



Guidelines for newborn screening of primary immunodeficiency diseases

Stephan Borte^{a,b,c}, Ulrika von Döbeln^d, and Lennart Hammarström^a

Purpose of review

Technical possibilities to screen for inborn errors of immune function at the neonatal stage have been rapidly progressing, whereas the guidelines that apply for the evaluation of benefits and concerns on expanding screening panels have not been broadly discussed for primary immunodeficiency diseases (PID). This review reflects on the assessment of severe combined immunodeficiencies (SCID), primary agammaglobulinaemias (such as X-linked agammaglobulinaemia) and inherited haemophagocytic syndromes (such as familial haemophagocytic lymphohistiocytosis) to be included in newborn screening (NBS) programmes.

Recent findings

Screening programmes in several federal states in the United States have been supplemented with the T-cell receptor excision circle assay during the past few years to identify children with SCID. The reported experience indicates that an efficient and validated screening approach for SCID is feasible on a population-based scale.

Summary

In the light of recent advances, severe PID ought to be discussed for their rapid implementation in national NBS programmes based upon clinical, social and economical criteria as consolidated in the extended 22-item Wilson–Jungner framework. Although SCID currently most favourably fulfils these screening guidelines, other strong candidates can be identified among primary immunodeficiency disorders. Future efforts of healthcare professionals and policy makers are essential to improve the concept of neonatal screening for PID.

Keywords

newborn screening, primary immunodeficiency diseases, severe combined immunodeficiency, Wilson–Jungner criteria, X-linked agammaglobulinaemia

INTRODUCTION

Primary immunodeficiency diseases (PID) represent a heterogeneous group of inborn defects of protective immunity that result in recurrent and severe infectious complications, and, furthermore, predispose to autoimmunity, inflammation and malignancy. Most PID can be characterized by a functional impairment of the tightly regulated capacity of leukocytes to clear infections, yet some disorders are due to limited differentiation of cells of the lymphoid lineage or increased lymphocyte apoptosis [1].

To date, more than 18 monogenetic defects have been identified that cause severe combined immunodeficiency (SCID), a paediatric emergency that originates from the life-threatening consequences of absent T cells, as well as B and natural killer cells in some patients [2]. Most newborns with SCID appear healthy at birth and remain clinically ‘silent’

during the first months of life until the inborn inability to develop T lymphocytes cannot be compensated by antibody-mediated maternal

^aDivision of Clinical Immunology and Transfusion Medicine, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital Huddinge, Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiencies, Stockholm, Sweden, ^bTranslational Centre for Regenerative Medicine (TRM), University of Leipzig, Leipzig, Germany, ^cImmunoDeficiencyCenter Leipzig (IDCL), Hospital St. Georg gGmbH Leipzig, Jeffrey Modell Diagnostic and Research Center for Primary Immunodeficiencies Leipzig, Leipzig, Germany and ^dDivision of Metabolic Diseases, Department of Laboratory Medicine, Karolinska Institutet, Karolinska University Hospital Solna, Stockholm, Sweden

Correspondence to Stephan Borte, Division of Clinical Immunology, Karolinska Institutet, Karolinska University Hospital, Huddinge, Stockholm SE-14186, Sweden. Tel: +46 8 5248 3586; fax: +46 8 5248 3588; e-mail: stephan.borte@ki.se

Curr Opin Hematol 2012, 19:000–000

DOI:10.1097/MOH.0b013e32835a9130

KEY POINTS

- Severe primary immunodeficiencies such as SCID, XLA and familial haemophagocytic lymphohistiocytosis fulfil clinical, therapeutic, social and economic criteria that should motivate serious evaluation of these disorders for implementation in population-based NBS programmes.
- The 22-item extended Wilson–Jungner framework of guidelines for mass screening provides a reliable basis for healthcare professionals, health insurers and policy makers when contemplating expansion of existing screening panels.
- Implementation of SCID into regular NBS programmes is favourably judged upon the extended Wilson–Jungner framework as well as the practical experience gained during the past few years in large-scale trials in the USA.

immunity. The most favourable overall outcome in SCID patients is achieved by curative haematopoietic stem cell transplantation (HSCT) or gene therapy, initiated before overwhelming infectious complications occur [3[¶]]. The clinical severity and the associated economic burden of a delayed diagnosis, as well as the potentially fatal outcome if not treated promptly, emphasize the importance of early identification of the affected newborns.

Although SCID represents one candidate group of disorders that features a profile particularly well fitted to be included in prospective newborn screening (NBS) programmes on a population-based scale, other severe PID may also fulfil the requirements for NBS. The rationale to evaluate other PID than SCID for inclusion in NBS trials is mainly motivated by: the clinical severity of the disease following the latent stage; the existence of a therapeutic consensus, at best of curative nature; a beneficial overall prognosis if diagnosed and treated early; and the cost-effectiveness of the screening approach in consideration of the incidence and the follow-up costs of late-diagnosed patients. Of the PID putatively fulfilling those criteria, familial haemophagocytic lymphohistiocytosis and primary agammaglobulinaemias, such as Bruton's disease (X-linked agammaglobulinaemia; XLA), have already been suggested as screening candidates [4[¶],5[¶],6].

HOW TO EVALUATE DISORDERS TO BE INTRODUCED TO NEWBORN SCREENING

Mass screening of newborn infants started in the early 1960s based on a method developed by Robert Guthrie and Ada Susi [7] for the screening of phenylketonuria. Peripheral blood from a heel stick was

blotted on filter paper at 2–5 days after birth, dried and sent by postal mail to centralized laboratories for analysis. Once this sampling procedure was established, new disorders were considered for NBS and it became necessary to create guidelines to help in deciding which diseases were suitable for a population-based screening. A conference arranged by the WHO, originally intended to discuss screening for cancer in adults, resulted in an article written by Wilson and Jungner [8] describing 10 criteria to be considered when a new disorder is proposed for mass screening.

The addition of new diseases in neonatal screening programmes has been critically determined by the development of methods suitable for the analysis of large numbers of samples up until the mid-1990s. The technological progress since then, allowing simultaneous determination of several hundreds of metabolites in dried blood spot samples (DBSS) from filter paper, together with novel techniques for the analysis of nucleic acids (DNA and RNA), revolutionized NBS. However, with these breakthrough technologies being broadly available, the choice of disorders to be included in NBS has to be carefully scrutinized with respect to cost–benefit considerations, the patient perspectives and social acceptance. The complex of problems associated with this process is illustrated by differing decisions made in different countries.

In the USA, the congress decided in 2003 to include 29 core and 25 secondary disorders in the NBS programme, adding up to a total of 54 inborn diseases that are screened for in newborns. This decision was motivated by thorough investigations, resulting in a priority list of disorders suitable for neonatal screening [9]. In recent years, more disorders have been included in this list of recommended diseases to be screened all over the United States, one of them being SCID [10]. At the other extreme, a health technology assessment performed in Great Britain in 2004 came to the conclusion that a substantial benefit of neonatal screening, based on the multianalyte technology, could only be shown for two disorders [11]. Thus, only five disorders are currently included in the general NBS programme in Great Britain. A recent investigation of the various approaches towards NBS evaluation in different European countries illustrates the considerable heterogeneity of the included disorders, as well as the diversity of the organizational structure of the NBS programmes [12[¶]]. Even given that different concepts for NBS evaluation exist, most countries require compliance of the proposed diseases with the original Wilson–Jungner criteria for mass screening, consisting of the following 10 items:

- (1) The condition sought should be an important health problem.
- (2) There should be an accepted treatment for patients with recognized diseases.
- (3) Facilities for diagnosis and treatment should be available.
- (4) There should be a recognizable latent or early symptomatic stage.
- (5) There should be a suitable test or examination.
- (6) The test should be acceptable to the population.
- (7) The natural history of the condition, including development from latent to declared disease, should be adequately understood.
- (8) There should be an agreed policy on whom to treat.
- (9) The cost of case finding (including diagnosis) should be economically balanced in relation to possible expenditure on medical healthcare as a whole.
- (10) Case finding should be a continuing process and not a 'once and for all' project.
- (21) Treatment risks and the impact of a false-positive test are explained to parents/guardians.
- (22) The limitations of screening and risks of a false-negative test are explained to parents/guardians.

To demonstrate the practical guidance that is given by the extended Wilson–Jungner framework, a brief evaluation of two severe PID, currently not included in NBS programmes, is shown in Table 1 [5[•],15–18], whereas the setting for SCID is discussed in more detail in the sections below.

SEVERE COMBINED IMMUNODEFICIENCY IS A LIFE-THREATENING GROUP OF DISORDERS, WHICH OUGHT TO BE INCLUDED IN NEONATAL SCREENING

SCID represent an important health problem as children born with these disorders lack cellular and, in some cases, humoral immunity and are prone to recurrent severe infections. Representing the most severe form of inherited primary immunodeficiencies, SCID is considered a life-threatening paediatric emergency. Although the condition is rare, precise estimates of its frequency based on large population studies are lacking. Early data from pilot screening programmes in the USA suggest that one in 30 000–50 000 infants may be born with SCID, whereas data from Turkey, Saudi Arabia and Kuwait indicate higher rates [19[•]]. Although incidence rates are not strictly part of the extended Wilson–Jungner evaluation framework, SCID may well be detected at frequencies similar to disorders of fatty acid or amino acid metabolism that are screened for in numerous national NBS programmes.

The benefit of a neonatal screening for SCID is best exemplified by the extremely effective treatment for SCID, with approximately 95% survival following HSCT when transplantation is performed before 3.5 months of age [20]. For some patients, gene therapy is an option when a suitable HSCT donor is not available. However, if SCID is not screened for at birth, patients will suffer from recurrent infections and the survival is severely compromised [3[•]]. Furthermore, following onset of infectious periods, the pharmacological preparation for stem cell transplantation has to be adjusted and becomes more difficult, often resulting in less successful engraftment. Thus, early intervention not only reduces the disease-related mortality and morbidity but is also curative, in contrast to the dietary therapeutic approaches that have to be taken in the metabolic diseases currently screened for.

The heterogeneity of the molecular basis and inheritance modes of SCID has prompted the

As the framework of the Wilson–Jungner criteria was not strictly designed to fit the demands of a neonatal screening evaluation, several countries have made more specific amendments [12[•]]. Moreover, to improve the ability of decision makers and public health practitioners to stratify disease-specific aspects in the context of an NBS setting, it was suggested to expand the Wilson–Jungner framework by the following supplemental items [13,14^{••}]:

- (11) There should be scientific evidence of screening programme effectiveness and the benefits of screening should be shown to outweigh the harm.
- (12) The test may be multiplexed or overlaid onto an existing structure or system.
- (13) The 'diagnostic odyssey' for the patient/family may be reduced or eliminated.
- (14) Adverse outcome(s) are rare with a false-positive test.
- (15) Treatment costs may be covered by third parties (either private or public).
- (16) Testing may be declined by parents/guardians.
- (17) Adequate pretesting information or counselling is available to parents/guardians.
- (18) Screening in the newborn period is critical for prompt diagnosis and treatment.
- (19) Public health infrastructure is in place to support all phases of the testing, diagnosis and interventions.
- (20) If carriers are identified, genetic counselling is provided.

Table 1. Evaluation of severe primary immunodeficiency diseases based on the extended Wilson ± Jungner framework

Criteria advocating newborn screening	Agammaglobulinaemias (e.g. Bruton's disease)		Familial haemophagocytic lymphohistiocytosis	
	Scientific evidence available	Uncertain or inconclusive	Scientific evidence available	Uncertain or inconclusive
1	Important health problem	X [15]	X [16]	
2	Accepted treatment	X [17]	X [16]	
3	Facilities for diagnosis and treatment exist	X [18]	X [18]	
4	Recognizable latent stage	X [5*]		X
5	Suitable test system available	X [5*]		X
6	Test acceptable to the population		X	X
7	Natural disease history understood	X [15]		X [16]
8	Agreed policy on whom to treat	X [15]		X
9	Cost of case finding is economically balanced		X	X
10	Case finding is a continuous process	X [15]		X [16]
11	Scientific evidence for screening efficacy		X	X
12	Test can be overlaid with existing systems	X [5*]		X
13	Reduced diagnostic odyssey	X [15]		X [16]
14	Adverse outcome rare in false-positives		X	X
15	Treatment costs covered	X		X
16	Testing may be declined	X		X
17	Pretesting information is available	X		X
18	Screening in newborn period is critical		X	X
19	Public health infrastructure existing		X	X
20	Genetic counselling is possible	X		X
21	Treatment risks explainable	X		X
22	Limitations of the screening explainable		X	X

Application of the 22-item extended framework of Wilson–Jungner criteria to advocate agammaglobulinaemias (such as X-linked agammaglobulinaemia; Bruton's disease) and inherited hemophagocytic syndromes (such as familial hemophagocytic lymphohistiocytosis) to be considered for implementation in newborn screening programmes.

establishment of a network of dedicated PID centres in recent decades. As a result of a clinical consensus, all children with SCID will be treated with HSCT or gene therapy according to agreed protocols. As with other disorders included in NBS programmes, there will be single patient cases that have to be discussed with respect to the natural history and the policy of whom to treat. The specialist centres for the diagnosis and treatment, according to current protocols that are updated regularly by the network of specialists themselves, may well serve as the required infrastructure for diagnosis and treatment in population-based NBS programmes. The confirmatory tests for SCID usually comprise differential white blood counts and lymphocyte subset enumeration by flow cytometry, which is a widely established routine method. When a preliminary diagnosis of SCID is made, the genotyping of causative mutations is both debatable for HSCT and helpful to allow genetic counselling [2].

Most children with SCID are clinically silent during the first weeks of life (latency phase), as passively transferred maternal immunoglobulins confer a partial protection against infections [21]. However, as soon as this passive barrier wanes, the lack of endogenous immune development leads to severe infections with opportunistic, viral, fungal and bacterial pathogens. As the pathogenesis of SCID is already fully developed at birth, suitable NBS assays would allow detection of affected children. Diagnosis at birth would prevent the onset of early infections, which can otherwise lead to irreversible organ damage or premature death. Thus, NBS for SCID may well eliminate the diagnostic odyssey for patients and the family. Moreover, in a cohort study of SCID infants it was shown that approximately 35% of patients presenting acutely die at first presentation [3].

There has been a long-standing search for a suitable biomarker for normal T-cell development and for methods that allow NBS for SCID [22]. Newly arising technologies for quantitative DNA analysis in small blood volumes have enabled the development of a validated test that can be used for NBS of DBSS [23]. Although SCID can arise from a variety of genetic defects, there is an abnormality of T-cell development in the thymus in all cases. During normal thymic processing, T cells undergo receptor gene splicing and rearrangement, leading to intracellular accumulation of DNA by-products known as T-cell receptor excision circles (TRECs). As TRECs do not replicate in dividing cells, they are found only in naive T cells that have recently left the thymus. When used in NBS assays, TRECs offer the potential to be a surrogate marker of newborns' capability to produce T cells, which is severely

hampered in SCID patients. Suitable TREC assays are based on DNA extracted from a regular dried blood spot collected at birth and can be determined either by quantitative or end-point PCR. These methods have to be newly introduced in most NBS laboratories, yet they offer the possibility to further expand screening panels by multiplexing. A severe T lymphopenia at birth is, however, not strictly disease-specific for SCID, and other disorders affecting lymphocyte maturation, such as trisomy 21 or DiGeorge syndrome, are partially identified in the TREC assay [6]. In contrast, some leaky, variant or delayed-onset forms of SCID are not detected at birth based on the TREC assay, yet the addition of other screening markers such as κ -deleting recombination excision circles (KRECs) may well help to overcome the diagnostic hurdles for such cases. This is particularly true for SCID patients with hypomorphic mutations in genes of relevance for DNA repair or cellular metabolism, such as in adenosine deaminase (ADA) deficiency. The increase of toxic metabolites might well be tolerated to a certain degree by dividing T cells, whereas B cells seem to be more vulnerable for genomic stress, as exemplified by patients with delayed-onset ADA SCID [24]. Ideally, a combined TREC–KREC testing strategy would be followed by second-tier tests for trisomy 21 and 22q11 microdeletion syndromes (such as DiGeorge syndrome), as depicted in Fig. 1 [5,6].

A number of US states have already adopted SCID NBS, based on the TREC assay only. The success of these programmes so far has led to a nationwide recommendation of NBS for SCID by the US Department of Health and Human Services [10]. In Wisconsin, SCID NBS was initiated with a pilot trial in 2008 [19]. During the past 3 years, 207 696 babies had been screened. Out of these, 72 infants were investigated clinically and 35 had SCID or another primary severe T lymphocytopenia (positive predictive value 48%). In California, more than 500 000 babies have been screened for SCID to date [22]. A total of eight per 10 000 initial samples had to be retested, with the majority being preterm or term infants already medically treated. Fifty infants (one in 10 000) were recalled for clinical investigation and 20 of these were confirmed to continuously feature low T-cell numbers (positive predictive value 40%). Based on the broad experience gained with the TREC assay for SCID screening, parents or guardians can be readily supplied with adequate pretesting information regarding test characteristics, limitations of the screening and treatment risks.

The cost-effectiveness of case finding in SCID screening is likely to be economically balanced in relation to the expenditures on medical healthcare for this group of disorders altogether. As one of the

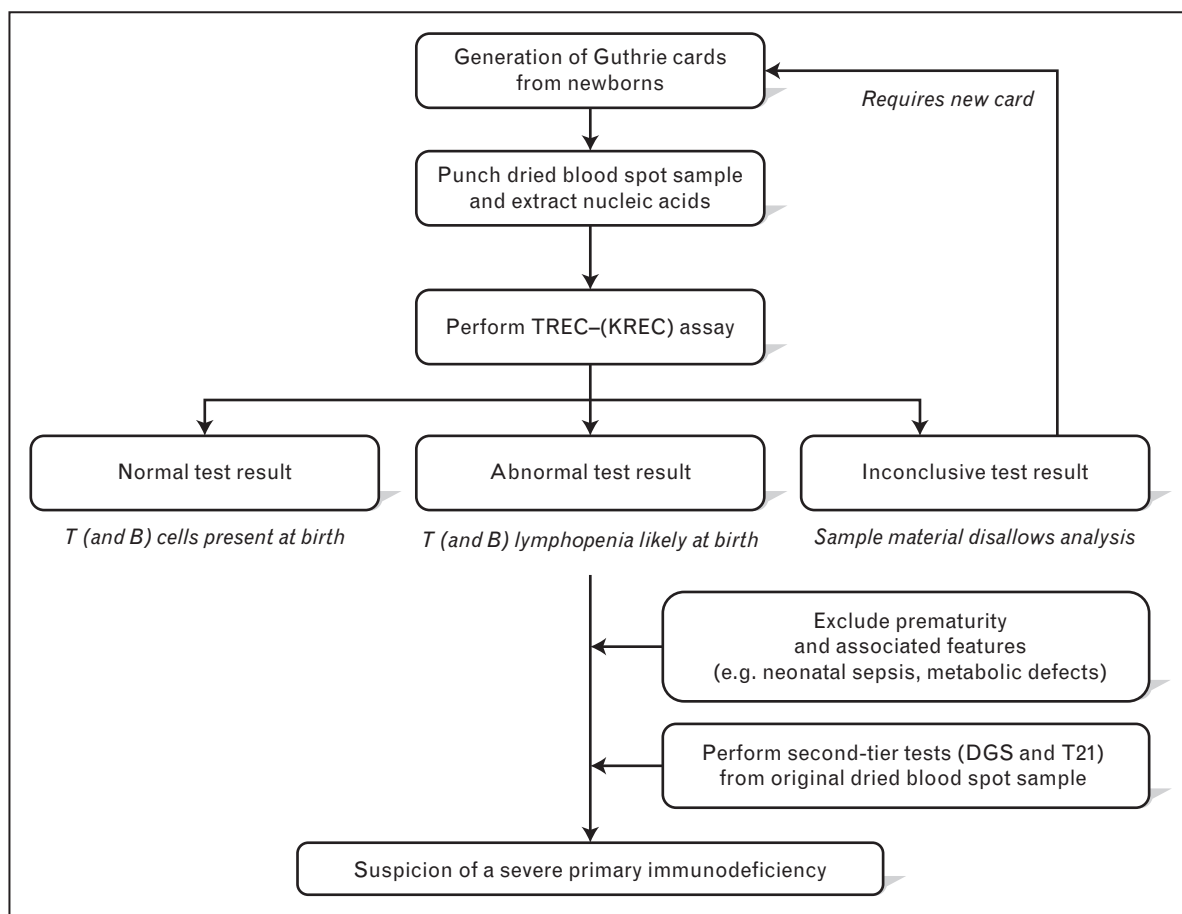


FIGURE 1. Testing strategy for excision circle assays in neonatal screening. Flowchart depicting the testing strategies for results returned by the combined TREC–KREC assay, including second-tier tests for patients with trisomy 21 (T21) or DiGeorge syndrome (DGS), in order to streamline the time to diagnosis for newborns with severe primary immunodeficiency disease.

most important determining factors, detection of SCID at birth allows for a child to be given a curative treatment before complications occur that often require protracted and expensive intensive care management that markedly increases healthcare costs. The rapid access to a therapeutic cure also negates the social burden, tremendous impairment in quality-of-life and the inestimable social costs associated with an undiagnosed SCID. Today, there is no published prospective study providing evidence on the cost-effectiveness of neonatal screening for SCID, yet theoretical calculations have been favourable [25,26].

As the benefits of a screening programme should outweigh the harm, the life-saving nature of an NBS programme for children with SCID is unquestionably of preferential importance. As described above, the performance of the TREC assay is satisfactory for population-based screening. Considering the effects of false-positive screening results on parental stress and the parent–child relationship, a well prepared screening programme

with information to the community, parents-to-be and all the medical staff involved in the diagnosis and treatment of SCID children will diminish these side effects [27].

CONCLUSION

The development of an efficient, reasonably competitive and validated method for a population-based SCID screening of newborns during the past few years has changed the prospect of survival towards a normal life for patients with this severe primary immunodeficiency. Despite a favourable evaluation of SCID upon screening guidelines, national implementation is pending in most countries which have a long-lasting tradition of NBS programmes. However, the paradigm of SCID screening itself will promote future research on preventive medicine for other severe primary immunodeficiencies, including, but not limited to, inherited agammaglobulinaemias and haemophagocytic syndromes.

Acknowledgements

This work was supported in part by the German Federal Ministry of Education and Research (BMBF, PtJ-Bio 0315883), ALF (the regional agreement on medical training and clinical research between Stockholm county council and the Karolinska Institutet), and the Jeffrey Modell Foundation.

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. Al-Herz W, Bousfiha A, Casanova JL, *et al.* Primary immunodeficiency diseases: an update on the classification from the international union of immunological societies expert committee for primary immunodeficiency. *Front Immunol* 2011; 2:54.
 2. Cossu F. Genetics of SCID. *Ital J Pediatr* 2010; 36:76.
 3. Brown L, Xu-Bayford J, Allwood Z, *et al.* Neonatal diagnosis of severe combined immunodeficiency leads to significantly improved survival outcome: the case for newborn screening. *Blood* 2011; 117:3243–3246.
- This retrospective study highlights the importance of a SCID diagnosis being made before 3.5 months of age to reduce the rate of infectious complications and to improve the overall survival and general outcome.
4. Meeths M, Chiang SC, Wood SM, *et al.* Familial hemophagocytic lymphohistiocytosis type 3 (FHL3) caused by deep intronic mutation and inversion in *UNC13D*. *Blood* 2011; 118:5783–5793.
- Analysis of patients with haemophagocytic lymphohistiocytosis (FHL) has revealed a frequent inversion in the *UNC13D* gene due to a common founder effect, potentially allowing specific NBS for this often-fatal severe PID.
5. Borte S, von Döbeln U, Fasth A, *et al.* Neonatal screening for severe primary immunodeficiency diseases using high-throughput triplex real-time PCR. *Blood* 2012; 119:2552–2555.
- This is the first study reporting on simultaneous measurement of TREC and KREC copy numbers in DBSSs from newborns. The presented methodology allows one multiplex assay of several DNA excision products to be applied to high-throughput NBS programmes.
6. Borte S, Wang N, Oskarsdóttir S, *et al.* Newborn screening for primary immunodeficiencies: beyond SCID and XLA. *Ann N Y Acad Sci* 2011; 1246:118–130.
 7. Guthrie R, Susi A. A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. *Pediatrics* 1963; 32:338–343.
 8. Wilson JMG, Jungner G. The principles and practice of screening for disease. WHO Chronicle Geneva 1968; 22:473.
 9. Watson M, Mann M, Lloyd-Puryear M, *et al.* Newborn screening: toward a uniform screening panel and system. *Pediatrics* 2006; 117:296–307.
 10. Accetta Pedersen DJ, Verbsky J, Routes JM. Screening newborns for primary T-cell immunodeficiencies: consensus and controversy. *Expert Rev Clin Immunol* 2011; 7:761–768.
 11. Pandor A, Eastham J, Beverley C, *et al.* Clinical effectiveness and cost-effectiveness of neonatal screening for inborn errors of metabolism using tandem mass spectrometry: a systematic review. *Health Technol Assess* 2004; 8:1–121.

12. Loeber G, Burgard P, Cornel M, *et al.* Newborn screening programmes in Europe; arguments and efforts regarding harmonization. Part 2: from screening laboratory results to treatment, follow-up and quality assurance. *J Inher Metab Dis* 2012; 35:613–625.

The survey results from European screening centres depicting considerable differences, but also similarities, in the organization of national NBS programmes, which imply the European trend towards PID screening. The article also presents disorders proposed by the EUNENBS network to be considered for gradual implementation in neonatal screening practice.

13. Andermann A, Blanquaert I, Beauchamp S, Déry V. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. *Bull World Health Org* 2008; 86:317–319.
14. Petros M. Revisiting the Wilson–Jungner criteria: how can supplemental criteria guide public health in the era of genetic screening? *Genet Med* 2012; 14:129–134.

This study reviews the applicability of the original Wilson–Jungner criteria for evaluation of disorders to be screened at the newborn stage. An amendment of 11 additional criteria is proposed to improve the decision-making process for the expansion of newborn screening programmes.

15. Plebani A, Soresina A, Rondelli R, *et al.* Clinical, immunological, and molecular analysis in a large cohort of patients with X-linked agammaglobulinemia: an Italian multicenter study. *Clin Immunol* 2002; 104:221–230.
16. Gholam C, Grigoriadou S, Gilmour KC, Gaspar HB. Familial haemophagocytic lymphohistiocytosis: advances in the genetic basis, diagnosis and management. *Clin Exp Immunol* 2011; 163:271–283.
17. Maarschalk-Ellerbroek LJ, Hoepelman IM, Ellerbroek PM. Immunoglobulin treatment in primary antibody deficiency. *Int J Antimicrob Agents* 2011; 37:396–404.
18. Bousfiha AA, Jeddane L, Ailal F, *et al.* Primary immunodeficiency diseases worldwide: more common than generally thought. *J Clin Immunol* 2012. doi: 10.1007/s10875-012-9751-7. [Epub ahead of print]
19. Verbsky J, Thakar M, Routes J. The Wisconsin approach to newborn screening for severe combined immunodeficiency. *J Allergy Clin Immunol* 2012; 129:622–627.

The retrospective analysis of NBS data from the Wisconsin group, which started SCID screening based on the TREC assay in 2008, provides important insight on the expected clinical impact and practical recommendations for population-based NBS for severe PID.

20. Buckley RH, Schiff SE, Schiff RI, *et al.* Hematopoietic stem-cell transplantation for the treatment of severe combined immunodeficiency. *N Engl J Med* 1999; 340:508–516.
21. Borte S, Janzi M, Pan-Hammarström O, *et al.* Placental transfer of maternally-derived IgA precludes the use of Guthrie card eluates as a screening tool for primary immunodeficiency diseases. *PLoS ONE* 2012; 7:e43419.
22. Puck JM. Laboratory technology for population-based screening for severe combined immunodeficiency in neonates: the winner is T-cell receptor excision circles. *J Allergy Clin Immunol* 2012; 129:607–616.

This study collects and analyses the test characteristics of the TREC assay to screen for newborns with severe T-cell lymphopenia, supporting broad implementation of this excision circle assay.

23. Baker MW, Grossman WJ, Laessig RH, *et al.* Development of a routine newborn screening protocol for severe combined immunodeficiency. *J Allergy Clin Immunol* 2009; 124:522–527.
24. Speckmann C, Neumann C, Borte S, *et al.* Delayed-onset adenosine deaminase deficiency: Strategies for an early diagnosis. *J Allergy Clin Immunol* 2012; 130:991–994.

This case report illustrates the diagnostic approach and outcome in children with delayed-onset SCID caused by hypomorphic mutations in the *ADA* gene. The single TREC assay would not have identified these newborns, yet importantly, abnormal results were returned for the KREC marker in the combined TREC–KREC assay.

25. McGhee SA, Stiehm ER, McCabe ER. Potential costs and benefits of newborn screening for severe combined immunodeficiency. *J Pediatr* 2005; 147:603–608.
26. Chan K, Davis J, Pai SY, *et al.* A Markov model to analyze cost-effectiveness of screening for severe combined immunodeficiency (SCID). *Mol Genet Metab* 2011; 104:383–389.

The cost-effectiveness of population-based NBS for severe T-cell lymphocytopenia in the United States is evaluated in the context of decision-making trees that include expenditures to be made for the screening test, savings returned from earlier diagnoses achieved and the quality-adjusted life years to be attributed to newborns identified by a screening programme for SCID.

27. Gurian E, Kinnamon D, Henry J, Waisbren S. Expanded newborn screening for biochemical disorders: the effect of a false-positive result. *Pediatrics* 2006; 117:1915–1921.