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ITALIAN NATIONAL COMMITTEE FOR BIOSECURITY, BIOTECHNOLOGY AND LIFE SCIENCES

LONG-TERM STORAGE OF RESIDUAL DOT BLOT SPOTS FROM NEONATAL SCREENING

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PREMISE

The NBC and the NCBBLS felt it was appropriate – with this Document – to focus attention on a particular aspect of the neonatal "screening", that is compulsory for some curable congenital diseases and carried out on a few drops of blood collected at birth on a suitable card (Guthrie card or neonatal screening card). This aspect is represented by the long-term storage of residual dot blot spots (DBS) from neonatal screening prescribed by the regulations in force. In particular, we propose to define the criteria for a uniform storage of the residual DBS, to be adopted in the Italian screening Centres. The storage is proposed compulsory for a relatively short time and voluntary for a prolonged time, according to the objectives we intend to achieve and available resources.

In the present Document, the aim is not to tackle the issue – broader from an ethical and legal point of view – of the genetic investigation carried out on underage children (newborns in particular) for medical-healthcare purposes, an issue that the two Committees might eventually tackle in the future. At the moment, we propose a completion of a national law which has proved beneficial, in many ways, for the protection of human health, but is managed with substantial differences in its organisation and diversity in the behaviour of the various Screening Centres – with regards to the preservation of the original samples – which is questionable from many points of view.

This variation can be inconvenient for the newborn if, in case of a "technical" need to replicate the analysis, the sample has been destroyed immediately after the initial tests; but it is also the source of a worrying loss of knowledge, at least potentially, and scientific and clinical information which could derive from the samples afterwards.

Therefore, as already performed in other Countries, also in Italy there is the need to define the rules for the long-term preservation of residual DBS, applicable in all Screening Centres, with times and criteria proportionate to the aims intended.

In this context, an accurate reflection on the criteria to adopt for a safe storage of the samples appears necessary, also in order to avoid possible abuses in the use of this biological material.[1]

[1] As known, it has been highlighted, in Texas, an episode of non-agreed use of 800 Guthrie spot samples by a military laboratory, aiming to build a database on the mitochondrial DNA.

Document's structure

We have felt it appropriate to divide the document into two chapters.

The first, includes brief general information on neonatal screening carried out in many Countries today and on the criteria of feasibility that have allowed their regular use (1st paragraph). In the second paragraph we describe – briefly – the current situation in Italy.

The second chapter examines the specific problems linked to the prolonged storage of the residual neonatal screening DBS, with regards to the objectives, criteria of healthcare, legal and bioethical protection of the newborns whose samples were taken. In this chapter the hypotheses of short-term (mostly for aims in the newborn's interest) and long-term storage are described. In particular the newborn is considered as a sample's "donor" whose residual DBS can be used for biomedical research purposes.

In the "Appendix", finally, we include and discuss mostly "technical" aspects, which illustrate hypotheses about how to accomplish the directives of the two Committees, according to the respective competences, hoping that the problems of reorganising and strengthening this area are tackled by the appropriate establishments.

CHAPTER 1

GENERAL OVERVIEW OF NEONATAL SCREENING

The term “neonatal screening” refers to the programmes of secondary preventive medicine, started on a large scale in the first days of life, aimed at performing the early selection and quick treatment of newborns at high risk of some congenital and curable pathologies, characterised mainly by high early mortality rate and/or severe morbility in the affected subjects.

1) Examples of programmes and relative techniques

More than fifty years ago, in 1963, an American researcher, Robert Guthrie, carried out the first laboratory test (which still today has his name: “Guthrie test”), which allowed to measure the concentration of the amino-acid phenylalanine in a drop of blood, collected by simply pricking the newborn’s heel, and then absorbed by and dried on a special card (Guthrie card or neonatal screening card). This test, which is low cost and easy to carry out on a large scale, allowed the realisation of the first campaigns of neonatal screening aimed at identifying newborns affected by phenylketonuria (PKU), the most common metabolic congenital disorder, characterised by a severe mental impairment.

In a historically important temporal succession of events, phenylketonuria was followed, amongst the pathologies absolutely indicated for neonatal screening, by congenital hypothyroidism and, in the following forty years, many other diseases mostly genetic, like endocrinopathies, congenital metabolic disorders, hemoglobinopathies, were found to be suitable for neonatal screening, because of their clinical characteristics.

Traditionally, neonatal screening programmes use, as pathology biomarkers, analytes – mostly haematic – the quantity and quality of which allows, with sufficient accuracy, the selection of high risk individuals in the neonatal population. In some programmes, the laboratory analysis is aimed at measuring the substrata accumulated in biological liquids, in different ways: a) altered use or transformation of a substratum due to an enzymatic deficit in a biochemical process (PKU: Phenylalanine; Galactosemia: Galactose; Congenital Adrenal Hyperplasia: 17-alpha Hydroxyprogesterone); b) mechanical obstruction (Cystic Fibrosis: Immunoreactive Trypsin); c) physiological activation of a feedback phenomenon (Hypothyroidism: TSH). In other cases, the biomarker is represented by the lack/decrease of a substratum

(Hypothyroidism: T4) or the presence of abnormal metabolites, absent in normal conditions (Hemoglobinopathies). Finally, the screening can be carried out by measuring or qualitatively assessing a specific enzymatic activity (Galactosemia: activity of the enzyme Galattosio-1-P-uridltransferasi). In the first days of life there is a "chronology" of concentrations of biomarkers, which are strongly affected by biological changes that happen in the delicate perinatal period. During this period processes of biochemical adjustment to autonomous life occur. Therefore time at which the neonatal screening sample is collected, must be carefully chosen in windows of time that guarantee, in the presence of the pathology, optimal levels to measure/assess the analyte, in order to reach the system's maximum efficiency (sensitivity + specificity).

2) Neonatal screening in the National Health System "policy of prevention"

There is today a large international consensus in considering mass neonatal screening as a model for many screenings of the population. These are generally believed to be an essential intervention, together with prevention programmes against malnutrition and infection, to ensure a better "outcome", in terms of health, of the neonatal population. With evident positive effects for the following development and health of the individual who – if affected by the pathology under investigation – can be quickly cured.

There is, then, certainly a parallel between the importance of neonatal screening and economic and social evolution.

The more the economic and social level of the population is developed, the more neonatal screening is important: the more family structure evolves from an extended family model, highly prone to illnesses and infant mortality (typical of less developed countries) to a smaller model (low reproductive index), the more preventive interventions from birth are stimulated to ensure a better infant survival and development. Among these interventions there are also metabolic screening programmes.

3) *The evolution of technology and the broadening of the screening potential*

Enzymatic methods, used at the beginning of the collection of the newborns' blood sample on a card (which is easy to preserve and not very bulky) are still used to diagnose phenylketonuria, congenital hypothyroidism and cystic fibrosis in many Countries.

However, it must be highlighted that since the 1960s technological development has offered increasing opportunities to broaden laboratory techniques applied to neonatal screening, giving the possibility to use –

on a large scale – a growing number of biomarkers to identify illnesses. In the 1990s, the possibility to overcome the concept "a marker, an illness" becomes progressively achievable, in order to reach the technical-organisational model "more simultaneous markers for many illnesses": a new multi-parametric vision or analytical platform in carrying out laboratory tests, which tends to change the structure of neonatal screening programmes. The technology that today better responds to this vision is *tandem mass spectrometry*, which was developed thanks to researchers from the Duke University and the "Newborn Screening Laboratory" (North Carolina, USA), at the end of the 1980s. The new vision of neonatal screening - introduced internationally in 2006 through the joint work of the American College of Medical Genetics (ACMG) and the American Academy of Paediatrics (AAP) – has in fact started the new revolutionary phase of the so-called " expanded newborn screening". This is a dynamic programme of neonatal screening aimed at identifying, in particular through the use of *tandem mass* and/or of "multiplex" platforms, an increasing number of "rare" diseases, in which early postnatal diagnosis represents the fundamental cornerstone of a medical and social intervention in favour of the affected individual, his/her family and the community.

The interesting aspect in highlighting these technological aspects of expanded screening is represented by the fact that *tandem mass spectrometry* can be used for samples eluted from blood spots absorbed on a Guthrie card, as a growing analytical literature is confirming.

4) The card for the collection of neonatal blood destined to screening tests

It is useful to complete this brief analysis of the technical aspects, with a short description of the tool that allows the collection both of personal data and the neonatal blood samples. We expect, in the Italian Screening Centres, an adjustment process that will lead to use a DBS card in which the two parts (personal data and blood samples) can be easily separated. The use of this support would allow, after the diagnostic process, to "anonymise" the samples. The unused parts of the card (residual DBS) can be stored according to the "short-term" or "long-term" methods that will be described later.

In fact, the card for the collection ofDBS used in neonatal screening is composed of two parts: a) a form for the collection of the newborn's demographic and medical data; b) a card for the collection of the capillary blood samples , obtained – usually – by pricking the heel. The card is highly standardised (in particular, with regards to its characteristics of capacity and volume when absorbing blood) and meets the specifications outlined by the American Clinical and Laboratory

Standards Institute (CLSI, 2007). In line with the specifications of the above mentioned document, the U.S. Food and Drug Administration has currently only two registered types of card for the collection of neonatal blood, as Class II implements: “Filter Paper Whatman 903” made by Whatman International LTD – UK and “Filter Paper Ahlstrom 226” made by Ahlstrom Corporation, Finland.

The different batches undergo comparative technical assessments by the Center for Disease Control and Prevention (CDC) in Atlanta (USA), while in Europe the screening cards must obtain, the CE mark required by the European Union directive 98/79/EC, in order to be used for medical purposes for in vitro diagnosis.

5) Neonatal screening in Italy: compulsory programmes and public screening centres

In Italy neonatal screening (available since 1972 thanks to local or regional initiatives) is compulsory since 1992, in line with art. 6, subsection 2 of Law 104/1992 and the subsequent applicative decree DPCM 9/07/1999 (Appendix 1) for the following congenital pathologies: congenital hypothyroidism (CH), cystic fibrosis (CF), phenylketonuria (PKU). On the basis of the autonomy of initiative given to the Regions, are currently active in Italy also screening programmes for: Galactosemia, Leucinosis, Biotinidase Deficiency, Homocystinuria, Congenital Adrenal Hyperplasia, glucose-6-phosphate Dehydrogenase deficiency, generally in universities or research institutes or some hospitals.

With regards to the programmes that are compulsory by law and as highlighted in the annual reports of the Italian Society for Neonatal Screening (SISN), in our Country there are a total of 32 neonatal Screening Centres. These serve a field of users diversified according to area size (Sub-Regional, Regional, Inter-regional) and number of samples (from 5,000 born/year to 100,000 born/year), and currently follow the different programmes available, depending on what has been decided by the competent regional Authorities (Appendix 2). Since the middle of the 1990s, the Italian nationwide programme of neonatal screening guarantees total coverage of the newborns (year 2007; 580,700) for two of the pathologies compulsory by law (PKU, IC), whilst the information about the coverage of the other diseases is variable and in any case it involves less than 90% of the newborns, including the programme for Cystic Fibrosis (compulsory by law).

The international evolution of the information to activate programmes of neonatal screening (in particular aimed no longer at the single pathology but at homogeneous groups of pathologies, like metabolic congenital disorders) has promoted also in Italy programmes,

mostly experimental or pilot, for the early screening and diagnosis of new diseases.

This evolution has been possible especially thanks to the application in neonatal screening of highly technical methods (mass spectrometry ESI-LC-MSMS or *tandem mass spectrometry*); currently there already are in our country some integrative programmes of extensive screening which can be applied to over 30 rare metabolic diseases.

In particular, the Italian Regions in which a programme of expanded newborn screening is active or assessment of feasibility projects is ongoing, are the following:

Tuscany: regional programme of expanded newborn screening, in line with the specific legislative decree of the Tuscany region.

Liguria: pilot regional programme of expanded newborn screening, with the collection of the parents' informed consent., approved by the regional healthcare authorities,

Lazio: pilot subregional programme of expanded newborn screening

Veneto, Emilia Romagna, Sicily: dissemination of regional deliberations for the activation in a near future of programmes of expanded newborn screening.

Lombardy, Marche, Campania: there are currently assessments of projects of feasibility, not yet approved or activated by the competent regional Authorities.

In the Appendix to this document, we give some information on the quantity of activity by the Centres, for a sample year.

Observations and suggestions felt to be appropriate to support the activity of the Centres will be given in the course of the document.

6) General criteria applicable to every screening also from the costs-benefits point of view

As known, to be feasible and efficient tools of healthcare policy, screening must be in line with the well defined demands resulting from a conspicuous international and national literature and also from the practical experience in many Countries. These general criteria are valid also for neonatal screening carried out in various Countries, and in Italy as well, with universal methods for all newborns and with compulsory procedures established by law.

We think that it is appropriate to recall in the note what has been recently decided by the Council of Europe in the *"Additional protocol to the Convention on human rights and biomedicine concerning genetic*

tests for medical purposes" in chapter 8, "Genetic screening: programmes for healthcare purposes (art. 19) (*SEE NOTE).[2]

They are, evidently framework regulations which must be taken into account also when we go "beyond" the screening tests and informing the parents of the results, and we feel that it is appropriate to start a long-term storage of the sample (as it will be clarified later).

Amongst the general aspects to consider, there is also the the cost-benefit evaluation . In fact, it is not enough to establish criteria of validity, efficiency, optimal access to programmes and adequate information of the population, but it is also necessary to verify the economic feasibility, with the cost-benefit criterion, which has become increasingly important with time.

We stress that the criteria of the Council of Europe are adopted in all the Countries that have not only promoted screening with a direct distribution of the results and the following destruction of the samples, but are applied also to the long-term preservation of the residual biological material. The cost/benefit evaluation has been deemed appropriate for universal application to newborn screening, but in Countries where healthcare is most developed, also for long-term storage, because of the availability of precious biological material both for basic genetic and applied research. We know that from the material preserved in the Danish newborn biobank, about ten GWA meta-analysis have been elaborated, obviously exploiting only a part of the numerous samples. If these studies will bring a real benefit in terms of increased health (well-being) of a class of patients (or individual citizens assumed to be healthy) remains – as for all these studies – to be seen. Further data will be given in the Appendix to this document.

[2] In chapter VIII "Genetic screening: programmes for aims that have medical purposes", reads: Chapter VIII- Genetic screening programmes for health purposes. Article 19- A health screening programme involving the use of genetic tests may only be implemented if it has been approved by the competent body. This approval may only be given after independent evaluation of its ethical acceptability and fulfilment of the following specific conditions: a) the programme is recognised for its health relevance for the whole population or section of population concerned; b) the scientific validity and effectiveness of the programme have been established; c) appropriate preventive or treatment measures in respect of the disease or disorder which is the subject of the screening, are available to the persons concerned; d) appropriate measures are provided to ensure equitable access to the programme; e) the programme provides measures to adequately inform the population or section of population concerned of the existence, purposes and means of accessing the screening programme as well as the voluntary nature of participation in it.

CHAPTER 2

LONG-TERM STORAGE

1) Premise

The long-term storage of residual DBS is now recognised, by the national and international scientific community, as a necessary tool to take full advantage of the high potentiality of the sample, pursuing two objectives:

1) A better protection of the growing child's health – if it is deemed necessary to integrate the initial analytical profile with new tests, or if the need to use the biological sample for medical-legal purposes arises, etc.

2) Giving value to the irreplaceable biological inheritance in order to improve scientific knowledge aimed at protecting public health.

As for many issues, the problems of biomedicine and bioethics must find a satisfactory mediation point between personal and public interests, according to the aphorism of the well-known Code of medicine and research regarding human rights, which reads:

"The interests and welfare of the human being shall prevail over the sole interest of society and science"[3]

However, the case we present is not about direct actions on the human body but the deferred use of samples deriving from the body, it is clear that the aphorism quoted above is valid in the way it has been translated in the Recommendation by the Council of Europe (REC (2006) 4) aimed by the Committee of Ministers to the member States, with regards to research utilising biological material of human origin.

This said, it is necessary to look first of all at the general advantages provided by long-term preservation, to then move on to investigating the application of such a directive in other Countries, and partially in Italy, and finish with the interests of the Community with regards to the eventual use of the samples for biomedical research.

[3] It is article 2 of the "Convention for the protection of human rights and dignity of the human being with regard to the application of biology and medicine" (Oviedo) 4/06/1997). The cited recommendation Rec (2006) 4 was adopted during the 958th Meeting of Ministers' delegates on March 2006.

2) Advantages of long-term preservation

Long-term preservation, according to the general objectives highlighted above, can have two variants:

a) Relatively short-term storage , believed to be necessary to fulfil the main precautionary obligations, exclusively for the benefit of the newborn's health.
   Suggested time: 2 years
   It could be called: COMPULSORY SHORT-TERM STORAGE

b) Long-term storage, for mainly scientific purposes.
   It could be called: VOLUNTARY LONG-TERM STORAGE.

Naturally, this is a general plan which can be illustrated as follows.

The main advantages deriving from long-term storage can be summarised in the following points:

1- Prevalent recourse to short-term storage:

a) Diagnostic assessment: the storage of residual DBS allows to confirm diagnosis of the pathologies that are the object of the screening and for the need of a broader diagnosis, due to new healthcare requirements.

b) Medico-legal aspects: the storage of the cards also allows to carry out analytical tests in answer to specific medico-legal requests, for example after the newborn's early death or any legal action undertaken by the family.

With regards to these two first objectives, we must highlight the advantages deriving from a non-immediate destruction of the sample or from the sample being preserved for even just a relatively short time. In particular cases, this practice puts the families of a newborn who has eventually died in the condition of having biological traces of potential interest, for example to have, afterwards, a clinical diagnosis that was not carried out when alive, or to perform family molecular investigations of interest to the parents, in the prospect of monitoring future pregnancies at risk. These opportunities have been trialled and used successfully, since the 1990s, and some Italian Centres of Medical Genetics demonstrated how a single Guthrie spot could be used after months or years from the

death of a little patient, who died without a diagnosis, to formulate a diagnosis or confirm, afterwards, a clinical suspicion and offer the family genetic advice in terms of reproductive risk and eventual control of pregnancies at risk. Illustration of this, are the examples of the identification of homozygous mutations of the SMN gene in newborns who die a few days after birth because of spinal muscular atrophy type 1 (Werdnig-Hoffman disease) or the diagnosis of deletion 22q11.2 in newborns who die because of DiGeorge/velocardiofacial syndrome. Another example – also repeatedly observed in Italy – regards the infant polycystic kidney. This autosomic recessive rare diseaseis transmitted from heterozygotic parents (healthy carriers) to, on average, 25% of their children, regardless of their sex. This is usually a lethal condition in the perinatal period. The diagnosis of the gene-disease is complex and, as such, it would make it difficult to directly study the gene-disease in the subsequent pregnancies of the parents at risk. This difficulty can be overcome by identifying, through the analysis of polymorphic markers flanking the gene-disease on the short branch of chromosome 6, the parental chromosome that carries the mutation, which, by definition, presents polymorphisms that are different from the chromosome without mutations. The reconstruction of the segregation phase requires the comparative analysis of the same markers in the parents and in the child. In the case of the deceased newborn, this analysis can be carried out on a Guthrie spot, allowing the family to acquire, after the event, critical information for the future monitoring of their pregnancies at risk.

2- Prevalent recourse to long-term storage.
We recall two fundamental objectives:

a) **The improvement of the methodology and development of new screening tests:** the long-term storage allows to use residual DBS for the improvement of already existing screening tests and the creation of new analytical strategies aimed at the screening.
It must be clarified that, in general, every card collects a variable number (from 4 to 6 or more) of spots of dehydrated blood, each one corresponding to a volume of about 50 - 75 microlitres. Generally, after carrying out the screening test, the residual biological material is represented on average by 1-2 spots of blood. The dehydration stabilises the analytes present in the blood for a preservation time that is variable from molecule to molecule, which can be further incremented with preservation at low temperatures (-20°C, -70°C). As previously stated, today there are numerous laboratory methods applicable to such biological material for the investigation and/or measure of a very large number of analytes, used as physiopathological markers in various laboratory medical fields

(clinical biochemistry, endocrinology, virology, haematology, etc.), and the residual spots have been in numerous cases used to confirm previous methodologies or introduce new and more selective ones.

b) **Biomedical research. The field already explored at the moment is vast, therefore, here we will give only some examples of it.**
As well as the specific and uncommon potential applications of these residual samples for diagnostic purposes, which could be relevant to some families, the availability of an anonymised DNA bank offers unique opportunities of scientific research for its extraordinary size. It is known that 1-3% of the inter-individual genome is different due to the presence of mutations, which are mostly relatively common (polymorphisms). These variations explain the differences among people, not only from a clinical phenotype point of view, but also from that of the susceptibility or resistance to common diseases and the individual response to drugs (pharmacogenetics). One of the most significant acquisitions of the so-called genetic revolution regards the development of technologies able to analyse on a large scale the entire genome to find variations linked to complex phenotypes. All this research has to take into account the knowledge of the distribution and frequency of individual polymorphisms within a population. Therefore, specific deviations can then be linked to diseases, creating the foundation for the assessment of the individual risk and resistance, at the basis of personalised medicine.
The possibility of extracting nucleic acids (DNA in particular) from this biological material allows to use methods of molecular genetics both for diagnostic as well as research purposes [Hollegaard 2007].
In addition, it has been very recently proven that blood spots on cards, stored at room temperature, can be used also for RNA extraction, making possible, in such a way, the quantitative analysis of genic expression. (Haak 2009).

As an example, we will recall that the long-term storage of residual DBS is certainly an essential resource to carry out “genome-wide associated studies”, aimed at researching new markers of susceptibility to the rare pathologies that are the object of the screening, but also for other rare pathologies for which a screening test is not yet available, for the most common pathologies affecting population (both in childhood and adulthood). In fact, the most recent genotypisation techniques and the possibility of obtaining adequate quantities of genomic material with procedures of

genomic amplification from DNA extracted from a single spot, make the study of various genetic variants possible [Green 2006, Paynter 2006, Sorensen 2007], and allow the linking of such data with clinical events, offering the chance to carry out focused etiological studies (Hollegaard, 2009).

The long-term storage of residual DBS also allows population studies aimed at deeping knowledge of the "gene-environment interaction" in multifactorial pathologies, researching both polymorphisms in genes codifying enzymes involved in the metabolism of chemical substances, the presence of which is associated to neonatal pathologies [Olshan AF 2005), as well as measuring the presence of these substances on blood spot . In fact, it has been demonstrated that the residual blood spots provide biological material that is suitable and sufficient for the identification and/or quantification of markers of exposure (heavy metals, residues of pesticides or industrial activity, endocrine disruptors in general) potentially involved in the etiopathogenesis of some diseases [Olshan AF 2007]. Using bidimensional chromatography and tandem mass spectrometry of atomic absorption, in studies of environmental research it is possible to also document traces of metals, in order to quantify harmful prenatal exposures happened even many years earlier, with negative effects on health (Olshan 2007; Chaudury, 2009).

Finally, on blood spots preserved at low temperatures it is possible to detect protein markers or infective agents (Croom 2006, McfDade 2007) with microanalytical techniques that use volumes less than 100 nl (Philips 2001).

In conclusion, it is important to stress the great importance that such studies can have, due to the fact that the high number of residual samples allows both the assessment of a population's exposure to a certain substance, as well as the assessment of the exposure's temporal trend by analysing, for a sufficiently representative observation period, the blood spots of newborns coming from the same geographic area [Spliethoff HM 2008].

3) International situation of the long-term storage of residual screening DBS

Because of its characteristics, residual screening DBS representsan extremely important biological resource for its possible use in biomedicine, toxicology and forensics. There is therefore a growing

awareness of the potential of this material also for new research purposes. This leads to an increasing focus of the scientific community and a tendency to realise projects and structures for the long-term preservation of such a material. However, at present, administrative and legal procedures for residual DBS storage are not yet defined or, if they exist, are very dissimilar in different Nations. In New Zealand and in some of the US States, like North Carolina and Michigan, the cards are stored for an indefinite time [www.nsu.govt.nz/index.asp]; in the United Kingdom the preservation time varies from county to county (from 2 months to an indeterminate time), even though on average the cards are preserved for 5 years [http://newbornbloodspot.screening.nhs.uk/]; also in Australia the preservation time varies from region to region (from 2 years in Western Australia to an indeterminate time in the State of Victoria) [www.genetics.edu.au]; in France they are stored for at least 1 year, in Holland for 5 years [Couzin-Frankel 2009], in Germany for at least 3 months up to 5 years [www.screening-dgns.de/index.php].

In a much diversified international situation, the oldest and broadest experience of residual screening DBS storage is represented by the national Danish experience with the creation, in 1982, of the *"Danish Newborn Screening Biobank"*, which currently preserves about 1.8 millions of neonatal samples. In this biobank, all the samples are linked to a database represented by a National Register *(Central Personal Register)*, which identifies the subjects through a distinctive code and which, linking to other national pathology registers, allows finding information on the occurrence of a variety of diseases. Through the years, this system has allowed the realisation of numerous etiologic and epidemiologic studies, after the approval by an ad hoc ethics and scientific committee (Steering Committee for Scientific Use of the NBS-Biobank), [Norgaard-Pedersen 2007]. It is important to stress that in the *"Danish Newborn Screening Biobank"* the parents authorise the preservation of residual screening DBS. In fact, before the collection of the sample (which is taken between the fifth and seventh day of life), parents are informed by appointed healthcare personnel about the screening programme and the aims of long-term residual DBS storage. These essentially regard the possibility of using biological samples after the screening to (in order of importance) a) repeat the screening test and for their subsequent diagnostic use in childhood, b) ensure quality and the improvement of the methods of analysis, and c) use specimens for research purposes. Once they have received this information, parents can choose whether to sign the authorisation for the preservation of the samples at the moment of screening, or whether to express their consent afterwards.

With regards to the release by parents of an authorisation for the long-term preservation of residual DBS, there is not much information available and – in any case – the position of the various Countries about this seems highly diversified (Table 1). For example, in Australia or in the United Kingdom screening programmes do not require the release of a parental consent for residual DBS storage. Differently in Holland the cards are preserved for 5 years and parents are offered the choice of signing a "non-assent" for the long-term preservation *(opting out)*. Finally, in New Zealand a new recommendation proposal has recently been issued by the local Health Ministry, which anticipates a preservation time of at least 16 years, with a parental consent [NSU, 2008].

From what we have said so far, it seems clear that there is a growing trend towards the long-term preservation of such material. This has led the *American College of Medical Genetics*, in April 2009, to issue their own recommendations for the long-term preservation of more than four millions of cards concerning newborns screened every year, as residual material of neonatal screening in the USA [www.acmg.net].

Table 1. International situation of the long-term preservation of residual material from screening tests

COUNTRY	PRESERVATION TIME	PARENTAL CONSENT TO LONG-TERM PRESERVATION	PARENTAL NO-CONSENT TO LONG-TERM PRESERVATION (*opting out*)
Denmark[1]	Indeterminate time	**YES**	NO
New Zealand[2]	Indeterminate time (at least 16 years)	**YES**	NO
North Carolina[3]	Indeterminate time	NO	NO
Michigan[3]	Indeterminate time	NO	NO
UK[4]	variable time depending on the county (on average 5 years)	NO	NO
Australia[2]	variable time depending on the region (2 months-indeterminate time)	NO	NO
Germany[2,5]	variable time depending on the region (3 months – 5 years)	Information unavailable	Information unavailable
Italy[6]	variable time depending on the region	NO	NO
Israel[2]	1 month	Information unavailable	Information unavailable
France[3]	At least 1 year	Information unavailable	Information unavailable
Holland[3]	5 years	NO	**YES**

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2. www.nsu.govt.nz/index.asp
3. Science 2009, 324:166
4. http://newbornbloodspot.screening.nhs.uk/
5. www.screening-dgns.de/index.php
6. www.sismme.it/sisn

4) Current situation on residual screening DBS in Italy

Even though it is consolidated practice in many Screening Centres to preserve the residual screening DBS for long time, at present there is neither a specific national regulation, or national or regional guidelines to which managers of Regional Screening Centres can refer for what concerns methods to perform long-term storage of residual DBS.

In 2006, the Italian Society of Neonatal Screening (SISN) proposed the preservation of the cards for a post-screening period of at least 5 years [SISN Technical Report 2007]. This recommendation was issued after carrying out, in the same year, a first nation-wide survey to document, on the basis of the statements made by the Managers of each Screening Centre, what really happened in the various Italian Centres with regards to this issue. On 26 Centres consulted, the results were the following:

1 Centre destroyed samples immediately after the screening test
4 Centres preserved samples for 1-2 years (in 2 cases due to company policies)
12 Centres preserved samples up to 5 years after the test
3 Centres preserved samples up to 10 years
6 Centres preserved for an indeterminate time

In the same year the SISN carried out a further consultation of the Centres to verify, amongst other things, how many required an informed consent from the parents to authorise the long-term preservation of the cards. On 15 Centres answering the questionnaire, it was found that not one required an informed consent from the parents, or clearly declared that the residual material would be preserved for a long period of time.

In consideration of the situation described above, also in Italy – where there is a programme of neonatal screening that provides total coverage of newborn population (about 580,000 a year), it seems necessary to start an evaluation and a broad discussion about the possibility to realise the long-term storage of residual screening DBS, with the aim of finding solutions that tackle ethical and practical-management issues and leading to the availability of a biological legacy, which is a unique instrument for scientific research and protection of public health.

5) Ethical-legal issues linked to the long-term storage of residual screening DBS

The long-term storage of residual screening DBS inevitably involves the need to find solutions for many ethical, legal and practical problems. As an example, we list some of them, which are currently the object of discussion in the scientific world.

The criteria to adopt, in any case, must conform to the general guidelines promoted by the Rec(2006) 4 of the Council of Europe (SEE NOTE[4]).

5.1 Protection of privacy

The cards are not anonymous, in fact they hold a record of the name, surname, some essential neonatal characteristics, address and town

[4] Indicatively, we report what anticipated in chapter IV "Collection of biological materials" in the abovementioned REC (2006) 4 of the Council of Europe.

CHAPTER IV

Article 14- Principles applicable to all collections of biological materials.

1. The person and/or institution responsible for the collection should be designated.

2. The purpose(s) of a collection should be specified. The principles of transparency and accountability should govern its management including access to and use and transfer of its biological materials and disclosure of information.

3. Each sample of biological material in the collection should be appropriately document, including information on any relevant consent or authorisation.

4. Clear conditions governing access to, and use of, the samples should be established.

5. Quality assurance measures should be in place, including conditions to ensure security and confidentiality during storage and handling of the biological materials.

Article 15 – Right to change the scope of, or to withdraw, consent or authorisation

1) When a person has provided consent to storage of identifiable biological materials for research purposes , the person retain the right to withdraw or alter the scope of that consent. The withdrawal or alteration of consent should not lead to any form of discrimination against the person concerned, in particular regarding the right to medical care.

When identifiable biological materials are stored for research purposes only, the person who has withdrawn consent should have the right to have, in the manner foreseen by national law, the materials either destroyed or rendered unlinked anonymised.

2. where authorisation has been given on behalf of a person not able to consent , the representative, authority, person or body provided for by law should have the rights referred to in paragraph 1 above.

3. Where a person on whose behalf authorisation has been given attains the capacity to give attains the capacity to give consent, that person should have the rights referred to in paragraph 1 above.

Article 16 – Transborder flows

Biological materials and associated personal data should only be transferred to another state ensures an adequate level of protection.

of residence, so that the parents can be contacted in case of positive screening.

In the case of the long-term storage of residual DBS, one of the main problems to tackle concerns the procedures to follow in order to guarantee the protection of the patient's privacy during the more or less prolonged time of storage.

Another matter about which scientific community and ethics committees in the whole world are questioning themselves, regards the issue of whether residual DBS must be made *completely anonymous* before their use in subsequent scientific research, or whether it is preferable to assign them a code which, although it does not allow an immediate recognition of the patient, still makes it possible to find him/her for the eventual communication of future research results (*temporarily anonymised cards*).

This aspect links to another important problem, that is, the need to respect the *right to know or not to know the outcome of the genetic test or of any other research aimed at defining/assessing the individual risk of a certain pathology.*

5.2 Information, Authorisation and Informed consent

The National Bioethics Committee, together with the National Committee for Biosafety, Biotechnologies and Life Sciences, through the drawing up of specific documents, have already highlighted the importance of residual screening DBS in biobanks for research purposes. These biobanks , with clinical information of each individual, represent a precious tool to highlight various aspects of clinical condition at birth comparing it after a few years with the current one , or the individual variability of genetic patterns within a certain population, or the effects of environmental factors, or other parameters to improve healthcare and cures . (NCBBLS 21/11/2008; NBC 9/6/2006; NCBBLS 19/4/2006).

These opportunities must be considered, in a cost/benefit evaluation of starting a system of long-term preservation of the samples, as one of the most convincing reasons in favour of long-term storage.

More recently the two Committees, stressing the concept that the biological samples belong to the donor who gives them with the general formula of "concession for use", and confirming in any case the principle of the free donation and the prohibition of personal discrimination, created a detailed and exhaustive model of informed consent. This guarantees a suitable instrument for protection of individual rights, promotes at the same time personal interest and collective interest [NBC, NCBBLS: "Collection of biological samples for research purposes: informed consent", 16/02/2009].

This model could be used also in the case of the long-term preservation of residual screening DBS. This procedure, as shown by the international experience matured in these last few years, has particular relevance for the healthcare of minors in the hypothesis of unforeseen pathologies (for example in the first 2-5 years of life) so much so that it is compulsory in many States; but long-term preservation is also extremely useful for the establishment of biobanks that could be of considerable help to the development of scientific research. It must be considered that taking the blood sample and carrying out the tests at birth is compulsory by law and it is therefore sufficient to give parents information that explains the reasons for the legal measure relating to the newborn's healthcare with regards to screened diseases, which is potentially valid for the first two years. Different is the case of the long-term preservation for diagnostic purposes that are not included in the screening, although they relate to the interest of the minor or to research purposes. In this hypothesis, for the principles already expressed in the abovementioned NBC and NCBBLS Documents, an explicit written authorisation must be given by the minor's legal representatives, which details the aims for allowing the "concession for use" of the biological material and the duration of the conservation period. Besides the protection of privacy and of all the guarantees imposed by the anonymisation, it must be ensured the possibility of checking the correct management of the preserved samples and the use of the relative information, and the right to withdraw, at any moment and for any reason, the authorisation given initially.

CONCLUSIONS

Before moving on to illustrate, in this document's Appendix, a hypothesis which can bring about the realisation of what has been explained so far, it seems appropriate to formulate some conclusions:

1) The need to overcome some current organisational problems of neonatal screening in Italy.

As highlighted in the annual reports of the Italian Society of Neonatal Screening – SISN, which is part today of the new SIMMESN Scientific Society (Italian Society for the Study of Hereditary Metabolic Diseases and Neonatal Screening), in our Country there are 32 active Centres of Neonatal Screening, with a very diversified pool of patients according to area size (Sub-regional, Regional, Inter-regional) and number of samples (from < 5,000 born/year to > 100,000 born/year),

which at the moment carry out different regional programmes, according to the local organisation, defined by the competent regional Authority.

Although recognising that the current organisation is an important tool of secondary preventive medicine, essential for children's healthcare, we cannot avoid stressing the strong dishomogeneity of the regional models of screening applied today. These, for the profound differences in the composition of the panel of screened pathologies, create in the Italian neonatal population (in possible violation of the universal criterion of the equality of all citizens) macroscopic differences with regards to healthcare, which is in effect mainly diversified by the newborn's place of birth.

It is also important to stress how the careful reading of the scientific documentation issued by the SISN-SISMME Society, highlights also a considerable, further dishomogeneity due to the organisation and technical management of each single regional screening programme, with numerous indicators (catchment area, cut-off values, recall index, analytical strategies of selection, efficiency index, etc.) which present a discretional variability that is not always justified by scientific evidence or by strictly economic-management reasons.

2) In this context, we must consider the regulations for the compulsory short-term/medium-term preservation of the samples, in the newborn's medical – legal interest.
With regards to this, the considerations expressed at various points in the text are still valid. According to these a positive cost/benefit coefficient – identifiable in the reduced cost of the preservation for two years – seems sufficient to meet the ethico-legal requirements (maybe even prevalent compared to the medico-diagnostic requirements) that the development of children's rights – also coherent with the fundamental framework of the right to health fixed in art. 32 of the national Constitution and promoted by solemn international Declarations – has by now "matured", not only in Italy but in various Countries, with concrete realisations, which has also happened in Italy for the spontaneous action of the managers and administrators of the activated Screening Centres.

3) The establishment of long-term collections of residual material from compulsory neonatal screening, to be used for research activities and to be organised following already identified international regulations – for example by the Council of Europe, the European Union or other international Institutions (see previous note 4) – must be considered in the opinion expressed by the NBC and by the NCBBLS – now "mature" from the point of view not only of an international but also Italian development of the regulation. These collections will have to be disciplined with

4) We deem appropriate to safeguard, with adequate regulations and whilst waiting for the solutions anticipated for the new acquisitions of neonatal biological material – the current collections present in some neonatal screening Centres to avoid their deterioration or destruction.
This measure seems now urgent, at least for some of the centres concerned.

5) In the NBC and NCBBLS opinion particular care should be reserved to occasions of periodical training of the staff operating in the various centres, according to initiatives of the competent Regions, with regards to not only the "technical" care of the samples (correct collection, correct use, appropriate preservation, etc.), but also of the rights of the newborn, child and family, inherent to appropriate information, confidentiality data protection and privacy**.**

APPENDIX

REFLECTIONS ON THE REORGANISATION OF NEONATAL SCREENING CENTRES AND THE INSTITUTION AND COORDINATION OF A NETWORK OF VOLUNTARY LONG-TERM COLLECTIONS OF RESIDUAL DOT BLOD SPOTS FROM COMPULSORY NEONATAL SCREENING

1) *Premise*

Without any expectation of elaborating models for a legal regulation, which is outside of the institutional duty of the NBC and of the NCBBLS, the two Committees feel that it is however appropriate to present some of the reflections – carried out during this work – regarding the organisational problems for the creation of a network aimed at the long-term storage of residual dot blood spots (DBS) from neonatal screening.

2) *Practical-management issues*

Not less important than the ethical aspects, described in the Document under examination, are the practical/management and economic problems that the Screening Centres have to tackle to guarantee the correct preservation, especially long-term, of residual DBS. With regards to this, we must take into account that in our Country *not all Screening Centres have "facilities" that are in line with the regulations in force and* such that they can guarantee the possibility of preserving, adequately and for a long time, of a high number of cards. The widespread and old technico-structural problems of the Centres, therefore, could be a practical impediment which cannot be overlooked for the realisation, in the short-medium term, of a nation-wide long-term DBS storage.
Another practical problem to consider is the *high number of Screening Centres found on the national territory* which, for the reasons mentioned above and for the small size of some of them, could not make it easy to carry out the adequate procedure necessary for the long-term preservation of the cards.
Finally, we must remember that, even in the presence of national scientific documents issued by the SISN and the SISMME for neonatal screening and the follow-up of genetic hyperphenylalaninemia, congenital hypothyroidism, congenital adrenal hyperplasia and for an extensive neonatal screening [Balsamo 1998, Antonozzi 2000, Romano 1997, SISMME-SISN 2008], at present there is not a uniform organisational vision for screening centres. In fact these use different models and formats of cards for neonatal blood collection, with a

typology of demographic and health data collection that is variable from centre to centre and sometimes not completely in line with the guidelines found in the DPCM 9/7/1999. This document represents the only one which provides organisational indications for carrying out nation-wide screening programmes. This dissimilarity causes a complete dishomogeneity in the management of residual DBS storage, in the typology of registration and care both of the samples themselves as well as the demographic and health data linked to them, and finally in the IT systems for laboratory management.

3) *Recommendations of a national framework of reference for the establishment and coordination of biobanks with the aim of developing a network of long-term collections of residual neonatal DBS*

Already in the National Healthcare Plan (NHP) 2006-2008, then used as reference in the State-Regions Agreement Conference on the 25th of March 2009, the implementation and coordination of "biobanks" was identified as a priority being one of the objectives to reach in order to have the constitutional guarantee of the right to health and other social and civil rights in healthcare.[5]

Given the relevance of such an objective and recognising in the creation of biobanks a determining factor for the development of care and research, this year the Ministry of Health – within the activity programme of the National Centre for the Prevention and Control of Diseases (CCM), which the Regions had an active role in defining – promoted support for the coordination and integration of *population biobanks* by backing strategic projects of national interest and identifying in them an important resource of diagnosis and research.

Therefore, the creation of a biobank for research purposes, collecting residual DBS from neonatal screening (otherwise destined for destruction) deriving from and representative of the Italian neonatal population (*neonatal biobank*), would fully fit within the main objectives of the Ministry of Health and the Regions, and would allow for the transformation of the Screening Centres, which in many cases already carry out the long-term storage of residual DBS from neonatal screening

[5] As stated in the aforesaid document, *"biobanks are units of service within public or private healthcare establishments, with no direct profit-making aims, finalised to the collection, manipulation, preservation, storage and distribution of human biological material, for the purpose of diagnostic investigation, research and therapeutic use… In healthcare there can be two different typologies of biobank:*

1. ***Biobanks** of human biological material used for diagnosis, studies on biodiversity and **research;***
2. ***Biobanks for therapeutic purposes** in which the biological material preserved is destined to applications on man."*

in the absence of definite regulations and operative homogeneity, in a *National Network of Regional Neonatal Biobanks*. These should be able to preserve a high number of samples in a standardised manner and make them available for diagnosis and research with uniform and shared methods.
The realisation of such a project necessarily implies achieving the following objectives:

1. Regulate the methods of preservation and use, for research purposes, of residual material from neonatal screening;
2. Define the elements of the Network in number not over one per Region*, verifying that each of them operates according to standardised criteria both in terms of the acquisition and donation of the samples, and in terms of quality and safety;
3. Define a *governance* system of the Network that identifies in the competent authorities (Ministry of Health and Istituto Superiore di Sanita') coordinating bodies for the collection, preservation and use of the residual material from neonatal screening, and for assessing whether the objectives have been achieved and aimed at public healthcare, thanks to the use of a biological inheritance that is extremely precious from the point of view of the potential for research.

We certainly do not hide the need to assess the realisation of such a project also from the point of view of the costs/benefits.
With regards to the benefits, real in the short-term and potential in the long-term, exhaustive information has already been given in the text of the document. However, we do not have at the moment an overall assessment of the costs for the current activity of the various screening Centres in the various Regions. Waiting for the anticipated communication of such data (see note[6]), we must however remember that the start of long-term preservation in the form of a national Network of regional neonatal biobanks should not be particularly expensive because the storage of residual DBS is performed at room temperature with a low humidity. Some data, communicated by Georges Dagher (georges.dagher@inserm.fr) in the preparatory phase of the project of European infrastructure *Biobanking and Biomolecular Research* – data presented informally at the *Working Group 7 Funding* – highlighted that the expenses of 73 European biobanks (20 Italian, 24 French, 7 German, 17 Dutch) reach an average cost of 440,000 euro per year, with 44% of the costs due to personnel, 17% to the annotation of the samples, 8% to functioning. These are, however, biobanks preserving samples in freezers

[6] The data for the Piedmont Region is currently available, and it indicates the average cost for the storing, preservation and study of some analytes of each biological sample to be around 40-50 euros.

or liquid nitrogen; consequently the cost of preserving the Guthrie spots at room temperature can be estimated to be less than a tenth of that of a traditional biobank.

4) *Particular aspects of the establishment, coordination and management of a national network of regional neonatal biobanks for the storage of residual DBS from neonatal screening.*

a) The National Network of Regional Neonatal Biobanks will have, as joint partners, the Istituto Superiore di Sanita' and the Regional Screening Centres identified by the competent regional authorities as structures that are suitable to become the location of the preservation of the residual material from neonatal screening on the basis of minimum requirements. The Istituto Superiore di Sanita' could be the main institution coordinating the Network and making itself available, where necessary, to preserve the biological material coming from those Centres that, due to intrinsic structural deficiencies are non adequate for the long-term preservation of the residual material from neonatal screening, or for those Centres that opt for a voluntary centralisation, in line with regional authorities.

b) In order to guarantee the protection of privacy for the newborn and his/her family, but also in order to use neonatal information collected in the Italian Screening Centres, and to link the biological samples to possibly existing pathologies registers , the cards will have to be used in *anonymised* form. In this case the Screening Centres, which will be the keepers of neonatal data that will be preserved in line with the regulations on the protection of personal data, will give a code to each sample that in this identification form will be preserved in the biobank.

c) The long-term preservation of the cards, in any case, should be carried out on the basis of definite organisational and management requirements. In addition, the long-term preservation of the biological samples should guarantee the availability of a minimum amount of residual material for the newborn's parents or guardians, who will be able to request it at any moment. Furthermore, the will of the parent who, at any moment, wants to withdraw the authorisation and ask that the donated sample is destroyed, is protected.

d) The preservation time will be established at the technical table discussed in the following point i) for the purpose of:

1) Fixing a compulsory preservation time needed to ensure the direct aim of the newborn's healthcare (for example repeating tests, complementary tests made necessary by the clinical history, medical-legal needs, etc…), valid for all centres.

2) Fixing the maximum preservation time for those centres that adhere to programmes of long-term preservation for biomedical research. For example, in order to allow the realisation of studies for the research of biomarkers pathologies with late onset.

e) The establishment of the prospective biobank should happen with the donation of the biological sample by the parents. In order for the donation to happen in full awareness of it, the parents should also be offered an appropriate oral information and an explicative note on the importance of long-term preservation, which should include the forms necessary to give their consent to the long-term preservation and the use of their child's biological material for research purposes (see Appendix 3).

f) Appropriate operative protocols, which are drawn also with the contribution of experts operating in the field of neonatal screening, will define unambiguous methods and procedures to follow nationally for the long-term preservation of the residual material of screening, including the methods to exercise the right to withdraw for motivated reasons (or for the transferral to another suitable healthcare establishment for analysis) the residual sample, or for the agreed end of the preservation or when the sample's owner has reached full decision-making capabilities.

g) It seems useful, in addition, to create an interdisciplinary scientific Coordination Group for the biobank, which will establish the priorities for the use of the cards and the most important research plans on the basis of agreed criteria.

h) With regards to the already existing collections found in many Italian Screening Centres, which gather biological material without parental consent, it is necessary that this material is used exclusively in an "*anonymised*" form, that is, with the impossibility of finding out the data of the individual who donated the biological sample. This practice will allow the realisation of broad epidemiological studies of social and healthcare relevance

i) We recommend the institution of a Technical Table at the Ministry of Health, aimed at monitoring the activities linked to the coordination, by the Istituto Superiore di Sanita', of the national network of regional biobanks, and to establish the methods for researchers to access previous collections or those implemented with new acquisitions, and finally to decide what else is necessary for the correct realisation of the project.

j) Finally, it seems appropriate to have an adequate probationary period entrusted to a much smaller number of Regions, for the "trial" of the agreed procedure.

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