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Concept Paper

Four Decades of Newborn Screening: A Historical Perspective of Laboratory Practices

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Abstract

The personal experience of 36 years in charge of the Newborn Screening Laboratory in Galicia and the consequences of the recent advancement of sampling from 3 days to 24 hours of the newborn's life are reviewed. The implication in the results of the assay of reducers in urine with the Mandelin reagent and its alternatives is commented. The implication in the effects of TSH in blood and the possible ways of dealing with the situation. Since 1978, the Newborn Screening Program in Galicia has maintained the urine sample on paper (Berry-Woolf specimen) and blood on paper (Guthrie specimen), so the possibilities of the urine sample are discussed, which are not being taken advantage of. The ignorance of LI Woolf's time at GOSH, where what brought us here was gestated, and his recommendations for preparing the diet, to which due attention was not paid. The contributions of the in vitro diagnostic industry, the concepts associated with Clinical Chemistry, and its professionals' work are discussed.

Keyword
Newborn screening; urine sample on paper; LI Woolf; in vitro diagnostic industry

1. Introduction

The editor-in-chief, on behalf of the guest editors, wrote me emails in February (Tuesday 21/02/2023 at 6:48 and before) requesting the sending of a paper based on my experience, which I had done previously. I must say that I have been retired for 10 years. I am still interested in research, but my activity is reduced to reviewing and commenting on articles and proposals for analytical procedures focused on neonatal screening.

2. Assay of Reducers

The inspiration came to me thinking about the visit I made to the Laboratory of Metabolopathies in mid-February, in which he informed me that they had advanced the sampling from 3 days to 24 hours of life of the newborn, with therefore, the assay of reducers in dried urine on adsorbent paper, with Mandelin's reagent, which we introduced in 1978 [1-7], for the detection of galactosemia and other inborn errors of carbohydrate metabolism, was unfeasible for them due to the high number of positive results, which required them to perform numerous cTLC of sugars with our procedure. They arranged to determine total galactose in blotter-dried blood with a procedure approach marketed by a company we took to the NBS field in 1985. The procedure is limited to the four types of galactosemia, so the possibility of detecting other disorders of carbohydrate metabolism, diabetes, and non-diabetic glycosuria will be lost. Having to change the procedure means considerably increasing the test cost.

The information provided by the manufacturing and marketing company says that *galactosemia is a hereditary disorder caused by the deficiency of one of the three enzymes responsible for the metabolism of α-D-galactose*, but four are involved. Type IV galactosemia is currently –galactose mutarotase (aldose-1-epimerase, GALM) deficiency–. It can detect it, as β-D-galactose is elevated with which GALK1 does not work, lacking GALM that catalyzes the move to α-D-galactose, with which GALK1 works. The first to act is GALM. The trade information continues, saying that *the most common form of the disease is galactose-1-phosphate uridyltransferase (GALT) deficiency, which occurs in approximately 1 in 30,000–60,000 live births and is often called classic galactosemia.* I add that it is Type I and that galactokinase (GALK1) deficiency originates from Type II. Type III is a consequence of epimerase deficiency (uridine diphosphate-galactose-4-epimerase).

3. Urine Sample Advantage

In our experience, using the urine sample may have an advantage over the blood sample if the newborn undergoes an exchange transfusion, which is very frequent in neonatal intensive care units in critical situations; the toxins and the accumulation of abnormal substrates and metabolites are quickly eliminated, as well as the defective enzymes that the correct ones replace; which prevents the diagnosis in the blood sample; we live this situation, and the fact that he

continued to excrete galactose in high amounts allowed us, with our usual procedure, to diagnose galactosemia, despite being receiving parenteral nutrition with glucose -our systems allow seeing galactose separated from glucose [5, 8, 9].

A similar situation occurred in an MSUD, which we diagnosed in urine by amino acid paper chromatography (which was part of the routine of our NBS Program, using urine and blood collected and analyzed simultaneously). BCAAs were still being excreted in high concentrations after exchange transfusion.

4. Galactosemia

To make the differential diagnosis of Types II and IV galactosemia, it will be helpful to evaluate α- and β-D-galactose separately and calculate the relationship between them. It can be done in urine and/or blood; when determining them in urine, the ratio will be the same no matter how concentrated that urine is (do not have to normalize to creatinine). Paper-dry urine does not allow epimerization to occur, and elution must be done in an eluent that prevents or slows it down; The galactose in these types will always be very high since all the steps that must be followed will be stopped. It is necessary to do so when typing with cTLC (see subsequent paragraph), it can be deduced that it is type II, but it could be type IV, that cTLC does not discriminate, in that cTLC only elevated galactose appears. With the MS/MS procedure that is being used with blood [10], they are not detected. Still, type III is in the blood sample, we verified in two cases that are not detectable with the assay of reducers in urine, as indicated, until one month of life (with milk feeding), when enough reducers accumulate. It can be used to determine α and β D-galactose, chromatography (separating other enantiomers with eluents that slow down epimerization, which has already been described), by column or TLC; enantiomeric chromatography with electrochemical detectors, etc. (this will not be necessary if GALK1 and GALM enzyme activities have been determined)

To make a differential diagnosis of Types I, II (or IV), and III (typing) galactosemias, the device described in the 2013 article [11] in which I participated could be used, if uridyl diphosphate galactose (UDP-Gal) in Type III, it is high enough in 1-day-old urine to detect it with such a device -- there are no cut-off data and we must normalize them concerning creatinine, something that was left pending in that article--; I did not have the opportunity to use it, due to my forced retirement for reasons of economic policy (budget cuts).

Typing using cTLC, separating: /Rt × 100/ Gal-1-P /5/, lactose /28/, galactose /59/, glucose /69/, UDP-Gal /87/, a 9 cm tank is used internal height, 2.5 or 2 mL of eluent and about 90 min of development, is done exclusively on the blood sample [5, 8, 9]. The background of this procedure can be consulted in communications [12, 13].

5. Urine Sample Use

The urine sample can be used to determine **pregnanetrialone** with a fluorometric method and to **detect Congenital Adrenal Hyperplasia (CAH)**, for which I proposed [14], which seems to have advantages over what is currently being done. Collecting the urine sample at 24 hours of life will allow it to arrive better in time to avoid harm to the newborn.

Urine also facilitates the work to **detect lysosomal disorders** since the biochemical markers are excreted in higher concentrations than what is in the blood, separated into three groups:

mucopolysaccharidosis (MPS), oligosaccharidosis, and glycosphingolipidoses [15-18]; the positive ones are subjected to cTLC or another method, to get closer to identifying markers or marker of the specific pathology, gradually; it is what analytical chemists call analytical gait, these works are commented [19] and are the origin of 'Project Find' for the early and accurate diagnosis of MPS [20], (biochemical markers in dried urine are more stable than enzymes in dried blood. This is a significant advantage).

6. Open Program

This procedure uses non-specific reagents for each group. I call it an open program, like opening the window and seeing who passes. If it is usual, the test result will be negative, the positive effect forces us to identify the stranger who does not present to us (it would be an unexpected finding; this is how we detected alkaptonuria, when a singular stain of homogentisic acid appeared in urine amino acid paper chromatography, sequentially stained with Pauli's reagent -diazotized sulfanilic acid-, not previously described) or if it is someone known who should not be present (these are the pathology markers that it was expected to be able to detect), this or these characters will define the current pathology or condition.

The procedures we developed and applied using the Berry-Woolf specimen [18] do not require special knowledge or expensive instrumentation. The results are easy to interpret and intuitive (consult monographs at the end)**. The same can be said of those proposed that use easy-to-use devices, reagents, and instrumentation and can be run manually or with automation.**

7. The in vitro Diagnostic Industry and CH

The **GSP®** instrument (PerkinElmer Life Sciences-*Wallac* Oy, Turku, Finland) used by the company that manufactures and markets the aforementioned analytical procedures allows fluorescence and absorbance measurements to be made adequately to adapt our proposals using urine on paper.

Taking the samples at 24 hours of life leads me to think about the high levels of thyrotropin (TSH) that physiologically present themselves, a consequence of the stress of birth, and how we introduced ***Wallac*** through **IZASA** (companies dedicated to *in vitro diagnostic* materials) in the NBS.

The Laboratory that initially dealt with the Detection of Congenital Hypothyroidism (CH) in Galicia was the Physiology Laboratory of the Faculty of Medicine (USC), to which we sent one of the two cards to collect the drops of dried blood on adsorbent paper used to principle. As in all the Laboratories that preceded us in Spain, we received the urine sample on blotting paper, obtained it simultaneously with the blood sample on blotting paper, and sent it to the Laboratory. In 1983 the pediatrics department settled in an attic, on the same 5th floor of the Hospital, where the Laboratory for the Detection of Genetic-Metabolic and Nutritional Alterations (today Metabolopathies) was located, the essentials to detect congenital hypothyroidism; I had passed the Radioactive Facilities Supervisor course, which only helped me to know that this was a radioactive facility and was outlawed, I had the idea of switching to non-radioactive procedures, which were beginning to be marketed and at the ***V National Meeting of the Spanish Society of Clinical Chemistry April 1985***, at the Faculty of Medicine of Santiago de Compostela (USC), there

was an exhibition of Laboratory Material, in which **IZASA** presented the **DELFIA®** System (PerkinElmer Life Sciences-*Wallac* Oy, Turku, Finland), then they only had commercialized applications to determine serum Hepatitis A Surface Antigen and Thyrotropin (TSH), the latter with a very high sensitivity, which made it possible to use a very small volume of serum, the lamp turned on immediately and I proposed adapting it to the blood sample on paper; we were surprised when, after a few weeks, without prior notice, they brought us the instrument they had at the exhibition, with a kit of consumables, adapted to the blood sample on paper; Dr. Colón, who had been doing tests with commercial RIA procedures for some time, which I was concerned about, when the personnel who had worked in the Physiology Laboratory left, who moved briefly to the new facilities, to see improvement the methodology used; he improved it in a few days, modifying the way of calibrating (which was later adopted by the manufacturer) [21], we used it for some time simultaneously with the RIA; later the instrument went to Seville (Virgen Macarena Hospital), it was the only one they had. Soon, we had another version of the tool available; with this second instrument, we began to detect Congenital Hypothyroidism routinely. With the introduction of the DELFIA method, the determination of TSH in duplicate was no longer carried out, which was done with the RIA, since the reproducibility of the results was improved and because the cost of the reagents made it unfeasible, even after acquiring the instrument and having agreed a reasonable price, which may not be that of the serum determination, since it is a Massive Public Health Program; which also simplified the NBS program.

In November 1986 we presented our experience [22] at the 6th International Neonatal Screening Symposium, Austin, Texas; at that meeting we were three laboratories, all European, who provided information on the use of DELFIA in the determination of TSH (a *Wallac* participant[1] attended, for whom what was done there was new), I remember that the colleagues from Copenhagen and West-Berlin was surprised that we used the S-S 903 paper, when in Europe the S-S 2992 of German manufacture was being used, the other was manufactured in the USA, it was also an anomaly in Europe to refer to the concentration in serum, in Europe it refers to whole blood, since the newborn's hematocrit is greater than 50%, the serum concentration is more than double, this comes from the fact that we made the comparison with an RIA procedure from the USA; This last circumstance was not known by many Spanish pediatric endocrinologists, which gave rise to misunderstandings; at a meeting in Spain (Granada 1996), which I attended, I had to clarify the situation, which was helped by Dr. T Torresani, also present, that he was not in favor of letting clinicians in on the cutoff numbers, which he told me privately.

In that ***V Meeting,*** the **Baxter's STRATUS System – DADE** [23] was also presented. The instrument that was in the exhibition had serial number 6 and went directly from the display to the Laboratory, it was not helpful for adaptation to the determination of neonatal TSH, but this instrument and the later version were very useful in the confirmation and follow-up of detected hypothyroidism, as they allowed the determination of TSH, T4 and sometime later, also free T4, in serum; in a few minutes, and can be used for a small number of samples or just one, Dr. Daisy E. Castiñeiras took care of this; until a few years later, the laboratories of all the hospitals had the means to do so, using alternative methods to the RIA, with which we stopped using it. The Central Laboratory carried out this analysis to confirm cases in the Health Area and confirmed and followed-up patients in our Hospital [23]**.**

[1] Jan Ostrup. Director of Engineering, Wallac Division of LKB. TURKU, Finland

We proposed modifications shortening the analysis time to just over two hours, reducing the reagent volume [24] from 200 µL to 100 µL, containing the same amount of tracer, for which we multiply its concentration and that of analyte by 2, until then, required overnight incubation in the refrigerator. Using the automated AutoDELFIA® system, we introduced another modification doubling the tracer content, whereby the tracer/analyte ratio is multiplied by 2, which is how we have done it since 1997; with this, we obtained the result the same morning that the samples enter the Laboratory, in time to notify to the clinician, to make an appointment with the newborn the day after the samples are received the laboratory; modification that we reported in 2011 at the same time as the use of a variable cut-off point [25].

We also adapted the DELFIA® immunoassay to determine free T4, in blood on paper [26], not marketed but used by others in Argentina [27]. The adaptation that we later made to AutoDELFIA®, we baptized it in the Galician language, the Galician: T4 ceiba. Later, other immunoassays were marketed to determine free T4 in this specimen in Japan [28, 29]. The determination of free T4 makes it possible to detect congenital hypothyroidism of any origin with certainty. It has been used for years in some laboratories [30] when TSH is elevated to reduce the number of sampling repetitions and bring forward the start date of treatment [31], as it is a method with excellent discriminant efficacy. This becomes important when taking the sample at 24 hours of life, which causes many elevated TSH results.

The DELFIA® immunoassay kit to determine serum-free T4, was discontinued.

8. Clinical Chemistry

In the "Introduction to Clinical Chemistry" Course at USC from 09/11 to 9/24/1986, I gave the inaugural lecture ‹‹Concept of Clinical Chemistry. Current Vision››, which I said to end: *Clinical Chemistry is also a vital industry, which has contributed and still contributes a lot to its development.*

Most of the industrial, chemical, and pharmaceutical firms are dedicated, either directly or through subsidiaries, to the field of clinical chemistry, developing, adapting, and marketing reagents, equipment, and instruments for Clinical Chemistry laboratories. We must be grateful that the synthesis and industrial preparation of complex reagents, substrates, coenzymes, enzymes, antibodies, marked products... and the commercialization of simplified equipment have allowed Clinical Chemistry to be taken to all corners. Today it is already next to the patient, using solid phase reagents and cassettes combined with automatic reading instruments.

The industry has produced, and the trend is increasing, almost "black boxes" "push buttons", which can only be used with reagents from the same manufacturer, which produces a total dependency (prices, equipment modifications, etc.; the businessman decides everything). This also has advantages: High quality and reliability can be used by low-skilled labor (low wages or in underdeveloped contexts); they can be handy in an emergency laboratory and next to the patient.

On the other hand, laboratories have appeared, which are actual industries, recipients of samples, and producers of results (sometimes sponsored or owned by the industry that produces the materials).

Seeing the panorama in this way, it seems that clinical Chemistry could be like Pharmacy at the beginning of the 20th century, which moved from the apothecary to industry, and this will be and is so in many aspects.

This sad panorama is even more so if we consider that Spain is only an important market; the primary client, almost the only one, is the National Health System, and it must be asked to dedicate efforts so that it stops being just a buyer, either manufacturing directly, or contracting or participating in companies dedicated to the manufacture of Clinical Chemistry materials, which in sometimes they are complicated to manufacture, but others are simple solutions, at most freeze-dried.

This would require a prior normalization or standardization of the methodology within the Institution.

Doctors and graduates who work in laboratories would not resign themselves to being just push buttons that produce results and would participate in industrial development by proposing and promoting it. This would lower costs.

Clinical Chemistry must evolve according to the clinic's needs and take into account the advances in science, which will open new horizons. In no case should needs be created due to technical progress, falling into a consumerism fostered by the industry. The requirements will be defined in agreement with the clinician, clearly setting priorities.

The picture seems pessimistic, but we must recognize the problem if we want to solve it.

For the consolidation and development of Clinical Chemistry as a science, it is decisive that it can be promoted as a pure provision of services or as a lucrative business. Let us remember that it is an applied (translational) *science with a precise orientation, the benefit of the patient or potential patient.*

Those of us who experienced the birth of the NBS Laboratories are witnesses of how, in a short time, we went from pure craftsmanship (a lot of this was done by HK Berry, HW Baird, ML Efron, V Shih, CR Schriver and R Guthrie) to the industrialization of our work. The appearance of "laboratory accreditation" also contributes to promoting the use of procedures, reagents, systems ..., approved by official bodies. In the European Union, *in vitro diagnostic* materials must bear the CE mark, which is subject to regulations.

When, in 1997, we made the modifications described above in the commercial procedures (and the first ones in 1985 when we decided to change the way of calibrating the TSH determination that the manufacturer did with 3 points and linear adjustment, as it did in serum), initially, we did not need any permission, still, when we suffered a hack in the laboratory's computer network. We had to reprogram the AutoDELFIA®, the 1997 modification, with which we obtained results that perfectly complied with the quality controls. After much insisting, they made it easy for us to replace it only as an investigation. It was already an officially approved procedure.

9. LI WOOLF

Remembering how **LI WOOLF** started this path at The Hospital for Sick Children, Great Ormond Street, London (**GOSH**), along which many of us, including **R GUTHRIE**, made a pilgrimage, we see that the evolution from craftsmanship has been one of continuous success; "we made the way by walking". Those of us who do not abandon the dried urine sample on paper, collected at the time the blood sample is obtained, placing the blotting paper on the genitals, held by the diaper, and then puncturing the heel to deposit the blood drops in their corresponding form, putting it to dry –*Guthrie specimen*– and removing the form with the urine (as a consequence of the newborn urinating by reflex action, when receiving the puncture) leaving it to dry –*Berry-Woolf specimen*–;

we would like the industry to look at that sample and test the possibility of developing marketable processes. Some perhaps abandoned that urine sample because they did not know what to do with it, which is very useful.

On his 100th birthday [32], some knew LI Woolf's time at GOSH, in London at the end of the 1940s, where everything that brought us here was gestated.

According to the minutes of the Hospital Research Committee meetings, reviewed by its archivist, Mr. Nicholas Baldwin, *‹‹Dr. Woolf was appointed as Imperial Chemical Industries' Research Fellow by the Institute of Child Health's Academic Board on 27 October 1947. The ICI funding ended in 1950, but the Research Committee meeting on 14 September 1950 decided that it was "essential" to extend the post. On 15 January 1953 the Committee extended the post for a further year from April 1st. Another year was granted at the 18 February 1954, with Woolf's salary noted as being £990 per annum. At the Committee meeting on 16 July 1953 an additional grant of £450 was awarded for 6 months to study two cases of Phenylketonuria, including visits by Dr. Ruth Griffiths. A further £500 was granted from 17 June 1954. Dr. Woolf's post was extended for a further year on 20 January 1955, with his salary raised to £1100. At the Committee meeting on 19 January 1956 another year's extension was granted, and salary raised to £1200, with note that the 'whole question' of the work of Woolf's laboratory should be looked at in the event of his leaving. He visited Canada as a lecturer at the University of British Columbia, giving a lecture on 'The Biochemistry of Mitochondrial Diseases'. At the Committee meeting on 16 January 1958 his post was extended for a final year, with note that it was "discussed to the proposed integration of the Organic Chemical lab. with the main Biochemistry lab." The meeting on 16 October 1958 reported that Ruth Griffith was to hand over the intelligence testing of PKU cases to Dr. Coates››.*

Baldwin attaches 16 scanned pages, including LI Woolf's Report to the Research Committee on the visit to Canada and the US from June 18 to July 3.

I copied the first paragraph of the phenylketonuria section, from the 1956 report: ***‹‹****Phenylketonuria. This condition is being diagnosed with increasing frequency as the ferric chloride test is more widely applied. Several of the patients are being treated with the diet. One patient (the sister of an older phenylketonuric) was diagnosed at seventeen days, the youngest over-recorded. This child presented many problems since a diet suitable for older phenylketonurics could not be fed to an infant of her age. These problems were solved jointly with Miss Dilliston, and not unpleasant synthetic milk is being used. Dosages of the vitamins and minerals presented special problems since the literature contains no reliable data as, e.g. the folic acid and vitamin B12 content of human milk, nor on the minimum daily requirements of a young infant for these two vitamins. In general, the aim has been to get a composition as close to that of human milk as possible; the fat is added as double cream, which also provides half the daily protein intake, the other half being added as cow's milk while the bulk of the nitrogen is present as the special casein hydrolysate****››.***

In the interview with Woolf, in the newsletter of the University of British Columbia, UBC Reports 1972 [33], he refers to this case, saying that the diet[2] should be supplied in liquid form,

[2] ***Information on Some Details of the Elaboration of the Therapeutic Diet.**

A Tiselius in Uppsala, was the first, in 1940, to carry out adsorption chromatography on columns with stationary phase of **pulverized activated carbon (ground vegetable charcoal)**, he began by applying it to the separation of sugars, the same as he did later, for the separation of amino acids and it served to eliminate phenylalanine from the casein hydrolyzate that inspired **LI Woolf** to prepare the therapeutic diet for the treatment of phenylketonuria (PKU).

with the aggravating circumstance that human milk has a high proportion of fat *(plus the colostrum)*. They tried "horrible" formulas, complex for children to tolerate, based on corn oil and emulsifying agents.

So, a GOSH dietitian solved what presented many problems since with an adequate diet for older phenylketonurics it was not possible to feed a baby her age. These problems were worked out together with **Miss Dilliston**, who uses not-so-unpleasant synthetic milk.

The doses of vitamins and minerals presented special problems since the literature does not contain reliable data, e.g., breast milk's folic acid and vitamin B12 content, nor the minimum daily needs of a small infant for these two vitamins. In general, the objective has been to achieve a composition as close as possible to that of breast milk; the fat is added as double cream, which also provides half of the daily protein intake, the other half is added as cow's milk, while most of the nitrogen is present as special casein hydrolyzate. Dilliston suggested using whipped cream ("double cream in UK") as a fat base, making milk that the girls readily accepted. The older sister who received late treatment did not exceed an IQ of 20, the phenylketonuric twin reached 90, and her disease-free twin sister reached an IQ of 110. This led to an excellent demonstration of the effectiveness of your diet. In that interview, Dr. Woolf commented, "It must have taken great courage for the doctor [38] to give her a completely unproven diet. I warned that some of the amino acid would have to be given to her in the form of normal food [39] otherwise, the child

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Tiselius in 1941 [34] says, after citing two authors that they find that many amino acids are remarkably adsorbed by carbon. That these last authors found that carbon can catalyze the oxidation of amino acids. To avoid difficulties of this type, traces of HCN were added to all solutions investigated in the present work, and only air-free water was used as solvent.

Schramm and Primosigh in 1943 [35], review amino acid chromatography, in the context of studies of viral proteins, remember that A Tiselius [34], introduced a form of analysis, by adsorption chromatography. A safe and reliable microanalytical method is proposed to determine amino acids in quantities of a few milligrams. Using adsorption through activated carbon, as stationary phase and 5% acetic acid as mobile phase. It was promising to attempt a quantitative chromatographic separation of aromatic from aliphatic amino acids and was possible in a simple manner and with high precision. They used granulated activated carbon (Schering), which they pulverized and sieved to a suitable grain size, for the desired flow. The coal was boiled with acetic acid to remove small amounts of substances that contain nitrogen. He says that it is necessary, according to Tiselius's proposal, to poison the coal with cyanide, otherwise notable alterations are produced, due to the oxidation of amino acids; I do not have documentation, nor do I have any other information, but when trying to apply it to the preparation of food, the cyanide had to be replaced by another compound that prevents oxidation (could it be ascorbic acid –Vitamin C-?). After filling the tube with carbon, 5% acetic acid is passed, to reduce the adsorption capacity of the carbon to a useful level; without this pretreatment, a quantitative separation of amino acids is not possible, since otherwise a certain part of them is stubbornly retained and continuous elution for a long time leads to blurring of the boundaries between aliphatic and aromatic amino acids. The totality of the aliphatic amino acids is eluted by washing with acetic acid, while the aromatic amino acids, phenylalanine, tyrosine, and tryptophan, are not eluted with this wash, a safe separation of these two groups of amino acids is guaranteed, under the experimental conditions that are describe. Adsorption on carbon is nonpolar, independent of the presence of electrical charges, and is strongly influenced by constitutional properties and stereochemistry. LI Woolf needed to move from the microanalytical to the preparative scale, throughout the entire procedure, including pretreatments; all of which is described in this work in German. In addition, he had to eliminate the acetic acid from the eluate, to obtain the mixture of aliphatic amino acids in solid and dry form, to which he adds tryptophan and tyrosine, the latter being an essential amino acid, for phenylketonurics.

Block and Bolling's book, in its second edition of 1951 (not so the first of 1945),

The amino acid Composition of Proteins and Foods. Analytical Methods and Results, in **Chapter IX Part IV Chromatographic Adsorption**, page 400-401 lists the above procedures and more [36].

Anne Green's article [37] gives some information on how the first therapeutic diet for PKU was prepared but does not mention how to avoid amino acid oxidation.

I don't know how they did it and do it in the industry.

wouldn't be able to grow. In the first few years of this treatment for PKU this advice either wasn't known or wasn't followed by some other doctors and the results were disastrous". Based on Woolf's work, the British company Trufood developed Minafen specifically for newborns [37].

This phenylketonuric, the first treated early with diet, in the year 2022, he turned 66 years old.

In 2008, Brosco et al. [40] wrote about the adverse medical outcomes of newborn screening programs for phenylketonuria. They came to say something like what we express in Spain with the phrase *"The remedy was worse than the disease."* They say among other things that there were many case reports of infants receiving excessively strict treatment for PKU in the 1950s and 1960s …; Moncrieff and Wilkinson [41], for example, information in 1961 the case of a child in the GOSH, London, UK **(1958 was the last year Woolf was on the GOSH and Moncrieff signed the 1955 paper [39] formulating the diet, he must have paid no attention to its preparation, nor did he discuss with Woolf the following year how he prepared the diet for a newborn 17 days; he was the clinician running the clinic and was probably not interested in the details of the preparation)**; the child had "indeterminate –inconclusive" neonatal screening results and was treated for PKU, due to high serum phenylalanine levels, which normalized with a restricted diet; however, the infant continued to have poor growth, restlessness, and a rash despite weeks of close follow-up, including dietary adjustments and careful attention to serum and urine test results; all the symptoms disappeared when the "whipped cream" was started and during the following months the child grew and maintained normal serum phenylalanine levels despite the lack of a restrictive diet; what Brosco et al. say is **not** true, as they expose it, the diet continued to be restrictive in phenylalanine; the use of whipped cream in the diet was already introduced by Woolf in the first infant with phenylketonuria treated in 1956 and already warned of malnutrition problems if some whole milk was not added to the diet [39]. Reviewing the article by Moncrieff and Wilkinson, the nonsense that the child lacked a restrictive diet is not deduced; what is supposed is that the lipid intake -whipped cream- is essential for the correct development of the infant.

It seems that the contribution of LI Woolf in the composition of diets [39], also for infants, has not been given the importance it deserves. Even in the GOSH, it was unknown what he had done in this regard and that he was the first to prepare a proper diet for a baby [37].

Prof. Mayor Zaragoza was with Prof. Krebs in Oxford in 1959. LI Woolf was already in Oxford then, but it was in the sabbatical year 1966-67 when they met. This meeting was decisive for Prof. Mayor Zaragoza to introduce the NBS in Spain.

When I took it upon myself to claim **LI WOOLF'**s participation in the preparation of the therapeutic diet for PKU and his involvement in the beginnings of NBS, I learned that those who knew him in Oxford and brought and accompanied him in Spain were unaware of his time in the GOSH (where the great **Sir Archibald Garrod** had been part of his staff, who was an active member of the staff, from 1892 to 1913, when he described some "Inborn Errors of Metabolism -**IEM**-" and introduced this concept), where and when what brought us here was conceived. This means that Woolf did not. He told them his vicissitudes to put his ideas into practice, which we followed.

10. Healthcare Professions

Returning to the concept of Clinical Chemistry, let us remember that it is an applied (translational) science that must design and develop the use of laboratory methods and

procedures so that they serve as support for medical decisions and a better knowledge of the biochemical basis of diagnosis, monitoring and prevention of the disease. If this is the case in all disorders, in congenital ones: IEM, endocrine, and others, including infectious diseases, this is its essence, and the laboratories that deal with the NBS are not conceived as other hospital laboratories or "industrial to use". In these laboratories, when "the Case" appears, keep in mind that the usual thing is that the newborns analyzed are healthy, and those affected are rare; the opposite of what the doctor sees in the Hospital, who are all sick, with very few exceptions, so that the perception of "the Case" by some and others is very different; in addition, "the Case", almost always, must be located and confirmed urgently or very urgently, to establish treatment as soon as possible, a phase in which the corresponding Unit of the Hospital acts as an emergency of those who are admitted every day; In this, the NBS Laboratory also differs from the Central Laboratory of a Hospital, which receives samples from admitted patients, to help establish a diagnosis or follow-up.

According to the Spanish regulation of Healthcare Professions, the NBS Laboratory should be a Specific Training Area (ACE); this ACE is not yet a reality (a Royal Decree of January 11, published on January 31, 1984, mentions creating *"specialties and areas of specific training that scientific and technological progress advises following health needs"*); this ACE could include the Laboratories that deal with Rare Diseases and of course, those that run the NBS Programs. In the case of Galicia, this Laboratory is at the Hospital Clínico de Santiago de Compostela (CHUS - SERGAS) and is also in charge of the diagnoses and follow-up of IEM. This implies some very peculiar knowledge that must be considered when recruiting personnel, who must have this knowledge so as not to constantly discover gunpowder. Those of us who grew up at the same time as this discipline must leave the witness to whoever arrives, who knows where we are and continues running from there that point. Urine and blood sampling, in the solid phase, is also peculiar in the NBS in such a way that it conditions the design, development, and use of laboratory methods and procedures, which, in my opinion, should be open, as I indicated, the so-called multiplex they are; others that lead to a positive for a group of conditions that oblige, without the need for new sampling, using the same or another aliquot, to continue analyzing, according to an analytical process, until the biochemical marker responsible for the positive is known, are equally so.

11. The NBS Today in My Environment

In the ten years that have elapsed since I retired, the pathologies or conditions detected have increased up to what is being done now, which can be consulted in the Galician language at the link:

https://www.sergas.es/Saude-publica/Documents/7090/Listado_enfermedades_gallego.pdf.

Currently, there are 15 NBS laboratories in Spain, although a recent work [42] incorrectly says there are 20 (they were in 2003). In this work, you can see the differences between the programs and laboratories.

Below are the covers of the two monographs from which I took the texts that make up this article. The second is being prepared, and this early sampling at 24 hours of life forces us to reconsider what is contained therein. To freely access these volumes (Figure 1), go to **ResearchGate** where the author's cited works are also found.

https://doi.org/10.13140/RG.2.2.29898.72641/1 & https://doi.org/10.13140/RG.2.2.12678.65605.

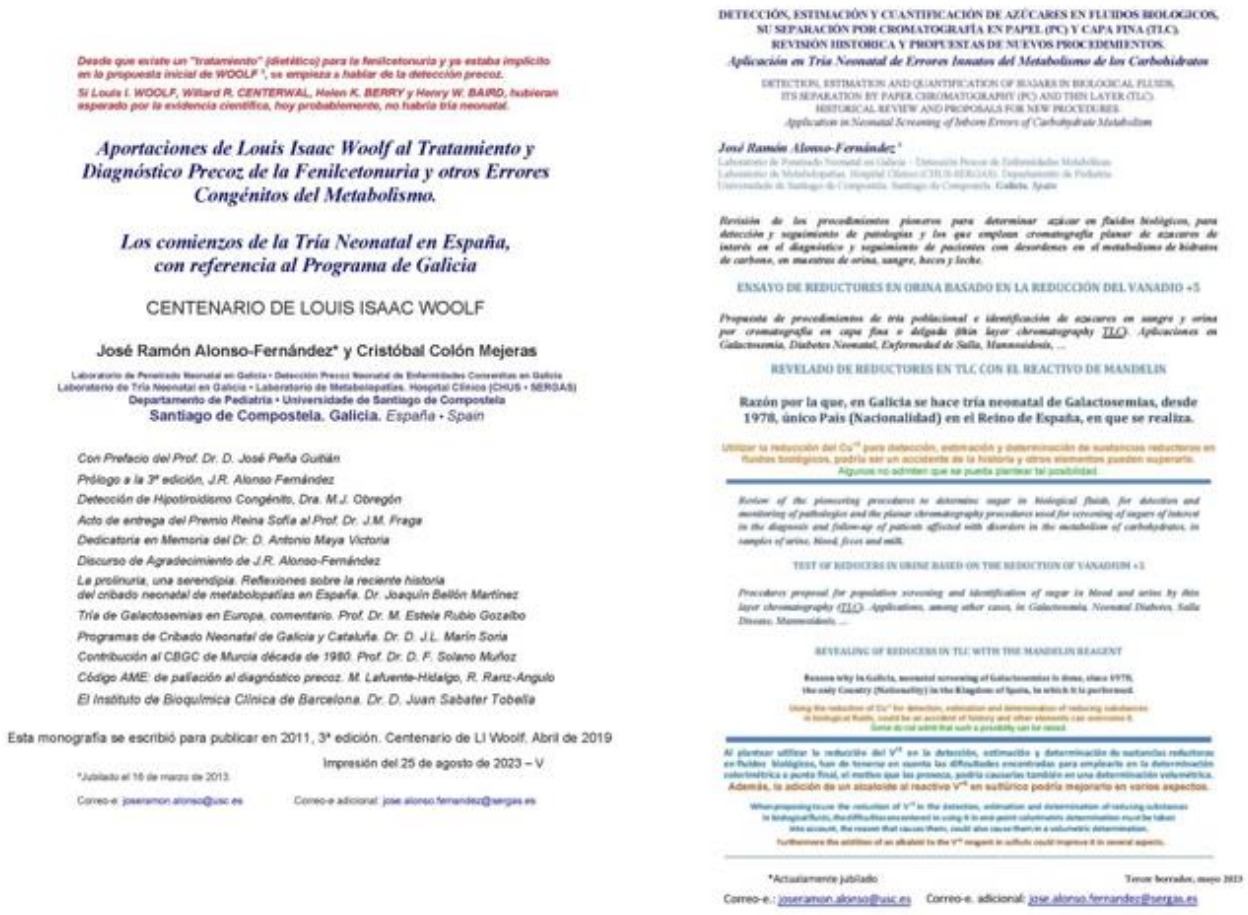

Figure 1 Covers of the monographs

12. Conclusion

This article is not intended to review what has happened in newborn screening since LI Woolf introduced it until today. It only refers to personal experience. However, in the peer review, I learned of the recent advancements in the NBS such as the detection of PDE (pyridoxal-dependent epilepsy) which can be done both in urine and in blood [43-45]. This is interesting, and other developments will have gone unnoticed in the 10 years I have retired. The writing assumes that the reader is informed of what neonatal screening is and does not include references, for example, referring to the detection of galactosemias, and is limited to those related to personal experience, including unknown aspects, the reason for which those of the author are in the majority throughout the entire account of the facts.

I hope that this reminder of personal experience and the questions I leave open are of interest and that what I have learned in almost 40 years dedicated to the NBS laboratory in GALICIA serves to discover some details and that interest in the URINE SAMPLE resurfaces.

I consider it my duty to make you aware of the letter from Prof. Federico Mayor Zaragoza to H.E Mr. Volker Turk, High Commissioner for Human Rights Office of the United Nations, last February 1st 2023. Section 2, at the end, refers to our matter.

https://www.docdroid.net/dJPCwVj/carta-mayor-zaragoza-0038-volker-turk-high-commisioner-for-human-rights-un-pdf.

Author Contributions

The author did all the research work of this study.

Competing Interests

The authors have declared that no competing interests exist.

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