capacity to scan or photograph by directly illuminating with selected wavelengths, including fluorescence capture and transillumination, combined with the contributions of **digital image** processing, which allow, among other things, recording, creating digital archives, identification, triggering alerts, quantification, normalization, etc., in which we have experience, opens up a large range of possibilities. **The main problem with the PC is the price.** It is too cheap. It lacks prestige, and sellers are not motivated to offer it. **Perhaps, with these proposals, interest will increase.***

Evaporative paper chromatography, *developed at the same time as conventional chromatography (17 h), improves this process, compacting the spots and obtaining very sharp separations* [8].

In what follows, specificity and sensitivity refer to the analytical methodology, in no case to that of the NBS

*The PC test for organic acids is exclusive to our laboratory [8]•pp 120-123, [9]. So too is the MANDELIN reagent test for reducing agents in urine and the cTLC (**without PYRIDINE**) for sugars, which is mentioned in this text.

Nonspecific Reagents (Non Target Analysis). Nonspecific Biomarkers

Nonspecific reagents can react with unknown markers or are unexpected, leading to freak findings.

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These are also what I call open procedures. Cl_3Fe reacted with the PKU marker phenylpyruvic acid when Føling was added to the urine, which was unexpected.

In the same way, the reaction of groups of biomarkers -sulfatides, glycosphingolipids, glycosaminoglycans or oligosaccharides- with a reagent that produces, with any of the biomarkers of one of these groups, a detectable and measurable signal with analytical methodology will allow the detection of lysosomal storage diseases. Initially, a single agent could give a positive result for one of these groups of diseases; additionally, a single reagent could give different reactions with markers of each of these groups. In this manner, we could obtain a positive result and identify which group it belonged to in a single step.

If the sample is **dry urine** (DUS-dry urine sample; Berry-Woolf specimen-BWS-), in which the concentration of these compounds increases more rapidly than in blood, possible biomarkers are discarded in aliquots of the same eluate until the one responsible for one of these pathologies is identified. This process is similar to the use of successive levels (or chromatography of one or a few aliquots) and can be combined with blood enzyme measurements followed by DNA sequencing.

Such an approach would lead to the identification of a biomarker and an unforeseen or unknown pathology, which Føling did in 1934.

I once saw it written that we still use urine samples; not only do we still use them, but we use them more. There are people who throw them away because they do not know what to do with them, but that is not the case [12–22].

Using urine, we were able to make the first detection of leucinosis, "maple syrup" or MSUD; it was detected in that sample. He had been transfused -very common in critical pathologies- in the neonatal ICU, where it quickly eliminates the toxin, so the blood is not useful. The BCAAs continue to be excreted in urine for some time; similarly, in galactosemia, galactose continues to be excreted when it has already been eliminated in blood; in this case, the transfused blood also provides the enzyme that is deficient in the newborn, which masks an enzymatic diagnosis; that sample with our procedures allowed us to detect an exanguinotransfused case and parenteral feeding with glucose. We are the only ones in Spain that detect all types of galactosemia and other carbohydrate IEMs using the MANDELIN reagent [4] to detect reducing sugars and cTLC with MP without pyridine [19–21].

We did not abandon the dried urine sample on paper; we collected it at the time of obtaining the blood sample, placed the blotting paper on the genitals, held it with the diaper, and then pricked the heel to deposit the drops of blood on the corresponding paper, letting it dry, and removed the paper with the urine (because of the newborn urinating by reflex when receiving the puncture), letting it dry, would like the industry to look at that sample and test the possibility of developing marketable processes.

Enzymatic reagents *and immunoassay reagents* ***–antibodies-*** *are specific and react with only a single biomarker molecule substrate and antigen, respectively; the procedures that employ them involve the antithesis of open procedures. However, the fact that these reagents detect only a single disease marker does not always prevent them from being considered open if that* ***biomarker is associated with multiple diseases non-specific biomarker.***

Moreover, when ***nonspecific reagents*** *are used to detect* ***non-specific biomarker,*** *additional* ***windows can be opened****, and the horizons of* ***detectable diseases*** *can be* ***widened****. Using a marker that is altered in several diseases* ***expands the range of pathologies*** *that can be detected. If we can* ***assess several of these nonspecific biomarkers****, which are* ***common to several diseases****, via a single analytical procedure, the* ***diagnostic landscape can be enormous.***

When a ***biomarker is an enzyme*** *that must act on a specific substrate and measure a product, in principle,* ***the procedure is not open****; rather, it involves detecting a single disease. To approximate what would be a* ***half-open procedure****, we must use the simultaneous* ***analysis of several enzymatic biomarkers****, which act on their respective substrates and* ***measure the products in a single assay****.*

A recent method, **reactomics**, provides a powerful way to quickly study many enzymatic reactions at once in dried blood spot samples, creating a complete metabolic profile [22]. The

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disadvantage of this approach is that the enzymatic activity is labile, which impairs sample preservation, unlike more stable molecular biomarkers.

Open procedures could be used to determine total **oxysterols** (**non-specific and specific biomarkers**), which are known to be more abundant in the urine of newborns than in that of individuals of other ages, unlike in blood, where they are more abundant at older ages [23]. Pediatric liver disease patients had significantly greater total oxysterol levels in both the urine and serum than did healthy children. The levels of oxysterols, particularly 24(S)-hydroxycholesterol, are greater in the urine than in the serum in patients with liver disease. This article [23] is possibly the first to propose the use of oxysterols as biomarkers of disease (also IEM, sometimes secondary to liver damage); there are currently many other articles on this topic. A reagent that reacts with all **oxysterols** can be used, and separation methods (TLC) to identify the altered oxysterol(s).

Enzymes in enzymatic methods and antibodies in immunoassays are more labile and more expensive reagents than chemical reagents are; additionally, they are generally stable molecules and simpler to synthesize than enzymes and antibodies obtained from living beings.

The best biomarker is the one that best discriminates between affected and unaffected values. The further apart these values are and the sooner this wide difference occurs, the better and earlier it will be.

The enzyme activity marker is expressed earlier than the accumulation of an unmetabolized substrate or the appearance of an abnormal metabolite. Obviously, the enzyme marker must be present in the samples received and be stable under the conditions of the route traveled.

When the biomarker is an antigen determined with an immunoassay, the epitopes can change their conformation with the passage from the solid phase to the solution upon elution, and environmental conditions can affect the conformation; additionally, reactive antibodies also require delicate handling; (lipoproteins are difficult to elute, and their epitopes require polyclonal antibodies to determine them and thus react with any variant).

Analytical methodologies using absorbance or fluorescence readings offer several advantages over MS/MS, as the latter analyzes one sample after another using automated sampling. This results in a longer analysis time, which, in the case of newborn screening, increases with the number of newborns analyzed, unless a sampling failure halts the process or a sample flow obstruction interrupts the analysis, requiring continuous attention. Absorbance readings can be taken at the end of a reaction or by measuring the reaction's progress, providing a kinetic reading. Readings can be taken at different wavelengths and a large number of newborns can be analyzed simultaneously in 96-well microtiter plates using suitable robotic readers with temperature control and automatic plate loading. Reading times are short and require minimal attention. Multiple analytes can be analyzed in a single run by varying the conditions. Fluorescence readings are the most sensitive signals in analytical methods, and by varying the excitation and emission wavelengths, using the aforementioned plate readers, which allow absorbance and fluorescence readings in the same instrument, as well as time-resolved fluorescence measurement, and what was described in relation to biomarkers, reagents, analysis, etc., opens up infinite analytical possibilities at a lower cost than MS/MS.

The Methodology Used and the Pathologies Included

The professionals who created the laboratories and strived to maintain their criteria were primarily responsible for the methodology used. Today, there are still people with a restrictive mentality, which largely explains the disparities, or what they call inequities, in Spain.

The programs' possible pathologies to be included are linked to the methodology used, the samples received in the labs, and the scientific culture of the professionals who work there.

When we talk about iniquity in the Kingdom of Spain, we must go back to the beginnings of the NBS when some said that what **was not indicated was contraindicated**; we can see this in what was written in Memory of Antonio Maya [8]●pp 222-232, which was the cause of confrontation at the time and had a political background because it was a consequence of the decision of the professional (with

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a scientific training similar to mine) responsible for the NBS Laboratory in a Historical Nationality, to use the Guthrie BIA to detect only PKU (when years later I asked -via e-mail- a clinician who had participated in the initial organization of that Program why had they decided to only detect PKU?", he answered that they also did it for CH¿?), the person responsible for choosing that method became the political position and director of public health in that country. With what was given, that of maintaining it and not amending it, which continues to this day, in this case there is a chauvinistic component; it seems that only there was it done well, the rest of us were wrong. That *Director General* sent me a letter, in an aggressive tone, trying to disqualify me, when apparently, I dismantled his approaches without being present at a meeting of politicians in charge of public health, where my writings were brandished. He possibly attended with the idea that he would be the only one with knowledge of the matter to be discussed.

https://www.researchgate.net/publication/333996309_cobertura_de_los_programas_de_tria_neonatal_en_Espana_y_estrategias_de_toma_de_muestras

https://www.researchgate.net/publication/333996196_Cobertura_de_los_programas_de_Tria_Neonatal_en_Espana_y_estrategias_de_toma_de_muestra

It is misleading to say that when phenylketonuria is detected, other aminoacidopathies can occur. I heard this statement once, it is true if you use planar chromatography, but not if you use the Guthrie test which, on the other hand, can produce false negatives due to the almost systematic use of antibiotics in hospitalized newborns. The change to the fluorometric method meant avoiding this problem, having greater analytical sensitivity with respect to the BIA, obtaining numerical results, which seemed to provide greater security, and being able to advance the date of sample collection, which was then between 5 and 8 days of life. In Galicia, we kept this until the introduction of MS/MS, although we had already detected a PKU in a sample taken two days after birth (we have no record of any false negatives.) This approach in laboratories using PC and/or TLC represented a step backward, as they stopped detecting other pathologies. However, it was more expensive, and the providers made a great effort to introduce it.

In 2000, while we inaugurated the laboratory installation in a new hospital, **Prof. JM Fraga** successfully **introduced MS/MS -tandem mass spectrometry-** into the laboratory, thereby expanding the already growing NBS program, which did not significantly change its perception among pediatricians and the Galician population.

Galicia expanded the program. which was already broader and cheaper than others in Spain (which is unknown), made some regions interested in what we did; there was no interest in the previous procedures, which were cheap; in one, they introduced MS/MS; and when the political sign of their government changed, they stopped using it. I do not know what the contract with the suppliers was like; a journalist from a national newspaper published in Madrid (perhaps guided by some politician) asked me (by email) about the cost of the MS/MS analysis. I answered that it depended on what was done and how. I gave her the cost of the Galicia Laboratory per neonate (a low cost that did not interest him), had to explain to her that we did not buy kits, and the procedures that use MS/MS were developed by Drs. JA Cocho and D Castiñeiras. The consumables were obtained from various suppliers (not depending entirely on IVD companies allows costs to be reduced; at the 2013 Joint Meeting of the NBS and Genetic Testing Symposium and International Society for Neonatal Screening. 50 Years of Newborn screening, in Atlanta, USA, meeting, when I had been retired for just over a month, talking to a PerkinElmer salesman of Cuban origin, he did not understand how MS/MS was used in the NBS of Galicia, without using their kits); I told the journalist to ask the suppliers of the kits and not be surprised if the price was different depending on where it was sold.

Relationship of the Laboratory with the Administration Its Intervention in NBS Program

In Galicia, the NBS began with a research project at the University of Santiago de Compostela led by the pediatrician Prof. José Peña Guitian, in which I was the principal investigator. The laboratory was set up in Neonatology (congenital metabolic disease detection and treatment unit [UDyTEMC] and headed by Prof. JM Fraga at the hospital; now, *Clínico-CHUS*, the physical situation

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changed in 2000, the administrative situation changed several times. This is the first NBS laboratory in Spain to be established in pediatrics and in a hospital, so the problem of a lack of a pediatrician to take charge of the detected cases that others in Spain had will not occur (see [8]●pp 225-…)

The administration responsible for the NBS was transferred from the Ministry of Health of the Government of Spain to the *Xunta de Galicia* in 1983. He did not deal with the detections that were made and how they were made, in the case of Galicia, until 2003, when Dr. JM Barral Castro took charge of the General Directorate of Public Health. They were in charge of the laboratory staff, instruments, and logistics, but the laboratory staff scientists and pediatricians had been leading the NBS field until then. The details are given in [8]●pg 49.

Since then, the head of the newly created Population Screening Programs Service (**SPPC**) has become interested in what we did and how we did it. She was pleased with everything, and nothing changed.

On 02/08/2005, a coalition government came to govern Galicia, and the person who had been there in a previous period took over as head of the Service. I had a wonderful personal relationship with him (outside of work and teaching, as a medical student), and he knew perfectly well everything about the laboratory and the NBS in Galicia, but we did something wrong in the relationship with the SPPC.

At the meetings held by those responsible for the **laboratories in Spain**, we discussed the incorporation of new diseases, but the administrations barely participated in the decision-making process; on pages 223 to 232 of the aforementioned monograph [8], in the dedication in memory of Antonio Maya Victoria, an account of these meetings was given. From 1986 onward, the meetings were held annually, coinciding with the SEQC (Spanish Society of Clinical Chemistry) Congresses and sometimes coinciding with international events. Since 1995, it has rotated between different laboratory locations. At one time, there were 22 (today 15). The Royal Board of Disability sponsored the annual meeting starting in 1996. In 2006, it was held in Santiago de Compostela, Galicia.

Systematic Review and Scientific Evidence

On 20/11/2008, I participated in the first meeting of the working group for the preparation of the service charter for metabolic disorders, according to Decree 117/2008, of 22 May, regulating the service charters of the *Xunta de Galicia* and the Quality Observatory of the Electronic Administration of Galicia (DOG 12/06/2008; 113: 11268-11279), to which I was not initially invited because they did not transmit my name from the UDyTEMC of the CHUS to the SPPC. I asked the SPPC to participate, and it managed my participation with the UDyTEMC. t was the first time it had been applied to a service that was not purely administrative, a public health program. *The work was distorted, resulting in an evaluation of health technology in light of scientific evidence, which is not possible when we do is evidence-generating medicine. The decree insists on the participation of the affected population, and on this occasion, the participation of those affected -family, relatives, etc.- was avoided. After much work and bibliographical review, on 28/06/2010, when the 15th and last meeting was held, the last of the conclusions were reached. The working group views the tasks assigned to it as completed. The Directorate General of Public Health and Planning may call for a meeting to reach a consensus if there are important discrepancies in the evaluation of the final documents. The SPPC concluded, without the work group, what was included in the ["Report on the review of compliance with criteria to establish a population screening programme by pathologies or conditions that are currently examined within the Galician Program for early detection of endocrine and metabolic diseases in the neonatal period"]. That report was not made public, and the working group learned about it from people outside it (I refuted in [8]●pp 19-22).*

At no point was there any interest in evaluating the Program or Laboratory's healthcare technology in light of our living evidence and our science. This is our unique approach, with very clear beneficial results for those affected, their families, and therefore society and the healthcare system. It is free of personal and social harm of any kind. It is fully incorporated into Galicia's healthcare culture and constitutes an acquired right of the Galician people. Its population coverage was growing to approximately 99%, and families facilitated the collection of urine and blood from

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newborns on paper. Pediatricians were also in agreement, collaborating without objection. **Cutting the NBS would be unethical.**

Those of us who ran the program resisted attempts to cut it. For a while it seemed that the desire to cut the NBS had calmed down.

The Galician government changed its political affiliation on 16/04/2009. In early 2015, I received news that the director of CHUS had said we were **doing too much** (Dr. Fraga has already retired). After attending the presentation of the *AVALIACIÓN da Estratexia Sergas* 2014 on February 23, I decided, after Rare Disease Day, the last day of February, to send a letter to the organizers Day, expressing my concern about the program's reduction and the elimination of urine testing. Additionally, I started a petition on *Change.org* addressed to the president of the *Xunta* to prevent these changes. It was successful on April 7.

At https://www.change.org/p/xunta-de-galicia-no-pongan-en-peligro-la-detección-de-enfermedades-en-galicia/u/10260701 address can be accessed through the petition on *Change.org* [accessed on 09/10/2025], and updates can be followed by going back to the beginning and forward to the press release where the Administration accepts my petition. Translated Inserting (Figure 4).

José Ramón Alonso Fernández
Santiago de Compostela, España
Abr 7 2015
Congratulations, you did it, with the collaboration of the media, the involvement of those who work and worked on the Program and the good judgment of the Authorities. I copy the press release from 4 pm:
Galicia includes 29 pathologies in its neonatal screening program The program includes the seven pathologies recommended by the Ministry One of them, sickle cell anemia, was not in our service portfolio until now It also includes 22 other pathologies that have already been carried out The General Directorate of Innovation and Public Health Management has come to meet with the professionals to work on improving the program and collect all their contributions.
Back Read Speaker Santiago, April 7, 2015.- The General Directorate of Innovation and Public Health Management of the Ministry of Health has collected the contributions of its clinicians in the design of the screening program for metabolic diseases, according to which the pathologies are maintained that have been screened until now, including the seven pathologies indicated by the Ministry of Health, Social Services and Equality. Once the instructions of the ministry were analyzed, the joint design of the program was worked on together with the professionals, so that its high quality is maintained, as demonstrated by the designation of the Santiago University Hospital Complex Unit as a reference center in Metabolopathies for all of Spain (CESUR). As announced, Galicia includes in the program the seven pathologies agreed upon for the National Health System and recommended by the Ministry of Health, Social Services and Equality, one of which is new to our portfolio of services - sickle cell disease. In addition, it will keep 22 pathologies additional to these recommendations in the program, making a total of 29 pathologies. Once the program has been agreed upon, the General Directorate of Innovation and Public Health Management will hold, as it has been doing until now, periodic meetings with the participants in the program to monitor the evolution and results. In this way, in the future it will be possible to continue improving the program in aspects such as communication, information to tutors, or the collection of samples. The Ministry of Health thus fulfills the objective of maintaining and improving the screening program for neonatal metabolic diseases to provide the best healthcare to its patients, collaborating with its professionals to achieve this objective.

Figure 4. The outcome of the Change.org petition to the president of the Xunta de Galicia, which aimed to prevent the program's reduction and elimination of urine, is shown.

In 2017, the issue was addressed in a similar way by the promoter of the development of the service charter, with the support of the Spanish Society of Epidemiology [24].

In the USA, where there is no universal public health system, these NBS programs are an anomaly in health. The Scientific and Patient Societies provided responses; this can be found in the cited material [8]●pp179-181 We can even speak of a **PUBLIC, UNIVERSAL SOCIO-HEALTH SYSTEM.**

A work from the UK [25] attempts to understand whether **differences between countries in recommendations on NBS for rare diseases** using newborn blood spot testing could be partly explained by the use of systematic review methods. In the introduction they review the current situation with expansive differences from one country to another; they do not know whether the differences between countries are due to genuine differences in disease prevalence, healthcare systems, and priorities, or differences in the process of reviewing the evidence used to generate policy, in particular the use of systematic reviews.

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They mention overdiagnosis as one of the main harms of NBS because the detection of a disease that would never cause symptoms in a particular person's life {this is not a disease or a false positive; it is a characteristic such as having black hair, and it does not seem easy for such a thing to occur; we can think of pentosuria}. The authors analyzed a country's policy decisions on which diseases to include in the NBS to determine whether systematic reviews were carried out and whether this was associated with the final recommendation to implement screening. The authors rated the accuracy of the test, the benefit of early detection and overdiagnosis, and the relationship with the decision made.

A total of **134 policy documents were identified**; 108 of them were from experts. A total of 41 studies were excluded, and the reasons for exclusion were provided. After the exclusions, **24 Spanish documents** remained, and 23 were from the USA; none of the other countries kept more than 8 documents. Of the only **60 recommendations** that used systematic review methods, 24 were from Spanish documents, and 21 covered test accuracy, the benefits of early detection, and overdiagnosis. They comment that the decision to carry out a systematic review could have been driven by factors internal to the country, and they can only draw tentative conclusions "It is curious that the 24 Spanish documents selected, all of them systematic reviews, top the list by country, surpassing other demographically much larger ones, and it is also curious that 9 are driven by Galicia, where I write, and another 9 by the Canary Islands, which demographically are below others, such as Andalusia or Catalonia; it would be necessary to consider what circumstances exist in them." In the monograph [8]●pp. 206-209, the article and the circumstances in Galicia and the disagreements that arose prior to the preparation of these studies, which did not change anything that already existed, are discussed in detail.

The researchers found that policy reports lacking systematic review methods were more likely to recommend screening, indicating that a rigorous assessment could reveal the absence or unreliability of the available evidence. Several studies have demonstrated discrepancies between expert opinion and research evidence; one study highlighted that professional treatment recommendations presented in review articles or textbooks often contradicted the best evidence from meta-analyses of trials available at the time of publication.

I see in this restrictive character something similar to what happened at the beginning of the NBS, when organized opposition to its implementation appeared; what was said about GALICIA and the CANARY ISLANDS, I relate to the presence in their administration, when the revisions were made, of "Dr. SAMUEL P. BESSMAN" [26]. I know the one from Galicia and I greeted the one from the Canary Islands once.

I conclude that we must avoid the work, expense (misappropriation of public funds), and wasted time on useless reviews, however robust they may be, and instead promote the creation of evidence, provided that the principle of beneficence is respected, it is ethically advisable, and it allows for the participation of those affected, their families, and close associates, who laid the groundwork for the start of NBS and continue to drive the expansion and research of the programs; a situation that is no longer debatable, since they constitute the best scientific evidence, evidence of efficacy, efficiency, effectiveness, evidence of need, and living evidence. This includes those who suffer adverse effects, false positives and negatives, overdiagnosis, or overtreatment.

On 06/10/2023, **Development of newborn screening policies in Spain from 2003– 2022, what do we actually need to reach an agreement? [27]** was published*, which is clear evidence of how systematic review articles searching for evidence, when published, are outdated. The error in writing that the number of NBS laboratories in Spain was 20, when there were 15, is, in my opinion, a palpable demonstration of what was said. Having counted on the participation of those involved and having published on 02/23/2021: [START, EVOLUTION AND CURRENT SITUATION OF NEONATAL SCREENING PROGRAMS IN SPAIN] [28], where it clearly states that there are 15 laboratories (in my case, I found out years before during a visit to the laboratory), it can be deduced that the wording is prior to its publication. The journal in which it is published contains more articles in which that number of laboratories is mentioned and the authors of the mentioned article [28] also write in that journal about NBS, so I do not understand the error.

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In 2003, there were 20 laboratories, but these numbers decreased according to the following report: [**Activity of neonatal screening programs in Spain. Review from its beginning until 2016**. According to the Ministry of Health, 2021] [29], on page 18: "in 2020, the reorganization carried out over the 50 years leaves 15 neonatal screening laboratories in operation in Spain, whose population coverage reaches 100% of newborns in Spain". The bibliography of the article [27]** is outdated in several sections, and the one cited in the next section is not included.

Rarologs

On another note, the work of epidemiologs who abuse scientific evidence but who do not offer the option of obtaining it, since we are evidence producers and, through open screening methods, we can detect unsuspected or unknown pathologies or conditions, must mutate into ‘a rarologs.

I now discuss the work of RAROLOGS, who, without knowledge, are beginning their work, driven by those affected, parents, relatives, etc.; possibly the first were those who suffer from Duchenne muscular dystrophy (**DMD**) to argue the relevance of the NBS, for which they articulate the form of participation of those concerned in decision making [30], to develop the evidence that supports the implementation of NBS for DMD. In this work, they review the organization and the effort put into what was done in the USA, where there is no universal public **SNS** (national health system). Another project [31] developed a new online method to include patients and their caregivers in this process, using the DMD care guidelines as an example. The expected completion date of the study was May 2020. In 2020 [32], the authors concluded that they developed a new online approach that makes it feasible and convenient to engage large numbers of patients and their caregivers in a rigorous and culturally appropriate manner, consistent with how clinicians engage in guideline development in clinical settings. The procedure produces results that, during the process, highlight the ability to facilitate learning and provide students with the opportunity to review their responses, which is important for achieving consensus.

*When I read this article [27] in ResarchGate (RG), I was correcting the galley proofs of the one referred to here with [4]; I cited it in that one with reference 45, indicating an error in the number of existing laboratories. Once published, I included it in RG and sent the authors some initial observations via RG, which I included as DATE. [accessed on 09/10/2025] https://www.researchgate.net/publication/395008864_Acabo_de_poner_en_RG_un_articulo_en_el_qee_os_cito..

Later, I elaborated more extensive comments, insisting on the obsolescence of its publication. Titled TRABAJOS DE RARÓLOGOS and sent by the same means; and in DATE https:///www.researchgate.net/publication/395005676_TRABAJOS_DE_RAROLOGOS [accessed on 09/10/2025].

**If these two documents were excluded for not meeting the epidemiological criteria for inclusion or were not sought by origin, the problem is of "Aurora borealis".

The best practices they identified could help engage patients and their caregivers in the guideline development process in other clinical areas, thus facilitating the work of guideline developers. A year earlier, they published an article [33] in the same vein. In the USA, the number and type of inherited disorders included in newborn screening currently vary from state to state, and as early as 2017 [34], an attempt was made to include all of the Recommended Uniform Screening Panel (**RUSP**).

However, this approach has not been adopted by all states, creating a geographic disparity in opportunities to receive timely intervention for life-threatening inherited conditions. Raralogs are doing significant work in this direction. The processes for reviewing evidence to add new conditions to state NBS panels rely on data from pilot studies that assess the potential benefits and harms of screening. However, consideration of the ethical, legal, and social implications (**ELSI**) of screening within this research has been limited. One article [35] described important ELSI-related issues in relation to newborn screening policies and practices as a resource to help researchers integrate the ELSI into NBS pilot studies. Integrating ELSI questions into pilot studies will help NBS programs better understand the potential impact of screening for a new condition on newborns and families

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and make critical policy decisions aimed at maximizing the benefits and mitigating potential negative medical or social implications of screening. This issue is discussed further in the cited monograph [8].

Current Situation in Galicia

The situation has changed in Galicia since "Dr BESSMAN" stopped leading the SPPC, and currently, the president of the *Xunta de Galicia* boasts are the ones who include the most pathologies in the NBS. In the twelve years that I have retired, the pathologies or conditions detected have been increasing, reaching what is being done now, which can be consulted in Galician at the link:

https://www.sergas.gal/Saude-publica/documents/7090/Listado_enfermedades_gallego.pdf

The methodology, procedures, and samples analyzed determine possible detectable congenital pathologies. In Galicia, urine and blood are analyzed simultaneously, and more than 60 diseases can be detected in samples taken between 24 and 48 hours after birth. Analytical procedures can be used to detect unforeseen or even unknown pathologies.

On 12/24/2023, the local newspaper *"El Correo Gallego"* reported that the Metabolopathies Laboratory is now able to detect 60 diseases with the NBS in babies from all over Galicia. Dr. JA Cocho, my successor at the head of the laboratory, stated in the interview that if any of the 34 included pathologies are detected, we can add three more pathologies, allowing up to 60 to appear on our list of diagnoses. This is practically double because "in the case of a child with a metabolic problem related to an amino acid, the cause of that problem can be more than one, and we very quickly look for the pathology that needs to be treated immediately, but if there is another, we also detect it". At the NBS, blood and urine samples on paper are analyzed in a very well-linked way so that, in two or three days, if a special test needs to be done, the diagnosis can be made, and the child can be treated. She says that when the baby is 24 hours old, the heel is pricked, and samples are soaked in absorbent blotting paper, which is sent by suitcase so that every day before 8 in the morning, they have a suitcase from each hospital with samples from those who were pricked the day before and were born a couple of days ago; the work lasts between one and three days, and normally between four and six days after the child is born, they have the diagnostic hypothesis.

https://www.elcorreogallego.es/santiago/2023/12/24/chus-logra-detectar-60-enfermedades-96207265.html [both accessed on 09/10/2025]

Current Situation in Spain

In Spain, there is a significant disparity in access to universal newborn screening (NBS) programs for newborns who are users of the public National Health System (SNS), depending on the nationality or region of residence.

When the SNS is universal, it avoids having to create the necessary [36] financial infrastructure in the USA to study the incorporation of new pathologies, and when unexpected findings or possible undescribed pathologies occur, the SNS is competent to study the case, and healthcare ethics committees channel it to achieve the maximum benefit without causing any harm; thus, express consent is not necessary for each pathology identified; there is already generic consent when consenting to the collection of the sample, which is reported at that time and described in the NBS program documentation.

The SNS benefits from detection by eliminating diagnostic difficulties and is also family spared from suffering and associated expenses. This allows the study of the natural history of disease and socio-health and psychological interventions, which will facilitate the performance of these public structures and the family.

We have experience with a patient with MSUD, using the diagnosis to modify the death certificate and offer genetic counseling.

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The family called us, truly grateful for having that information. The family can consider reproductive options they might not have otherwise. Where there are no SNSs universal, this possibility is not considered.

Those who complain about the lack of equity in NBSs in Spain have an effortless solution: by emulating those they consider to be in a better situation. If this does not improve the situation according to the others, they will have to prove the former wrong.

The differences in NBSs among populations, regions, nationalities, nations, or countries enhance our ability to evaluate various policies against one another in the spirit of healthy competition and progress. These differences serve as an incentive to enhance our understanding of the advantages and disadvantages of each population, region, nationality, nation, or country; in all cases, the ethical principles of beneficence and non-maleficence must be respected.

It is my duty to make known the letter from Prof. F. Mayor* to Mr. Volker Turk, High Commissioner for Human Rights of the United Nations Office, dated February 1, 2023. Finally, Section 2 refers to our matter. We have recently heard from you that you are working to include the NBS in the Universal Declaration of Human Rights. [accessed on 09/10/2025] https://www.docdroid.net/dJPCwVj/carta-mayor-zaragoza-0038-volker-turk-high-commisioner-for

Pursuing Global Equity in This, as in so Many Other Aspects, is Difficult to Achieve

Her Majesty Queen Letizia is interested in those suffering from rare diseases and supports their demands for the expansion of newborn screening

The text preceding the title of the monograph [8] is from the first draft, and the editor of an article [37] moved it to the abstract; inserting it translated (Figure 5).

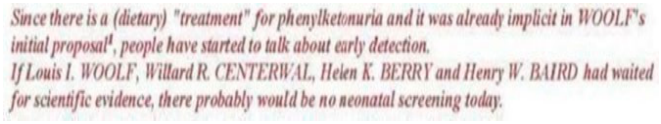
Since there is a (dietary) "treatment" for phenylketonuria and it was already implicit in WOOLF's initial proposal[1], people have started to talk about early detection.
If Louis I. WOOLF, Willard R. CENTERWAL, Helen K. BERRY and Henry W. BAIRD had waited for scientific evidence, there probably would be no neonatal screening today.

Figure 5. The text precedesthe monograph title.

The pursuit of equity in NBS programs cannot in any way restrict a program in which no harm is caused or for which the detection of a pathology or condition or group of them is questioned, as doing so is beneficial to the country.

***Professor Federico Mayor Zaragoza, who passed away on December 20, 2024, leaves us a final article [38]**, which, in the last paragraphs, refers to the fact that on October 30, 2024, he contacted Michelle Bachellet, former United Nations Commissioner for Human Rights and closely related to Volker Turk, requesting her help in processing this new human right.

I disagree with your approach to centralizing the decision to introduce a pathology in the Neonatal Screening Program to achieve uniformity. Programs; differences stimulate progress on the matter, as I have written

Differences in health outcomes cannot be expected between populations who benefit from an NBS program for a few disorders and those whose programs cover many conditions or pathologies. Since these are minority diseases and sufferers belong to the social minority, they go unnoticed. Professor Mayor Zaragoza states that a single affected child in every 10,000 has a 100% impact on both the family and the affected child.

Therefore, precisely because these are irreversible situations, there is no room for percentages. We must ensure that birth is considered, from both a medical and social care perspective, as an event at least as significant as death. We must continue to address the needs that cause suffering, regardless of their nature [8].

This subject is constantly evolving and rapidly evolving due to advances in analytical methodologies, such as genetics, chemistry and biochemistry, as well as in treatments such as personalized precision medicine (CRISPR/Cas9), enzyme replacement, and substrate restriction. In

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this case, it is not possible to obtain scientific evidence quickly, as was the case at the beginning of the COVID-19 pandemic when the evidence changed every hour; on the other hand, additional time is needed to allow the screening of large populations. Therefore, it will be necessary to work to create a tool for screening, for which it is essential to implement said screening (this demonstrates the great difference between an epidemic or pandemic and rare diseases; these are antithetical situations).

However, the emergence of new biomarkers, treatments and research introduces new evidence that is increasingly dynamic and can change over several days, weeks or months. This phenomenon causes the evidence to reverse and change rapidly.

The mentality, ideas and training of scientists, health professionals, administrators and political leaders (family members or close friends affected?) competent in the matter, together with social acceptance, as expressed by doctors in consultations and in the media, play a fundamental role when introducing a detection or a policy related to these programs (their treatment in the budget).

Epilogue

The ability of a newborn screening laboratory to detect pathologies depends on the samples received in the solid phase on adsorbent paper (desk blotting paper from the first experiences with this type of sample), urine, blood (serum, plasma, saliva, sweat, tears), and the methodology used to perform the analyses based on these samples.

If the methodology allows for the discovery and evaluation of multiple analytes -biomarkers for various diseases- with a single procedure in a single sample aliquot, chromatography, electrophoresis, or tandem mass spectrometry (MS/MS), etc., they are now called multiplex methods. These methods can detect multiple diseases and identify biomarkers that could indicate an unexpected or unknown disease or condition, not necessarily classified as such (such as pentosuria). If the methodology involves the use of sequential maneuvers, one or more analytes of a type or group of molecules that are biomarkers of possible pathologies in abnormal proportions are identified in successive steps, continuing with the same aliquot (acting on what was obtained in the previous step, or another aliquot) or another type of sample (blood, urine, etc.), these molecules or others are identified and assessed, depending on the sample.

This process is now called second-level testing, but successive levels can be concatenated, similar to what analytical chemists call analytical marching. This methodology is amenable to the detection of multiple pathologies, whether anticipated, unexpected or even unknown.

The markers and reagents that affect the robustness of the procedures are discussed. The importance of having a urine sample available was emphasized.

It is recommended to review the work of Efron et al. [39] to see how NBS was born with an open methodology; this can be improved with few changes and reduced costs.

After a diagnosis is reached, regardless of how disastrous the prognosis is always beneficial. Occasionally, the intervention of a psychologist will be necessary to determine how, when, and where to communicate. In some cases, the presymptomatic stage can be extended, and palliative and social care can be provided. The family benefits from being able to plan new pregnancies using assisted reproduction to prevent recurrence of the disease. It is not a question of looking for pathologies without treatmentbut rather those thatare found with open procedures.

Having a diagnosis is essential for receiving treatment; today, we are moving closer to genetic correction.

In one instance, we used laboratory results to alter the patient's death certificate. The family called to thank us for the information. They noticed a noticeable change in the doctors' attitude, from evasive and uncommunicative to providing all the necessary explanations and guidelines. This means that the doctors were also relieved. There is nothing left unsaid regarding the diagnostic odyssey, which disappears.

I know that my approaches may seem unorthodox, but I believe they can be valid, effective, and efficient

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This is a partial view from the **biochemical laboratory**, since only the aspects related to the **biochemical phenotype** are considered; I think that starting by doing these analyses is a good way to do it. The study did not address relevant laboratories of genetics, microbiology, immunology, etc. In addition, we must consider other aspects of the program, such as diagnostic confirmation, treatment, and follow-up.

Today, the author's techniques, methods, and procedures are no longer used in the Galician laboratory.

They continue to develop and use his procedures for urine analysis; the rest are purchased.

The test for reducing substances with the MANDELIN reagent could be performed by kinetic reading, quantification and normalization with creatinine *[4,40–42]****, which allows the detection of reducing agents in urine at 24 h of life.***

A non-reducing biomarker can also be sought in urine.

The BOLETÍN OFICIAL DE LAS CORTES GENERALES●CONGRESO DE LOS DIPUTADOS, [The OFFICIAL BULLETIN OF THE GENERAL PARLIAMENT●CONGRESS OF DEPUTIES]

reported that at its meeting on June 13, 2025, the Bureau of the Chamber agreed to admit for processing the document exercising the initial processing of the INICIATIVA LEGISLATIVA POPULAR (ILP), **[**POPULAR LEGISLATIVE INITIATIVE (PLI)**]**, and the Bill for the **Regulation of Universal and Equitable Neonatal Screening in Spain.**

The text of the proposal is two pages long, has eight articles and two final provisions, and does not contribute anything new. It proposes a mandatory minimum panel of diseases subject to neonatal screening throughout Spain, established by a Commission, reviewable at least every two years, using traditional criteria: scientific evidence, treatment, technical feasibility of screening, and cost-effectiveness. This approach does not address issues such as the methodology used in the laboratory, which constitutes the bulk of this article and would be difficult to introduce. Perhaps an addition could be made to "technical feasibility of screening, ***including no target analysis using non-specific reagents and biomarkers, analytical procedures open to detection of unforeseen or unknown pathologies or conditions***. The text still has a long way to go before it reaches its final draft.

Ethical Aspects

Detecting an unforeseen or unknown pathology or potential pathology as a result of using an open methodology can raise ethical issues. In some situations, communicating the findings to the pediatrician and family will present difficulties. In the first case, it does not seem to be overly problematic. Second, the intervention of a psychologist is required to try to maximize the benefit of detection and minimize harm, preparing the family for what lies ahead, especially when deterioration cannot be avoided. However, this is the first step toward obtaining some benefit, such as prolonging the presymptomatic stage and studying possible treatments. In any case, communicating to parents about the impact of the findings, which may be unknown or known to be heterogeneous, and the possible decisions to be made are complex.

This consequence, in some cases, may present the ethical dilemma of whether to inform the family of their genetic characteristics when no possible intervention that would improve the newborn's prognosis is in sight. By informing the family, they can consider reproductive options and consider having more children, which they might not have done without this information. They can also encourage the screening of relatives.

This is a clinical-care approach, and a Clinical-Care Ethics Committee will resolve each case (which will be rare), considering the right of those affected to remain unaware. Information can also be found pending advances in treatment or the emergence of a plausible treatment approach.

Close monitoring of these children will allow for the study of the natural history of the disease and attention to any emerging social and health needs.

Acknowledgments: To Magdalena Ugarte. I could not imagine that those who knew LI Woolf in Oxford were unaware of her time at GOSH in London, where the work that brought us here was conceived, as was expressed

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to me by M Ugarte, who was totally unaware of this circumstance. I became aware of the fact when (after the special issue was published, on the occasion of the 100th anniversary of LI WOOLF https://www.mdpi.com/journal/IJNS/special_issues/neonatal_screening which I had the honor of promoting and initiating, we exchanged e-mails to try to get her to participate in that issue to which she had not sent any work, an issue of which she had informed to Prof. Mayor), she told me in an e-mail that she had been with Charles Enrique Dent Colsa (I put the names in Spanish, because he was born in Burgos to a Spanish mother and that is his full name) in the Hospital where Sir Archibald Garrod was from 1892 to 1913, when he describes some "Innate Errors of Metabolism" and introduces this concept; I told her that it was where LI Woolf had done the initial work that brought us here and she was shocked, she had not heard anything from This means that Woolf did not comment on what she did in London before going to Oxford, which gives us an idea of her modesty. Charles Robert Scriver, a physician and researcher, acquired the chromatographic technique from Dent in London. He introduced new laboratory techniques in the USA. Later, along with others, he implemented a pioneering newborn screening program for congenital disorders in Quebec in 1969. *It was then that she sent me, among other documents, Woolf 's letter and the Health Department letter.* To the *"Asociación Enfermedades Raras Más Visibles"* ["More Visible Rare Disease Association"] and his spokesperson Pedro Lendinez Ortega, for allowing us to include its link that allows access to the documentary *"La vida en una gota"* ["Life in a drop"]. Supporting the research in Open Programs of NBS, advocated in this writing. www.masvisibles.com. To the editor of this magazine for shaping the presentation.

References

1. Green A. Sheila. In *BREWIN BOOKS*; Available online: https://www.espku.org/2020/10/12/sheila-unlocking-the-treatment-for-pku/ ISBN 978-1-85858-714-1.
2. Green A. The first treatment for PKU: The Pioneers-Birmingham 1951. *Int J Neonatal Screen* **2021**, *7*, 19. [Google Scholar] [CrossRef]
3. Alonso-Fernández JR. Dr. Louis Isaac Woolf: At the Forefront of Newborn Screeningand the Diet to Treat Phenylketonuria- Biography to Mark His 100th Birthday. *Int J Neonatal Screen* **2020**, *6*, 61. [Google Scholar] [CrossRef]
4. Alonso-Fernández JR. Four Decades of Newborn Screening: A Historical Perspective of Laboratory Practices. *OBM Genetics* **2023**, *7*. [Google Scholar] [CrossRef]
5. Moncrieff A, Wilkinson RH. Further experiences in the treatment of phenylketonuria. *Br Med J* **1961**, *1*, 763–767. [Google Scholar] [CrossRef] [PubMed]
6. Woolf, LI, Griffths R, Moncrieff A. Treatment of Phenylketonuria with a diet low in phenylalanine. *Br Med J.* **1955**, *8*, 57–64. [Google Scholar] [CrossRef]
7. Woolf LI. The heterozygote advantage of phenylketonuria. *Am J Hum Genet.* **1986**, *38*, 773–775. Available online: https://pmc.ncbi.nlm.nih.gov/articles/PMC1684820/pdf/ajhg00154-0177.pdf
8. Alonso-Fernández JR, Colón Mejeras C. Aportaciones de Louis Isaac Woolf al Tratamiento y Diagnóstico Precoz de la Fenilcetonuria y otros Errores Congénitos del Metabolismo. Los comienzos de la Tría Neonatal en España, con referencia al Programa de Galicia. CENTENARIO DE LOUIS ISAAC WOOLF. 2019. DOI: 10.13140/RG.2.2.17513.15201 https://doi.org/10.13140/RG.2.2.17513.15201 file:///I:/LIBRO_impresion2026-XVAportacionesLIWoolfTratamientoDiagnosticoPrecozdeFenilcetonuria%20(1).pdf DOI: 10.13140/RG.2.2.29898.72641/1 https://doi.org/10.13140/RG.2.2.29898.72641/1. file:///I:/2026-XVAportacionesLIWoolfTratamientoDiagnosticoPrecozdeFenilcetonuria%20(2).pdf file:///I:/2026-
9. Alonso-Fernández JR, Bóveda MD, Peña J, Fraga JM. Screening of organic acid disorders. The use of acridine dye as a neonatal screening method. A comparative study with fast garnet dye. International Meeting on Neonatal Screening. August1982. Tokio. Available online: https://www.researchgate.net/publication/397706092_Screening_of_organic_acid_disorders_The_use_of_A CRIDINE_dye_as_a_neonatal_screening_method_A_comparative_study_with_FAST_GARNET_dye.
10. Maya, A. Aportaciones al Diagnóstico Precoz de Alteraciones Metabólicas Congénitas en el Recién Nacido. Tesis Doctoral. Facultad de Farmacia. Universidad de Barcelona. 1974.

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Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 February 2026 doi:10.20944/preprints202511.1966.v4

https://www.researchgate.net/publication/379732142_LIBRO_Aportaciones_LI_Woolf_Tratamiento_Diagnostico_P recoz_de_Fenilcetonuria_2

11. Alonso-Fernández JR. Neonatal Screening for Metabolopthies in Less than an Hour by Pressure Paper Chromatography. November1991, 8th International Neonatal Screening Symposium and Inaugural Meeting of the International Society for Neonatal Screening. Leura Blue Mountains, N.S.W. Australia. Available online: https://www.researchgate.net/publication/318280742_Neonatal_Screening_for_Metabolopthies_in_Less_than_An_Hour_by_Pressure_Paper_Chromatogrphy.
12. Alonso-Fernández JR, Fidalgo López J. Review and Proposal of Alternative Technologies for Comprehensive and Reliable Newborn Screening Using Paper Borne Urine Samples for Lysosomal Storage Disorders: Glycosphingolipid Disorders. JIMS 2021, 9, 20200011. [Google Scholar] [CrossRef]
13. Alonso-Fernández JR. Pregnanetriolone in paper-borne urine for neonatal screening for 21-hydroxylase deficiency: The place of urine in neonatal screening. *Mol Genet Metab Rep*. **2016**, *18*, 99–102. [~~Google Scholar~~] [CrossRef]
14. Jiao X, Gong P, Niu Y, Xu Z, Zhou Z, Qin J. et al. Evaluating the suitability of 6-oxo-PIP as a novel biomarker for pyridoxine dependent epilepsy in multiple samples. *Research Square Platform LLC* **2024**. Available online: https://www.researchsquare.com/article/rs-3843855/v1. [CrossRef]
15. Merino-Hernández A, Sánchez-Barrio Pedro L, Villar-Castro S, Aguado-Del Hoyo A, Marsinyach-Ros I, Sánchez-Luna M. Cost effectiveness of a cytomegalovirus screening strategy in neonates born after 34 weeks small for gestational age. *An Pediatr (Engl Ed)* **2023**, *98*, 41–47. [Google Scholar] [CrossRef] [PubMed]
16. Rebollido M, Cocho JA, Castiñeiras DE, Boveda MD, Fraga JM. Aplicación de la Espectrometría de Masas en Tándem al análisis de aminoácidos, acilcarnitinas, acilglicinas y ácidos orgánicos en muestras de orina en papel. *Química Clínica* **2006**, *25*, 64–74. Available online: https://www.seqc.es/download/revista/102/345/627712070/1024/cms/QC_2006_64-74.pdf/.
17. Newborn Screening for Congenital Cytomegalovirus. *Canadian J Health Technologies* **2024**, *4*. Available online: https://www.cda-amc.ca/sites/default/files/pdf/htis/2024/RC1533_Final_Review.pdf.
18. Sheila CD, Mark RS, Scott DG. Public health and laboratory considerations regarding newborn screening for congenital cytomegalovirus. *JIMD* **2010**, *33*, 249–254. [Google Scholar] [CrossRef]
19. Alonso-Fernandez JR, Carpinteiro MI, Baleato J, Fidalgo J. Vertical Sandwich-Type Continuous/Evaporative TLC with Fixed Mobile Phase Volume for Separating Sugars of Clinical Relevance in Paper-Borne Urine and Blood Samples in Newborn Screening. *J Clin Lab Anal.* 2010, 24, 106–112. [Google Scholar] [CrossRef]
20. Alonso-Fernandez JR, Carpinteiro MI, Baleato J, Fidalgo J. Vertical Sandwich-Type Continuous/Evaporative TLC with Fixed Mobile Phase Volume for Separating Sugars of Clinical Relevance in Paper-Borne Urine and Blood Samples in Newborn Screening. *J Clin Lab Anal.* **2010**, *24*, 106–112. [Google Scholar] [CrossRef]
21. Alonso-Fernandez JR, Patel VB. Dietary sugars: TLC screening of sugars in urine and blood samples. In *book: Food and Nutritional Components in Focus. Dietary Sugars. Chemistry, Analysis, Function and Effects Edition: 1. Chapter: 12*; Royal Society of Chemistry Publishing, 2012. [Google Scholar] [CrossRef]
22. Alonso-Fernández JR. DETECCIÓN, ESTIMACIÓN Y CUANTIFICACIÓN DE AZÚCARES EN FLUIDOS BIOLOGICOS, SU SEPARACIÓN POR CROMATOGRAFÍA EN PAPEL (PC) Y CAPA FINA (TLC). REVISIÓN HISTORICA Y PROPUESTAS DENUEVOSPROCEDIMIENTOS. Aplicación en Tría Neonatal de Errores Innatos del Metabolismo de los Carbohidratos. https://doi.org/10.13140/RG.2.2.12678.65605. file:///I:/H-DETECCINESTIMACIONYCUANTIFICACIONDEAZUCARES%20(2).pdf [CrossRef]
23. Takaki Y, Mizuochi T, Takei H, Eda K, Konishi KI, Ishihara J, Kinoshita M, Hashizume N, Yamashita Y, Nittono H, Kimura A. Urinary and serum oxysterols in children: developmental pattern and potential biomarker for pediatric liver disease. *Sci Rep.* **2020**, *10*(1), 6752. [Google Scholar] [CrossRef] [PubMed] [PubMed Central].
24. Grupo de Trabajo de Cribado de la Sociedad Española de Epidemiología. Personas de contacto: *Raquel Zubizarreta*. CRIBADO NEONATAL DESDE LA PERSPECTIVA DE SALUD PÚBLICA. Situación y recomendaciones. Available online:

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Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 February 2026 doi:10.20944/preprints202511.1966.v4

https://www.seepidemiologia.es/documents/dummy/SEE%20Recomendaciones%20programas%20de%20c ribado%20pat.pdf.

25. Taylor-Phillips S, Stinton C, Ferrante di Ruffano L, Seedat F. Clarke A, Deeks JJ. Association between use of systematic reviews and national policy recommendations on screening newborn babies for rare diseases: Systematic review and metaanalysis. *BMJ* **2018**, *361*, k1612. [Google Scholar] [CrossRef] [PubMed]
26. Levy H., Robert Guthrie and the Trials and Tribulations of Newborn Screening. *Int J Neonatal Screen* **2021**, *7*, 5. [Google Scholar] [CrossRef]
27. Valcárcel-Nazco C, García-Pérez L, Linertová R, Guirado-Fuentes C, Hernández-Yumar A, Paz-Valiñas L, et al. Development of newborn screening policies in Spain 2003-2022: what do we actually need to reach an agreement? *Rare Dis Orphan Drugs J* **2023**, *2*, 19. [Google Scholar] [CrossRef]
28. Marín Soria JL, González de Aledo Castillo JM, Argudo Ramírez A, López Galera RM, Pajares García S, Ribes Rubió A, García Villoria J, et al. INICIO, EVOLUCIÓN Y SITUACIÓN ACTUAL DE LOS PROGRAMAS DE CRIBADO NEONATAL EN ESPAÑA. Rev Esp Salud Pública 2021, 95, 23, Available online: https://www.sanidad.gob.es/biblioPublic/publicaciones/recursos_propios/resp/revista_cdrom/VOL95/C_ESPECIALES/RS95C_202102041.pdf
29. Dulín Iñiguez E, Eguileor Gurtubai I, Espada Sáez-Torre M, Pàmpols Ros T, Zubizarreta Alberdi R. Actividad de los programas de cribado neonatal en España. Revisión desde sus inicios hasta 2016. Ministerio de Sanidad, 2021. Edita: © MINISTERIO DE SANIDAD CENTRO DE PUBLICACIONES PASEO DEL PRADO, 18-20. 28014. https://www.sanidad.gob.es/bibliotecaPub/repositorio/libros/28943_actividad_de_los_programas_de_cribado_ne onatal_en_Espana_-_revision_desde_sus_inicios_hasta_2016.pdf Available online: https://cpage.mpr.gob.es/
30. Al-Zaidy S.A, Lloyd-Puryear M, Kennedy A, Lopez V, Mendell JR. A Roadmap to Newborn Screening for Duchenne Muscular Dystrophy. *Int J Neonatal Screen* 2017, 3, 8. [Google Scholar] [CrossRef] [PubMed]
31. Khodyakov D, Kinnett K, Grant Lucas A, Martin A, Denger B, et al. Engaging Patients and Caregivers Managing Rare Diseases to Improve the Methods of Clinical Guideline Development: A Research Protocol. *JMIR Res Protoc*. 2017, 6, e57. [Google Scholar] [CrossRef]
32. Khodyakov D, Kinnett K, Denger B, Grant S, Armstrong C, Martin A, et al. Developing a Process for Getting Patient and Caregiver Input on Clinical Practice Guidelines [Internet]; Patient-Centered Outcomes Research Institute (PCORI): Washington (DC), 2020. [Google Scholar] [CrossRef]
33. Khodyakov D, Grant S, Denger B, Kinnett K, Martin A, Booth M, et al. Using an Online, Modified Delphi Approach to Engage Patients and Caregivers in Determining the Patient-Centeredness of Duchenne Muscular Dystrophy Care Considerations. *Med Decis Making* 2019, 39, 1019–1031. [Google Scholar] [CrossRef]
34. Starkweather A, Coleman B, Barcelona de Mendoza V, Fu, M, Taylor J, Henderson W, et al. Policy brief: Improve coverage of newborn genetic screening to include the Recommended Uniform Screening Panel and newborn screening registry. Nurs Outlook 2017, 65, 480–484. [Google Scholar] [CrossRef]
35. Goldenberg AJ, Lloyd-Puryear M, Brosco JP, Therrell B, Bush L, Berry S. Including ELSI research questions in newborn screening pilot studies. *Genet Med.* 2019, 21, P525–533. [Google Scholar] [CrossRef]
36. Kelly NR, Orsini JJ, Goldenberg AJ, Mulrooney NS, Boychuk NA, Clarke MJ, et al. ScreenPlus: A comprehensive, multidisorder newborn screening program. *Mol Gen Metab Reports* 2024, 38, 101037. [Google Scholar] [CrossRef]
37. Alonso-Fernández JR, Colón C. The contributions of Louis I Woolf to the treatment, early diagnosis and understanding of phenylketonuria. *J Med Screen* 2009, 16, 205–211. (accessed on 29 May 2024). Available online: https://www.researchgate.net/publication/40870034_The_contributions_of_Louis_I_Woolf_to_the_treatment_early_diagnosis_and_understanding_of_phenylketonuria https://doi.org/10.1258/jms.2009.009062 https://journals.sagepub.com/doi/10.1258/jms.2009.009062 [CrossRef]

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Preprints.org (www.preprints.org) | NOT PEER-REVIEWED | Posted: 27 February 2026 doi:10.20944/preprints202511.1966.v4

38. Mayor Zaragoza F. Prevención en el neonato de enfermedades que cursan con daño cerebral irreversible, derecho humano. Prevention of irreversible brain damage in newborns, a human right. *An RANM* 2024, 141, 80–85. https://analesranm.es/revista/2024/141_01/14101_rev09 [Google Scholar] [CrossRef]
39. **Efron, M,** Young D, Moser HW, Maccready RA. A SIMPLE CHROMATOGRAPHIC SCREENING TEST FOR THE DETECTION OF DISORDERS OF AMINO ACID METABOLISM. A Technic Using Whole Blood or Urine Collected on Filter Paper. *N Engl J Med.* 1964, 270(26), 1378–1383. https://doi.org/10.1056/NEJM196406252702602
40. Alonso-Fernández, JR. Tría neonatal de galactosemia: situación del ensayo en orina: Neonatal galactosaemia screening. Urine assay situation. *Rev Lab Clín* **2008**, *1*, 133–134. https://doi.org/10.1016/j.labcli.2008.08.001
41. Alonso-Fernández JR, Castiñeiras Ramos DE, Parrado C, Fraga J.M, Peña J. Galactose Newborn Screening: Test for Reducing Sugars in Urine samples Impregnated on Paper. Nov 1986. 6th Intern NBS Symp. 5° Nat NBS Symp. Austin, Texas. Available online: https://www.researchgate.net/publication/332780669_Galactose_Newborn_Screening_Test_for_Reducing_S ugars_in_Urine_samples_Impregnated_on_Paper.
42. Alonso-Fernández JR, Iglesias AJ, Bóveda M.D, Colón C. The vanadium test for reducing sugars in paper-borne urine samples: Conversion from qualitative test to quantitative assay, and application to the detection of galactosemias and other disorders of sugar metabolism. December 2009, 7th International and Latin American Congress Inborn Errors of Metabolism and Neonatal Screening, Cancun, Mexico. Available online: https://www.researchgate.net/publication/326319448_The_vanadium_test_for_reducing_sugars_in_paper_borne_urine_samples_Conversion_from_qualitative_test_to_quantitative_assay_and_application_to_the_detection_of_galactosemias_and_other_disorders_of_sugar

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