In-Service Evaluation (ISE) of Screening for Severe Combined Immunodeficiency (SCID)

Acknowledgements

There have been many individuals, teams and organisations who have supported this work so far and it would be impossible to name them all. However, it would not have been possible to complete the evaluation without the efforts of a wide range of individuals, teams, and key stakeholders working together particularly during some very challenging times.

We especially want to acknowledge the contribution of Rebecca Nightingale whose son Scott sadly lost his life to Severe Combined Immune Deficiency (SCID). Rebecca supported the evaluation with the design, consultation and delivery of a wide range of public and professional resources.

In addition we would also like to acknowledge the huge contribution of Dr Susan Walsh, CEO of Immunodeficiency UK, in providing the patient and public voice.

This NHS England SCID in-service evaluation report should be read in conjunction with the accompanying health economic evaluation report written by the School of Medicine & Population Health (SCHARR) at the University of Sheffield

Contents

Plain English summary

Severe combined immunodeficiencies (SCID) are a group of inherited disorders that cause a failure to combat infections. Babies with SCID are usually well for a short time after birth, but then develop severe infections, which are difficult to treat. Without treatment, most die before they reach their first birthday. Early treatment, before severe infections occur, improves outcomes. Babies who have a close relative with the condition are already identified and treated before they have any symptoms.

Newborn screening for SCID is already offered in most of the USA and some other countries. The screening test is not specific for SCID. It uses a small blood sample taken from the baby's heel to measure the number of T-cell receptor excision circles (TRECs). Low TREC levels can indicate a deficiency in T-cells, a type of white blood cell crucial for immunity, and may suggest a baby has SCID. This is the same sample, already taken at 5 days old, to test for 9 other conditions.

Many babies who have an abnormal screening test result do not have any problems with their immunity. Others may have a transient problem or another less severe problem that may or may not benefit from early detection.

In 2017, the UK National Screening Committee (UK NSC) recommended a formal in-service evaluation (ISE) of newborn screening for SCID in real world NHS services, to assess whether screening for SCID would do more good than harm at reasonable cost.

Live vaccines should not be given to babies with SCID. Therefore, after discussion with the Joint Committee on Vaccination and Immunisation (JCVI), it was agreed that, from the beginning of the ISE, the Bacillus Calmette-Guérin (BCG) vaccine should be delayed from the early neonatal period to when the screening result was available. The ISE evaluation period ran from 6 September 2021 to 1 March 2024. Screening has continued in the ISE areas since March 2024.

ISE results

At the beginning of the ISE, 2 screening methods were available: Immuno IVD (IIVD) SPOit and Perkin Elmer (now Revvity) Enlite. Enlite was replaced by a third method (EONISQ) towards the end of the ISE when it became apparent it did not perform as

well as IIVD and was to be discontinued by the manufacturers. The ISE tested a pathway for the screening, confirmatory testing, diagnosis and care of newborn babies with SCID. Of 955,507 babies screened, 568 had a 'SCID suspected' result, of whom 316 had a normal T-cell phenotype and 45 were proven (33) or assumed (12) to have a reversible abnormality. 12 babies with SCID were found, only 2 of whom would have been identified without screening. The positive predictive value (PPV) for SCID, for all methods combined, was 2%.

The ISE identified 56 babies with non-transient non-SCID T-cell lymphopenia. Early detection was beneficial for several of these babies. This is because their doctors then set a lower threshold for antibiotic use and avoidance of live vaccines. Of these babies 8 had idiopathic T-cell lymphopenia (unexplained abnormal T-cell profile). The management of 2 of these babies was influenced by this knowledge. The PPV for the test increases to 6.7% if all babies judged to have benefitted from screening are considered. Using the parameters set during the evaluation, the IIVD testing method performed better than the PE Enlite testing method. The results of using the EONISQ technology will be analysed.

The screening programme did not aim to detect babies with non-SCID lymphopenias. However, their numbers are significant, so an attempt should be made to factor in their costs and benefits. At this stage it has only been possible to factor in the costs.

The programme was stressful for parents of babies with 'SCID suspected' results, whatever their outcome. It also increased the burden on services. However, all welcomed the programme as an addition to the newborn screening offer.

Screening for SCID has disrupted the BCG programme. Inevitably, babies are now older when they receive the vaccine. The limited data available on vaccination uptake indicates that the uptake by 12 months of age has increased in some areas. There is no suggestion that cases of tuberculosis increased following the change to the vaccine schedule.

Modelling shows that the programme has a cost per quality adjusted life years (QALY) gained of £80-90,000, which is well above the threshold usually considered cost effective (£20-30,000). It is unlikely that any adjustments to the programme would make it cost effective as a stand-alone addition to screening. This is because the burden of costs resides predominantly with the cost of carrying out the screening test, including the cost of the test itself, the equipment and human resources.

Screening for SCID can be combined on the IIVD and EONISQ platforms used for SCID testing with the screening test for spinal muscular atrophy (SMA). Screening for SCID would become cost effective if screening for SMA was introduced, and the cost of SCID screening was considered as an incremental cost, or the cost could be split between the 2 programmes.

Main findings

The UK NSC recommended setting up the ISE to address a number of important questions. Here is a summary of the answers to those questions.

Cost of the TREC test: for the IIVD method, the TREC test cost a total of £7.58 per baby. This includes test reagents, equipment and human resources.

Incidence of SCID in England during the evaluation: 21 cases of SCID (10 via screening, 4 by family history and 9 presenting symptomatically) were found during the 30-month evaluation period. This amounts to a birth prevalence of approximately 1 in 71,000. This seemed low. The birth prevalence of SCID in the period 2010 to 2020 was 1 in 53,000.

In the 12 months immediately following the evaluation period, 16 cases of SCID were reported in the screened and unscreened areas combined. This amounts to a birth prevalence of 1 in 35,100. If combined with the cases from the evaluation, this amounts to 1 in 55,500, approximately equal to what was expected.

Post HSCT mortality rates in the early diagnosed population: the follow-up data from the evaluation is limited due both to the small numbers and the limited duration of follow-up. However, at the time of follow-up, only one of the 14 SCID babies who were screened or had a family history had died following Haematopoietic Stem Cell Transplantation (HSCT). In contrast, of the 7 babies who presented symptomatically, 2 died before definitive treatment could be initiated and 3 died despite transplantation. All 3 had a history of severe infection, including one with BCGosis.

Length of stay in hospital of the early diagnosed SCID patients: the follow-up data from the evaluation is limited due both to the small numbers and the limited duration of follow-up. However, the total length of stay and the use of intensive care units (ICUs) was much higher in the clinically presenting population.

Proportion of patients detected by family history in the absence of screening: this was 37/146 (25%) from the retrospective data, covering babies presenting

between 1 January 2010 and 6 September 2021. The number of babies detected by family history during the evaluation was 2/12 (17%) in those screened and 2/9 (22%) in those not screened, giving a total of 4/21 (19%) overall during the evaluation period. If this is combined with the cases reported in the 12 months after the evaluation period, there was a positive family history in 7/37 (19%) of cases.

Effect of the screening programme on participant families: a 'SCID suspected' result was stressful for families. This was compounded by the fact they had no recollection of being told SCID was one of the conditions being screened for, and so it came as a total surprise. Families of sick babies felt this even more. This effect wore off for families where there was a false positive result. Families where there was a significant immunodeficiency, whether SCID or non-SCID identified, were grateful to know sooner. The overwhelming view was that screening for SCID was welcomed. These findings are seen in other newborn screening programmes.

Capacity of NHS services: the large number of 'SCID suspected' babies put an extra workload on immunology services and added to the workload of the screening laboratories. Unlike the screening laboratories, no extra resources were provided for the immunology services (clinical and laboratory) and these services, especially the smaller ones, found this a difficult burden to carry.

Recommendations

The ISE board makes the following recommendations based on the results from the 30-month evaluation period:

- SCID screening cannot be recommended as a standalone programme as it is not cost effective
- screening for SCID should continue in the ISE areas as it is now until a decision is made about screening for SMA - in the meantime, work on further adjustments to the cut-offs and possible modification of the preterm pathway should proceed
- the use of reflex genomics should be considered in the work to reduce the number of false positives - babies with absent or extremely low levels of TRECs would be referred while those with higher levels, but below the cut-off,

would be referred for genomics - the panel chosen for this purpose could include just SCID genes or a broader range of actionable conditions

- follow-up of babies with SCID and Idiopathic T cell Lymphopenia (ITCL) should continue, and consideration should be given to follow-up of some other groups of babies - linkage to routine health and education data, enhanced by periods of rich clinical data collection, should be considered as methods for follow-up

- the content and mode of delivery of parent information should be reviewed for all newborn blood spot screening programmes, particularly as the number of conditions screened may increase substantially - any lessons learnt from the Generation Study should be fed into this work

- the outcome of the monitoring of the BCG programme, which has substantially improved, and of the epidemiology of childhood TB should be fed into reviews of the screening programme, if continued

- clinical protocols for the management of babies found to have non-SCID T-cell lymphopenia should be reviewed by relevant professional bodies to ensure there is consistency in management

Executive Summary

Introduction

Severe combined immunodeficiencies (SCID) are a group of inherited disorders causing a failure to combat infections. Patients with SCID are usually well for a short time after birth, but then develop severe infections, which are difficult to treat. The main treatment for SCID is Haematopoietic Stem Cell Transplantation (HSCT). This involves taking cells, usually from bone marrow or the umbilical cord, from a person who is closely matched with the patient. These cells are injected to replace the patient’s own bone marrow. In a minority of cases, alternative treatments, such as thymic transplantation or gene therapy, may be appropriate. Without one of these treatments, most patients die before they reach their first birthday. Early treatment, before severe infections have occurred, improves the outcome. This already occurs for children who have a close relative with the condition and are treated before they have any symptoms.

Newborn screening is already in place in most of the USA and some other countries. The screening test is not specific for SCID. It gives an indication of the maturation of part of the immune system – T-cells. Babies who have an abnormal screening test result may turn out to have no problems with their immunity. Others may have a transient problem or another less severe problem that may or may not benefit from early detection.

In June 2017, the UK National Screening Committee (UKNSC) recommended a formal in-service evaluation (ISE) of newborn screening for SCID, to address whether screening for SCID, in general, would do more good than harm at reasonable cost, and whether it would be appropriate in a UK setting.

After discussion with the Joint Committee on Vaccination and Immunisation (JCVI), it was agreed that the BCG vaccine should be delayed from the early neonatal period to approximately 28 days, as live vaccines were contraindicated in babies with SCID.

After being delayed by the COVID-19 pandemic, the ISE ran from 6 September 2021 to 1 March 2024.

A multidisciplinary board, including parent representation, was set up with a number of working groups (referred to in the appendices) to plan, co-ordinate and oversee the ISE.

Following the end of the formal evaluation period (1 March 2024) the purpose of the SCID Board was reviewed and revised to include monitoring of screening for SCID and programme continuity pending this report and a UKNSC recommendation.

Laboratory Findings

Two approved commercial kits were available for SCID screening at the start of the evaluation – the Perkin Elmer[1] Enlite™ Neonatal TREC kit (end-point PCR) and the Immuno IVD Spot-it™ kit (real time PCR). Six labs were chosen to participate in the evaluation, three evaluating each method. Cut-off values were set to balance the harms v benefits for families, while being sufficiently high to allow data to be collected to enable modelling of different scenarios. A third method using real time

[1] Part way through the evaluation, Perkin Elmer was reorganised into two separate companies, Revitty being the part that included newborn screening.

PCR (Perkin Elmer EONIS™ Q) was introduced, once available in November 2023. Evaluation of this method is ongoing.

Using the cut-offs set for the ISE, the positive predictive value (PPV) (the proportion of babies having a screening result requiring further investigation who are found to have the condition) ranged from 1.57% to 4.22%, depending on the method. This is very low in comparison with other conditions screened for using the newborn blood spot screening test. Modelling showed that, with the IIVD methodology, it would be possible to achieve a PPV of 10.83%. No cases of SCID were known to have been missed, despite checking many sources of information.

Clinical Findings

It is not possible to ascertain whether an individual baby has benefitted from T-cell receptor excision cell (TREC) screening based on the condition identified. The Diagnostic Review Group (DRG) ascertained from the condition and the clinical features whether each baby had benefitted from screening.

568 children (1 in 1,680 screened) were referred to regional immunology services for further assessment. Premature babies were over-represented in this group (23% <28 weeks gestation as opposed to 7.5% in the screen negative population). Of the 568 babies, the majority (56%, 316/568) had a normal flow cytometry or a proven or probably reversible condition (45). Of the remainder, a large group (76) died before investigations were completed, reflecting the high number of severely ill premature babies. Babies in these groups derived no benefit from screening. On the contrary, many families were put through the stress of being told their baby may have a life threatening disorder, when this turned out not to be true.

12 babies were identified with SCID. Ten of them, in the absence of screening, would not have presented until symptomatic and would therefore have been more difficult to treat.

Overall survival of babies with SCID in the screened group, whether or not they had a family history, was 92% (11/12). These babies spent fewer days in Paediatric intensive care (PICU) than those in the unscreened cohort, had no complications related to Bacillus Calmette-Guérin (BCG) or rotavirus vaccine and had predominantly good outcomes post-definitive treatment. Most stopped long-term immunoglobulin replacement and responded to vaccination.

In the non-screened cohort, overall survival of babies with SCID was poor at 20% (2/7). Two babies died before definitive therapy. Most experienced complications secondary to BCG and/or rotavirus infection and post-transplant outcomes were complicated, necessitating prolonged hospital admission or ongoing treatment.

Although the numbers are small, the magnitude of the benefit to children with SCID probably outweighs the disbenefit to the larger number of babies who were found to have no significant T-cell anomalies.

A small group of screened babies (48) had a variety of T-cell anomalies, about half of whom benefitted from early recognition.

In addition, eight babies had a persistent T-cell abnormality for which no cause was identified. In at least two, the management of these babies was altered because of the condition. These babies probably benefitted from screening, but it is unclear and only long-term follow-up of all will help clarify whether screening benefited this group. This is similar to the uncertainty inherent in the designation of a Cystic Fibrosis Screen Positive, Inconclusive Diagnosis (CFSPID) result following newborn screening for CF.

Utilities research

This research aimed to ascertain the effects of the SCID screening programme on families who experienced a range of results and on service providers, including midwives, laboratory staff, immunologists and clinical nurse specialists/health visitors. Receiving a suspicious screening result for SCID was distressing for parents. False positive screening results for SCID could cause parents to be over concerned about their child's vulnerability in the short term. However, exposing children to 'normal' infections in the first year of life provided evidence to parents that their child's immune system was functional, and this helped to reassure them. If newborn blood spot (NBS) screening for SCID is to be rolled out nationally, careful consideration needs to be given to the additional workload this triggers for laboratory and immunology teams and how this can best be managed given finite resources.

Both parents and clinicians are in favour of SCID being added to the national NBS screening programme and were able to provide recommendations to improve the success of this.

Health Economics (See full Health Economics report)

A health economics model was developed from the model used by the UKNSC to consider SCID screening in 2016/17. The model compares NBS screening for SCID against the pathway for these babies without screening. There were three primary sources of data for the health economic model:

- the NHS SCID Evaluation (including the prospective SCID data collection, the retrospective SCID cohort from Great Ormond Street Hospital for Children NHS Foundation Trust (GOSH) and Newcastle Upon Tyne Hospitals NHS Foundation Trust (NUTH) and routine Hospital Episode Statistics (HES) and Office for National Statistics (ONS) data)
- data from the parallel SCID Outcomes Research Study
- published and grey literature

The SCID Evaluation included a mix of screening technologies with algorithms designed to yield evidence for optimising practice. In contrast, the economic modelling contains projections of screening outcomes that would be obtained using a real time PCR screening technology operating with a screening algorithm optimised for practice in an annual UK birth population. These population screening outcomes are summarised in Section 4.7 below and reported fully in the accompanying SCID health economics report

The incremental costs of screening and subsequent diagnostic investigations are estimated at approximately £5.3m per year. Early detection and improved management of an annual cohort of SCID patients is estimated to save approximately £419k (£102k, £749k) in the first year and an additional £38k (-£369k, £438k) discounted over the lifetime of the annual cohort. The incremental total discounted cost of screening is therefore estimated at £4.8m (£4.3m, £5.4m) per year.

The cost effectiveness of screening for SCID compared to no screening is estimated at £87,813 per quality adjusted life years (QALY) gained. The UK 2022 population net monetary benefit of screening at a cost effectiveness threshold of £30,000 per QALY is -£3.2m (-£4.5m, -£2.0). The full economic report describes a range of sensitivity analyses. Sensitivity analyses examine two alternative approaches to

apportioning the cost of the screening. Firstly, the marginal impact of screening for SCID plus Spinal Muscular Atrophy (SMA) compared to screening for SMA alone is considered, that is 10% of the laboratory cost of screening. Secondly, apportioning of costs according to the relative birth prevalence of the two conditions is considered. The cost effectiveness of screening for SCID in these two scenarios is estimated at £4,409 and £8,062 per QALY gained respectively and the probability that it is cost effective at a threshold of £30,000 is 98% and 97% respectively.

Bacillus Calmette-Guérin (BCG)

The BCG programme undoubtedly underwent a major organisational change. The lack of good data prior to the change makes generalised comments difficult. It is reassuring that uptake in the areas where the offer of BCG was universal did not fall and that the detailed data from the West Midlands have shown that the timeliness of vaccination, although affected by the change, is improving as new systems settle in. However much it improves, it will not revert to what it was, but it is unclear whether this will affect the incidence of Tuberculosis (TB). The evidence is that screening for SCID will reduce the number of cases of BCGosis, but the magnitude of this change is difficult to predict. An undoubtedly beneficial effect associated with the change in the programme has been a much improved system of recording uptake, an essential component of any national vaccination programme.

Responses to UK NSC questions posed

Cost of the TREC test

For the IIVD method, this amounted in total to £7.58 per baby. This includes test reagents, equipment and human resources.

Incidence of SCID in England during the evaluation

21 cases of SCID (10 via screening, four by family history and 9 presenting symptomatically) were found during the 30-month evaluation period. This amounts to

a birth prevalence of approximately 1 in 71,000. This seemed low. The birth prevalence of SCID in the period 2010 to 2020 was 1 in 53,000.

In the 12 months immediately following the evaluation period, 16 cases of SCID were reported in the screened and unscreened areas combined. This amounts to a birth prevalence of 1 in 35,100. If combined with the cases from the evaluation, this amounts to 1 in 55,500, approximately equal to what was expected.

Post HSCT mortality rates in the early diagnosed population

The follow-up data from the evaluation is limited due both to the small numbers and the limited duration of follow-up. However, at the time of follow-up, only one of the 14 SCID cases that were screened or had a family history had died, and this was after HSCT. On the other hand, of the seven of the symptomatically presenting cases, two died before definitive treatment could be initiated and three died despite transplantation (one was a thymic transplant). All three had a history of severe infection, including one with BCGosis. This mortality probability of 71% (5/7) in the SCID patients symptomatically detected during the Evaluation period is markedly higher than the 22% (24/109) 2 year mortality over the retrospective data collection period. The explanation for this is not immediately apparent. The symptomatic mortality rate used in the economic modelling is based upon the larger retrospective cohort.

Length of stay in hospital of the early diagnosed SCID patients

The follow-up data from the evaluation is limited due both to the small numbers and the limited duration of follow-up. However, the total length of stay and the use of Intensive Care Units (ICUs) was much higher in the clinically presenting population. Heavy use was made of the retrospective data. See the economic evaluation for a full discussion of this.

Proportion of patients detected by family history in the absence of screening

This was 37/146 (25%) from the retrospective data. The numbers found during the evaluation were 2/12 (17%) in those screened and 2/9 (22%) in those not screened, giving a total of 4/21 (19%) overall during the evaluation period. If this is combined

with the cases reported in the 12 months after the evaluation, there was a positive family history in 7/37 (19%).

Effect of the screening programme on the participant families

A ‘SCID suspected’ result was undoubtedly stressful for families. This was compounded by the fact that by the time the result came through, they had no recollection of being told this was one of the conditions being screened for, so it came as a total surprise. Families of sick babies felt this even more. This effect wore off for families where there was a false positive result. Families where there was a significant immunodeficiency, whether SCID or non-SCID identified, were grateful to know sooner. The overwhelming view was that screening for SCID was welcomed. These findings are seen in other newborn screening programmes.

Capacity of NHS services

The large number of ‘SCID suspected’ babies put an extra workload on immunology services and added to the workload of the screening laboratories. Unlike the screening laboratories, no extra resources were provided for the immunology services (clinical and laboratory) and these services, especially the smaller ones, found this a difficult burden to carry.

Conclusions

The ISE tested a pathway for the screening, confirmatory testing, diagnosis and care of newborn babies with SCID. Of 955,507 babies screened, 568 had a ‘SCID suspected’ result, of whom 316 had a normal T-cell phenotype and 45 were proven (33) or assumed (12) to have a reversible abnormality. 12 babies with SCID were found, only two of whom would have been identified without screening. The PPV for SCID, for all methods combined, was 2%. Modelling showed that this could be increased to 11%, using the IIVD methodology.

56 babies were identified with non-transient non-SCID T-cell lymphopenia, several of whom would derive benefit from early detection, e.g. a lower threshold for antibiotic use and avoidance of live vaccines. 8 of these babies had idiopathic T-cell

lymphopenia (unexplained abnormal T-cell profile). The management of two of these babies was influenced by this knowledge. The PPV rises to 6.7% if taking into account all cases judged to have benefitted from screening. Using the parameters set during the evaluation, the IIVD method performed better than the PE Enlite method.

Babies with non-SCID lymphopenias are not the target of the screening programme. However, their numbers are such that an attempt should be made to factor in their costs and benefits. At this stage it has only been possible to factor in the costs.

Although the programme was stressful for parents of babies with 'SCID suspected' results, whatever their outcome, and was an increased burden on services, all welcomed the programme as an addition to the newborn screening offer.

Screening for SCID has disrupted the BCG programme. Inevitably, babies are now older when they receive the vaccine. The limited data available on vaccination uptake indicates that the uptake by 12 months of age has increased in some areas. There is no suggestion that TB has increased because of the change in the programme.

Modelling shows that the programme has a cost per QALY gained of £80-90k, which is well above the threshold usually considered cost effective (£20-30k). It is unlikely that any adjustments to the programme would make it cost effective as a stand-alone addition to screening. This is because the burden of costs resides predominantly with the cost of carrying out the screening test – the cost of the test itself, the equipment and human resources. Screening for SMA can be multiplexed on the IIVD and EONISQ platforms used for SCID testing. If screening for SMA were to be introduced, and the cost of SCID screening was considered as an incremental cost, or other apportionment of costs of the two programmes was made, screening for SCID would become cost effective.

Recommendations

SCID screening cannot be recommended as a standalone programme, as it is not cost effective:

- The programme should continue as it is now until a decision is made about screening for SMA.

- In the meantime, work on further adjustments to the cut-offs and possible modification of the preterm pathway should proceed.

- The use of reflex genomics should be considered in the work to reduce the number of false positives. Babies with absent or extremely low levels of TRECs would be referred while those with higher levels, but below the cut-off, would be referred for genomics. The panel chosen for this purpose could include just SCID genes or a broader range of actionable conditions.

- Follow-up of babies with SCID and Idiopathic T cell Lymphopenia (ITCL) should continue, and consideration should be given to follow-up of some other groups of babies. Linkage to routine health and education data, enhanced by periods of rich clinical data collection, should be considered as methods for follow-up.

- The content and mode of delivery of parent information should be reviewed for all NBS screening programmes, particularly as the number of conditions screened may increase substantially. Any lessons learnt from the Generation Study should be fed into this work.

- The outcome of the monitoring of the BCG programme, which has substantially improved, and of the epidemiology of childhood TB should be fed into reviews of the screening programme, if continued.

- Clinical protocols for the management of babies found to have non-SCID T-cell lymphopenia should be reviewed by relevant professional bodies to ensure there is consistency in management.

1. Introduction

1.1 The condition

Severe combined immunodeficiencies (SCID) are genetically determined errors of immunity, leading to failure of T-lymphocyte development, with or without failure of B-lymphocyte and Natural Killer (NK) cell development or function. Most forms of SCID are inherited in an autosomal recessive fashion, with one, interleukin-2 (IL-2) receptor gamma chain (IL-2Rg) deficiency, that has X-linked inheritance. Over 20 genes have been described which cause SCID – for a few patients (~ 5%), a genetic

diagnosis remains elusive. Regardless of genotype, patients classically present with a similar spectrum of clinical features, usually at several months of age with a wasted, malnourished infant, with persistent gastrointestinal and/or respiratory viral infection. In the modern era in the UK, persistent vaccine-strain rotavirus enteritis is common, and many infants require total parenteral nutrition. Eligible infants who received the live Bacillus Calmette-Guérin (BCG) vaccine may present with disseminated BCG infection. Persistent viral respiratory infection is common, particularly with RSV or parainfluenzae viruses, and many infants have Pneumocystis jirovecii pneumonia (PCP), in severe cases requiring invasive mechanical ventilation to support respiratory failure. Other presentations rarely occur. For most infants, transplantation of allogeneic haematopoietic stem cells (HSCT) is the standard of care, and in the modern era, survival of 85-90% can be expected, and is curative. For adenosine deaminase (ADA)-deficient SCID, gene addition therapy is a recognised alternative therapy. This is licensed and available for treatment in Milan, funded by the NHS. Specifically for ADA SCID, enzyme replacement therapy (elapegademase) is available as a bridging therapy. For a few other genetic variants, gene addition therapy clinical trials are underway. For some patients' thymic transplantation may be appropriate.

Approximately 10% of patients with SCID have genetic defects (most commonly complete DiGeorge Syndrome, caused by a 22q11 chromosomal deletion) which inhibit the normal development of the thymus gland, resulting in congenital athymia. The thymus gland is essential for the final maturation steps of T cells development, and therefore patients with congenital athymia have the same absence of functional T-cells in blood, despite having normal bone marrow function. Patients with congenital athymia are preferentially treated with thymic transplantation rather than bone marrow transplant. Great Ormond Street Hospital, London is one of two centres in the world which offer this procedure.

For many years, it has been recognised that patients who are diagnosed early before they contract infection, (usually on the basis of a family history of the condition) have better therapeutic outcomes with a lower mortality risk, than patients who have pre-existing and persistent infection. This recognition led to the development of a screening test to detect T-lymphocyte receptor excision circles (TRECs) on the newborn blood spot screening card. TRECs are a by-product of successful T-lymphocyte receptor formation and are absent, or extremely low, in patients with SCID. First piloted in the USA, usually using the already collected bloodspots, TREC screening has now been adopted in many countries worldwide and has been

demonstrated to result in superior neurological outcomes and better overall survival in SCID populations, compared to a non-screened population.

1.2. The pathway to the UKNSC decision to have an ISE

In 2003 and, again in 2013, the UKNSC considered evidence on newborn screening for SCID found insufficient evidence to recommend it.

A further review presented to the UKNSC meeting in June 2017, following a workshop with relevant stakeholders, suggested that screening for SCID might be a candidate for adding to the national newborn blood spot (NBS) screening programme. However, the model found that cost effectiveness was reliant on data about the:

- cost of the TREC test
- incidence of SCID in UK
- post HSCT mortality rates in the early diagnosed population
- length of stay in hospital of the early diagnosed SCID patients
- proportion of patients detected by family history in the absence of screening
- effect of the screening programme on the participant families
- benefits and costs to those with non-SCID immunological problems
- capacity of NHS services

At its meeting, the UKNSC recommended a formal in-service evaluation (ISE), subject to formal consultation. Documents on the SCID recommendation web page (https://view-health-screening-recommendations.service.gov.uk/scid/) include the evidence summary, SCID economics consultation document and the cover sheet that went to the committee.

Responses to a public consultation in late 2017 were favourable and work started on planning the ISE.

It was noted that BCG and rotavirus vaccines were contraindicated in a baby with SCID. After discussion with the Joint Committee on Vaccination and Immunisation (JCVI), it was agreed that the BCG vaccine should be delayed from the early neonatal period to approximately 28 days, but this should be modelled and the decision reviewed (see JCVI minutes of for October 2018, October 2019 and February 2020 at https://app.box.com/s/iddfb4ppwkmtjusir2tc).

In February 2020, a Sheffield Centre for Health and Related Research (ScHARR) paper on the effect of delaying BCG was presented to the JCVI along with other data. The JCVI confirmed its previous decision to delay BCG, recognising that there was little reliable data on the benefits and harms of this decision.

Unfortunately, the start of the ISE was delayed by the COVID-19 pandemic.

The launch of the ISE on September 2021 was summarised in a UK National Screening Committee blog article.

2. Planning the In-Service Evaluation

The aim of the evaluation was to address the data gaps highlighted by the UKNSC (see section 1.2 above). Blood from the bloodspot card taken on day 5 was used to provide the sample. Six of the 13 bloodspot screening laboratories in England participated from the start of the evaluation. The laboratories were chosen to have a range of sociodemographic characteristics and cover about 50% of the newborn population. Initially two different screening methods were evaluated, in three laboratories each. Laboratory cut-off values and algorithms for screen positive cases were agreed and a referral pathway for screen positive cases was developed. Information was produced for families and professionals involved with the screening. Data was collected from laboratories, diagnostic immunology services and tertiary immunology services on outcomes for clinical cases. Since providing information on the benefits and costs to those with non-SCID immunological problems was one of the key aims of the evaluation, a process for agreeing which of these cases had benefitted from screening was developed. Additional information was obtained to support the economic analysis. At the end of the evaluation, data were collected from a number of sources – Child Mortality Data Base, ONS mortality statistics and Hospital Episode Statistics (HES) – to ensure that all cases of SCID were included.

2.1 Design of the evaluation

To plan, co-ordinate and oversee the In-Service Evaluation (ISE) a board was set up. The first meeting was held on 18 December 2017. In addition to the screening programme staff, members included workstream chairs, specialist clinical immunologists and diagnostic SCID laboratory representatives, maternity and health visiting services, devolved nations, patient and public representatives and Royal College of Midwives.

Details of the Board, membership and workstreams are in Appendices 1-6. The Terms of Reference were reviewed and updated following the abolition of Public Health England (PHE) in 2021 and again in 2024 following the end of recruitment to the evaluation.

The overall design of the evaluation was considered within the full board. Detailed aspects were discussed by the working groups and their recommendations agreed by the board. The scope of the evaluation and questions to be answered were discussed in detail. There was tension between obtaining the maximum possible information from the evaluation and keeping a tight focus on the UKNSC questions to be answered. Early in the study design the board agreed that rather than select one of the two laboratory kits which were commercially available at the time to measure TRECs, both kits would be used, and the two compared. This would allow the findings to inform and recommend on criteria/standards which kits should meet.

During the evaluation it became apparent that one of the screening methods would be replaced by the manufacturers, with an improved alternative. Towards the end of 2023, 2 of the 3 laboratories using the original method changed to this alternative and a seventh laboratory was added, to help evaluate this new screening test.

Following the end of the formal evaluation period (1st March 2024) the purpose of the SCID Board was reviewed to include monitoring of screening for SCID and maintenance of stability during the interim phase and until the UKNSC has advised ministers on the outcome.

NHSE Antenatal and Newborn screening national portfolio commissions screening for SCID during this time and until there is a formal outcome from the evaluation.

Working groups were set up. These reported to the SCID board on progress.

2.2 Laboratory working group

The Laboratory Working Group considered all screening lab aspects, including equipment to use, cut-off points, algorithm, repeat testing, verification and validation, training of staff. Following discussion of the cut off and algorithm to be used for testing, a board decision was made to aim for very high sensitivity, to allow impact of this to be assessed, and, at the end of the evaluation, to model lower cut offs.

2.3 Patient Information, Communication & Training (PICT)

The patient information, communication and training (PICT) workstream included families affected by SCID and parent information groups as well as clinical teams and programme staff. The PICT workstream consulted with families and healthcare professionals on the implementation of adding SCID screening as a new condition for NBS screening. The group devised, communicated and published public and professional resources prior to and during the course of the evaluation. [2,3]

A series of online training sessions were held for maternity and neonatal services, newborn blood spot sample takers, child health information services (CHIS), quality assurance teams and regional commissioners. These were hosted by the NBS screening programme team and the directors of the regional NBS laboratories.

Families were consulted through a series of focus groups about the design and content of information leaflet.[4] This describes the evaluation and explains the option to choose screening for SCID or to decline.

2.4 Clinical Referral Management

This workstream devised and implemented an immunology diagnostic referral pathway for regional clinical immunology teams, to manage screen positive cases.

[2] Resources for healthcare professionals Newborn blood spot screening programme: supporting publications - GOV.UK

[3] Screening tests for you and your baby (STFYAYB) - GOV.UK

[4] SCID screening: helping you decide if you want this for your baby - GOV.UK

These extended from receipt of a ‘SCID suspected’ result to the definitive diagnosis, where available.

Resources were shared and published online[5,6] Screening was performed as defined by the SCID screening algorithm (see laboratory section of the report).

Screen positive SCID babies, i.e. those with a ‘SCID suspected’ result, were referred to a designated Consultant Paediatric Immunologist or their deputy, according to locally agreed and documented procedures, within one working day of the definitive result being available. Contact was then made with the family and it was explained that the test result indicated that there might be a problem, and further blood tests were indicated. The team contacting the family (a member of the immunology team) ensured that the family understood the need to come to the regional immunology centre for urgent tests, generally the following day. Interpreters were used if the family did not speak English. The family were signposted to information about SCID on-line.[7] Families were generally seen within 48 hours of the result, unless it was a weekend or bank holiday, when they were contacted once they were able to attend an appointment the following day.

At the appointment, a clinical history was taken, particularly looking for a notable family history, a history of consanguinity or evidence of maternal gestational diabetes or the use of immunosuppressive medication during pregnancy (which can cross the placenta and depress thymopoiesis, but which is reversible).

While most infants with SCID are normal at birth the infant was examined for stigmata, such as deafness, microcephaly or a rash. that might indicate a diagnosis. Other features such as congenital heart disease or cleft lip and/or palate may be associated with other causes of severe T-lymphocytopenia such as 22q11 deletion syndrome, or CHARGE syndrome. Blood was taken to measure numbers and types of lymphocyte using flow cytometry.

2.4.1 Normal result

Families with a normal flow cytometry result were informed of this, and a letter sent to the GP and family informing them of the result and explaining that vaccination

[5] Newborn blood spot screening programme: supporting publications - GOV.UK

[6] Clinical pathway for babies who screen positive for SCID - GOV.UK

[7] SCID screening: helping you decide if you want this for your baby - GOV.UK

could proceed as normal, including the live BCG vaccination if indicated. The letter explained that SCID had not been found ('not suspected'), but that other immunodeficiencies had not been looked for and if there were clinical concerns with the infant, further advice was recommended.

2.4.2 SCID

Families with a result indicating SCID were informed of the result and referred to one of the two specialists SCID services at the Great North Children's Hospital, Newcastle upon Tyne or Great Ormond Street Hospital, London, where they were seen within two days. Blood was sent to the genetic service at Great Ormond Street Hospital for rapid exome sequencing to determine the genetic cause of SCID and a result was generally available within 4 weeks of receiving the sample. The infant commenced co-trimoxazole and fluconazole prophylaxis. Breast feeding was encouraged unless the mother was cytomegalovirus (CMV) positive and the infant CMV negative (CMV transmission can occur through breast milk and CMV infection complicates the definitive treatment of stem cell or thymic transplantation). The family were advised to isolate the infant at home, unless the patient was unwell, in which case they were admitted to hospital for further management. The GP was informed of the diagnosis and advised that vaccination was contra-indicated, particularly with the live rotavirus and BCG vaccines, until after the definitive treatment. Definitive treatment, particularly haematopoietic stem cell transplantation was recommended to be performed by 3 months of age.

2.4.3 Non-SCID T-lymphocytopenia

A number of other conditions may be flagged by demonstrating low or absent TRECs on screening. Some of these are recognised inborn errors of immunity. If flow cytometry was abnormal, but not indicative of SCID, further investigations were performed to elucidate the cause, including exome sequencing. As a diagnosis of SCID had been excluded, exome sequencing results went through the routine channels and results took longer than 4 weeks to be received. Appropriate management was commenced, depending on the total T-lymphocyte count and phenotype and might include anti-bacterial and anti-fungal prophylaxis at the treating physician's discretion, advice about breast feeding depending on maternal CMV status, and avoidance of all or of live vaccines until a result was reached. Non-inherited causes of T-lymphocytopenia were sought if appropriate, including infant

related causes (severe sepsis, hydrops, lymphangiectasia) and maternal causes (gestational diabetes, maternal immunosuppressive medication) and managed appropriately.

2.4.4 Idiopathic T-lymphocytopenia

A small subset of infants picked up on newborn screening with low TREC had abnormally low lymphocyte phenotyping, not consistent with SCID and with no genetic or other cause identified. Appropriate management was commenced, depending on the total T-lymphocyte count and phenotype and might include anti-bacterial and anti-fungal prophylaxis at the treating physician's discretion, advice about breast feeding depending on maternal CMV status, and avoidance of all or of live vaccines. These infants were followed in an immunology clinic to monitor progress. Some of these will develop a normal lymphocyte phenotype over time and be discharged. The remainder will need to be followed.

2.5 Immunology network

A series of immunology network meetings were set up. These were initially held bimonthly and included clinical and laboratory immunology teams from across England (both SCID and non SCID areas). The group oversaw the plan of investigation of screen positive babies, including initial immunology and flow cytometry. They defined appropriate flow cytometry test cut-offs and turnaround times and fed back on workload for immunology labs and the impact of screen positive babies on immunology services.

Immunology colleagues supported the evaluation for the duration of the project via a number of routes and workstreams including SCID Board, the Data monitoring group, Diagnostic review panel, PICT and clinical referral management.

2.6 Data monitoring group (DMG)

The DMG agreed requirements for data collection from screening labs, immunology services and SCID services as well as the timepoints in the clinical pathway when data would be collected. The group had oversight of the database structure, data management and governance of identifiable data. As the ISE progressed, this group

reviewed the data for completeness and validity and maintained oversight of changes to data collection and management. The DMG supported the development of the Data Protection Impact Assessment (DPIA) and negotiated the information governance approvals to allow data linkage. Towards the end of the evaluation, DMG members considered and advised the Board on (1) the different options for archiving ISE data in an identifiable format to allow identification of late missed cases (false negatives) and longer-term follow-up of screen positive infants, including patient-reported outcomes and quality of life; and (2) the data requirements for monitoring a future SCID screening programme if implemented.

2.7 Diagnostic review panel

The Diagnostic Review Panel (DRP) was set up to provide an independent decision on the categorisation of each case where a condition suspected result on the basis of TREC screening was given. The chair was an international authority on screening for SCID and members were drawn from various disciplines, some of whom were not involved, otherwise, in the evaluation.

Cases were assigned a diagnosis, a categorisation of this diagnosis and whether the child had benefitted from screening. These decisions were made on the basis of all the available evidence, including flow cytometry, genetic analysis and clinical details.

Having arrived at a diagnosis, where possible, cases were then allocated to the categories of ‘normal T-cell subsets”, ‘SCID’ ‘syndromic T-cell lymphopenia’, ‘non-syndromic T-cell lymphopenia’, reversible T-cell lymphopenia and ‘inconclusive’ Appendix 7, 8, 9 At the end of the evaluation, when death certificate information became available, categorisation was changed in a few cases.

Benefit was defined as a change in management of the child as a result of diagnosis. This was a consensus view and was often difficult to ascribe. It was assigned on the basis of the diagnosis, whether it was ascertained through screening and whether it changed management. 6 categories were described – dis-benefit, no/neutral, benefit from earlier diagnosis of SCID, benefit from earlier diagnosis of non-SCID TCL, benefit from earlier diagnosis of another condition and unknown. See 4.2.1.

2.8 SCID operational implementation

During the period where screening for SCID is not a regionally commissioned service responsibility for commissioning lies with the antenatal and newborn Screening Operations, Vaccination and Screening Directorate, NHSE.

Regional screening and immunisation teams provided extensive support to the project to operationalise delivery including changes to the model of delivery for BCG.

2.9 Procurement

This group included project team and business and commercial colleagues to develop business cases, procure the services required in line with PHE and then NHSE requirements. This included contracts with suppliers for production and supply of reagents, contracts with screening laboratories for staff costs and equipment. In addition, contracts were drawn up with academic teams to deliver the economic model and the Utilities research.

2.10 Utilities

Qualitative research to understand the harms and benefits to families and to health service stakeholders was commissioned from an academic unit. The utilities working group developed the specification for this research, which was then carried out by independent researchers, recruited by competitive tender. Their research will continue until enrolled children reach their fifth birthday.

2.11 Health Economics

The health economics analysis was commissioned from the School of Health and Related Research (ScHARR). ScHARR were members of the SCID Board and DMG throughout the evaluation.

2.12 BCG

It was noted that BCG and rotavirus vaccines were contraindicated in a baby with SCID. After discussion with the Joint Committee on Vaccination and Immunisation (JCVI), it was agreed that the BCG vaccine should be delayed until the screening result was available. (see JCVI minutes for October 2018, October 2019 and February 2020).

Information was produced by UK Health Security Agency (UKHSA) and NHSE project team for clinicians and public on changes to the BCG programme. Close joint working arrangements were in place. This ensured timely changes were made to the BCG vaccine programme to coincide with the start of screening for SCID.

2.13 IT and clinical data flows

The group considered what data flows were needed to support the evaluation. New codes were set up to record the screening results, including a "not offered screening" code for babies in non-screening areas. Laboratory Information Management systems (LIMS), Child Health Information Services (CHIS) and the Newborn Failsafe System were modified to accommodate these codes.

Data flows were devised to ensure timely receipt of the outcome from screening for SCID. This ensures babies eligible for BCG vaccine receive the outcome of their SCID result prior to appointment for BCG vaccination. The Newborn Infant Physical Examination (NIPE) IT system (SMaRT for NIPE/S4N) was modified to allow mandatory entry of BCG eligibility. Timely data flow of the SCID result was expedited to ensure BCG providers had SCID screening codes for babies eligible for BCG.

This workstream reported directly to both the SCID Board and the UK Health Security Agency (UKHSA) BCG implementation Board. Close and joint working was key to the successful completion of this work

2.14 Links with devolved nations and cross boundary flows

The SCID screening evaluation was in England only, but the UKNSC recommendation it informs will be to all four nations. Representatives from the

devolved nations were on the board and so were informed of progress with the evaluation throughout.

The management of babies impacted by flows between SCID screening and non-SCID screening areas was planned and implemented. (See Appendix 10).

2.15 International links

International experiences of screening informed the design of the evaluation. A Board member from the Netherlands chaired the DRP. Screening laboratory personnel visited screening labs abroad to learn from their experiences. Regular meetings are held with services in Australia and New Zealand.

In 2021, the International Society for Newborn Screening (ISNS) hosted a virtual international conference on screening for SCID. Counties across the world who were screening for SCID shared their experiences of screening.

3. Timeline for the ISE

The pressures on the NHS, consequent on the COVID pandemic, meant that it was not possible to start recruiting to the evaluation until September 6th 2021. It had been planned to recruit for 2 years, but the numbers of babies found with SCID and the introduction of a new screening methodology meant it was extended for a further 6 months. Data continues to be collected on babies screened after this, so as to assess the new technology and estimate the incidence of SCID. Babies with SCID and idiopathic T-cell lymphopenia, in particular will be monitored to look at their longer-term outcome.

4. Findings

In this section we will describe the findings, problems encountered and what action was taken.

Refer to the Appendices for further information (**Laboratory Appendix 1-9**).

4.1 Screening laboratory findings

4.1.1 Laboratory TREC Tests – Basic Principle

TREC DNA is measured. If low, the measurement is repeated alongside measurement of a control gene.

Once the assay has been run, the levels of both the TRECs and the control gene can be used to identify screen-positive babies (those with low TRECs presumed to have SCID) and screen-negative babies (those whose TREC levels are above a cut-off value). The control gene is used to ensure that there are no problems with the spot itself, DNA extraction or analysis.

4.1.2 Alternative Methods Tested

Two approved commercial kits were available for SCID screening at the start of the evaluation – the Perkin Elmer Enlite™ Neonatal TREC kit and the Immuno IVD Spot-it™ kit.

4.1.3 The Plan

We agreed that six labs would participate in the evaluation, three evaluating each method. (see Laboratory Appendix 1: Options for Delivery of Laboratory Services for SCID Screening)

Over two years, it was estimated that this would result in 360k in the PE Enlite group and 415k in the ImmunoIVD group. The remaining unscreened population in the UK, over the two years, would be 712k.

4.1.4 Initial Algorithm and Choice of Cut-Offs

This was informed by:

- The initial validation work performed by the laboratories. (see Laboratory Appendix 2: Validation Protocol)

- Experience in other centres in Europe and Worldwide.
- The desire to set the cut-off sufficiently high to allow data to be collected that would enable modelling of different scenarios and recommendation of the option which optimises clinical sensitivity and specificity.
- The requirement to balance the need to avoid generating large numbers of false positive results whilst minimising the risk of missing a SCID case.

We were conscious that the level at which we set the cut-off value was crucial to balancing the harms v benefits for families when accepting the offer of screening.

See Laboratory Appendix 3: Considerations Relating to Establishing the TREC Cut-Off Value to be used when screening for SCID & Laboratory Appendix 4: Algorithm and Cut-Offs Implemented at the start of the Evaluation.

4.1.5 The issue of premature babies

Published data showed that a large proportion of babies with false positive results on TREC testing were premature.

In centres worldwide premature babies are handled in a multitude of different ways which include:

- Using identical cut-offs & pathway for term babies.
- Using term cut-offs but repeating any screen positive results at 37 weeks equivalent gestation.
- Using term cut-offs but repeating any screen positive results at two weekly intervals and referring only those that remain positive at 37 weeks equivalent gestation.
- Using separate cut-offs for premature babies.

We considered the approach of not referring premature babies with an abnormally low number of TRECs direct to flow cytometry (as would be the case for term babies) and instead retesting the babies after a specific interval had elapsed since there was evidence that this reduced the number of babies referred for flow cytometry and lowered the false positive rate. However, concern was voiced that, in theory, this

could mean that babies born prematurely with SCID, would be left for a number of weeks without specific treatment. Premature babies frequently suffer from infection and it would prove clinically difficult to distinguish this 'normal' state of affairs from an immunological problem. Therefore, we decided to opt for a lower cut-off for premature babies for immediate referral and to retest premature babies with TREC levels between this cut-off and the term cut-off at 37 weeks equivalent gestation.

4.1.6 Algorithm Modification

Almost immediately following implementation it was noted that the En-Lite TREC assay in Sheffield was running with a marked negative bias resulting in an unacceptable number of referrals. As a result, a decision was made to reduce the cut-off for this site only and the rationale for this is summarised in the paper "Suggested Amendment to TREC cut-off in Sheffield" in Laboratory Appendix 5. This lower cut-off was maintained although no explanation for the on-going and persistent bias was identified.

Initially, the combined referral rate was approximately 1:1500 which, whilst not unexpected, was proving stressful for families and demanding for the clinical teams. Therefore, in September 2022, the algorithm was modified.

The main adjustment to the algorithm related to the handling of duplicate results obtained following an initial result below the assigned cut-off. The lower of the duplicates was being used to determine subsequent action and a decision was made to replace this with the geometric mean of the two repeat measurements. Individual values below the Limit of the Blank (the highest apparent analyte concentration expected to be found when replicates of a blank sample containing no analyte are tested) were assigned a value of zero. This has the effect of reducing the impact of single elevated result or "flier" and thus minimising the potential for a missed case.

The new version of the algorithm and a paper which describes the rationale for the algorithm modifications in more detail can be found in Laboratory Appendices 6 & 7.

4.1.7 Introduction of a third method (Revvity EonisQ)

The Revvity Eonis Q method utilises real time dry QPCR technology. The use of dry QPCR technology is associated with a simplified workflow, shorter assay time and removes the requirement for a clean area.

It was agreed at the outset that it would be valuable to include EONIS Q in the evaluation once the technology became available for the following reasons:

- Evidence presented at an International SCID meeting which took place in January 2021 indicated that the number of false positives reported when using RTPCR methods was significantly lower than among PE Enlite users.
- It was thought to be highly likely EONIS Q would become a replacement for En-Lite and that Revvity may not support EnLite into the future. This was subsequently confirmed by Revvity.
- We had experienced some issues with reagent supply from ImmunoIVD (related to COVID and Brexit) and it seemed prudent for the programme not to be in the position of relying on a single RTPCR kit provider.
- Dry RTPCR technology potentially offers some advantages which it would be advantageous to explore.

It was agreed that EONIS Q should be introduced from Sept 2022 in 2 Enlite labs as a replacement, together with one additional lab of around 50k samples pa which was currently not part of the SCID screening evaluation. It was estimated that this would result in 244K in the EnLite group, 170K in the EONIS Q group and 430K in the ImmunoIVD group. The remaining unscreened population in the UK would be 449K.

Unfortunately, the development of the EONIS Q technology was delayed and when kits did finally become available early in 2023 the validation work performed by the screening labs demonstrated a requirement for further assay development and optimisation. The method was finally introduced at the end of November 2023, allowing only 3 months of data to be collected on its performance prior to the end of the evaluation in 2024. The cut-offs have been reviewed several times since implementation and have subsequently been adjusted in order to reduce the referral rate whilst ensuring detection of babies with SCID. See Laboratory Appendix 8 for the current EONIS Q algorithm.

4.1.8 Comparison of the three methodologies

A questionnaire was circulated to all labs regarding analytical performance, operational issues, specific problems with equipment and reagents and timeliness of response of supplier to any of these issues.

Summary data for each of the 3 methods and the 2 suppliers is provided in Laboratory Appendix 9

4.1.9 General Points & Lessons Learnt

- The qPCR methods are essentially semi-quantitative and cannot be assessed using conventional biochemical performance criteria. There is a lack of understanding regarding this not just by NBS labs but also to some extent by the kit manufacturers and some UKAS inspectors.

- Manufacturer's quoted Limits of Detection cannot be used to set cut-offs in the same way as they would be applied for biochemical assays.

- Units and technologies differ between kits and method specific cut-offs must be determined using clinical performance data.

- An understanding of specific laboratory configuration required for PCR methods in order to optimise performance and minimise contamination is essential.

- Careful consideration needs to be given to staffing levels and grades required for assay evaluation which is in excess of requirements for routine running of the method. There is a significant training resource requirement in order to make staff competent to work flexibly across PCR and biochemical NBS assays.

- There were more problems experienced with the PE En-lite end point PCR method than the IIVD real-time PCR method and none of the 3 labs would recommend it. The Revvity EONISQ (formerly PE) real time PCR method was unfortunately only available for the last few months of the evaluation and assessment of its performance is on-going.

- Key considerations in the choice of PCR kits includes instrumentation availability & support, kit supply, shelf life & lot to lot variation, frequency of plate failures and manufacturer's response to method performance and kit supply issues and other problems.

- Ability to make timely changes to cut-offs and testing algorithm at a reasonable cost is important.

Table 1: Summary of responses to survey **(See lab appendix 9 for full table)**

	ImmunoIVD	EnLite	EONISQ
General Comments	At the outset the method appeared to be quite complex with numerous steps but has proved reliable. Analysis can be undertaken by Band 4/5 staff. Method is susceptible to Lithium Heparin interference which caused a high number of repeats requiring liaison with and education of staff within the neonatal units.	None of the 3 labs would recommend this method – problems cited include variability in performance, too many plate failures, susceptibility to contamination despite workflow being configured to meet initial PE recommendations.	Method and workflow are good. Assay was marketed as fully developed – in fact labs supported Revvity in required further development work in order to make the method fit for purpose. Refinement of cut-offs is on-going but the assay has potential.
General Experience with supplier	Supplier scored highly in all categories	Same supplier for EnLite and EONISQ. Generally satisfactory but occasional issues with timeliness of response.	

Table 2: Clinical Performance – Descriptive Data and Comparison of Methodologies

Number of babies referred for flow cytometry after a positive screen:

A) By sex

Sex	**Number of babies referred**
Female	219
Male	344
Indeterminate or not known	5
Total	**568**

B) By ethnicity

Ethnicity (Census 2011 group categories)	**Number of babies referred (%*)**	**Number not referred (%*)**
Asian, Asian British, Asian Welsh	89 (17%)	151,172 (16%)
Black, Black British, Black Welsh, Caribbean or African	44 (8%)	66,527 (7%)
Mixed or Multiple	35 (6%)	81,426 (9%)
Other ethnic group	22 (4%)	2,9,281 (3%)
White	324 (57%)	593,218 (62%)
Not stated	54 (10%)	33,315 (4%)

Please Note: *Percentages do not amount to exactly 100% due to rounding.

C) By gestation

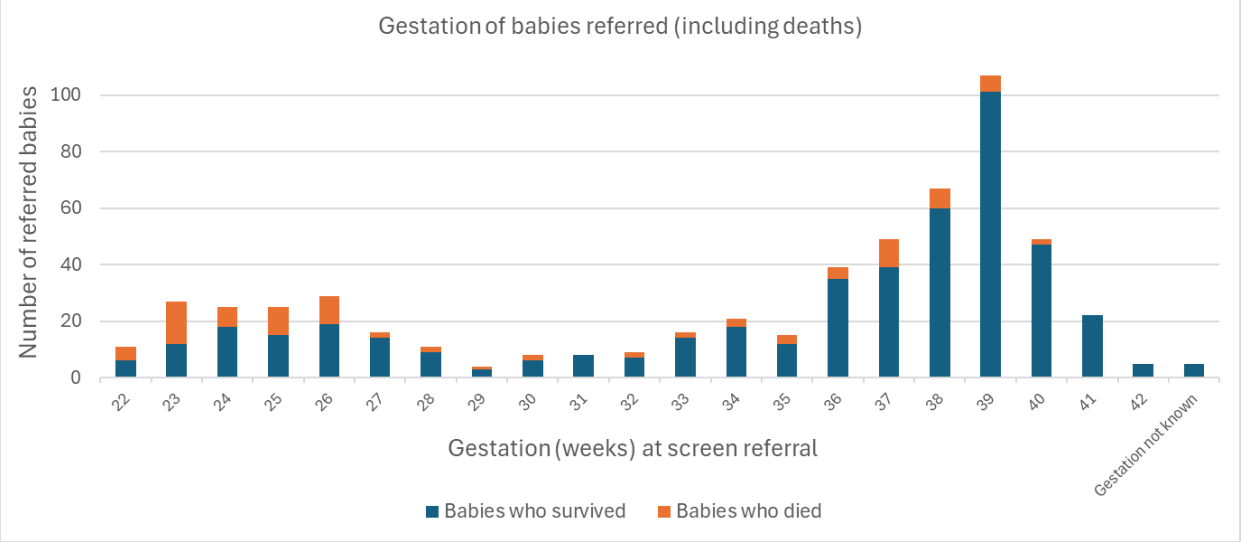
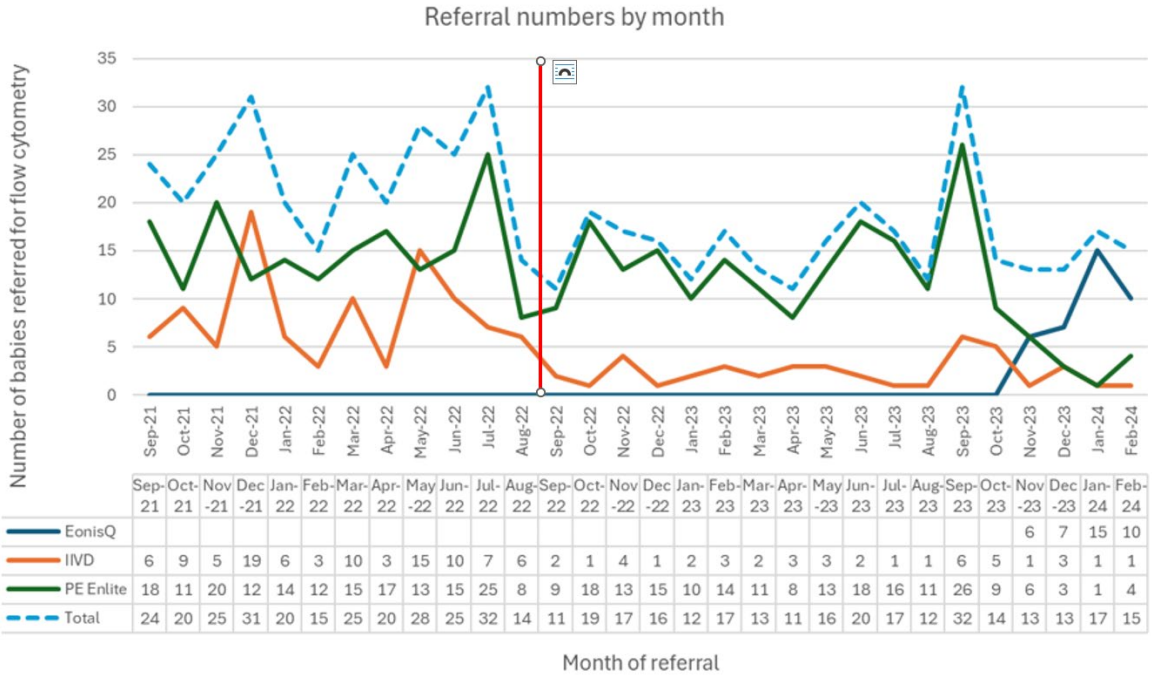


Table 3 By test method

Test method	Total number of babies (number)	Screen negative babies (number)	Screen positive babies (number)	Screen positive rate (per 10,000)
IIVD	485,775	485,633	142	2.92
PE Enlite	434,518	434,131	387	8.90
EonisQ	35,213	35,175	38	10.8
Unknown	1	-	1	-
Total	**955,507**	**954,939**	**568**	**5.94**



Screening test performance

NOTE: We have 1 positive screen by 'unknown' method – this is not included in the numerators below however any negative screens by 'unknown' method will be in the denominator as we can't separate these out. Aggregate data denominators and the number of inconclusives have been confirmed.

Table 4: SCID only – methods comparison table

In this scenario, all non-SCID screen positives are false positives.

	TP	FP	TN	FN	Sensitivity	Specificity	PPV
All methods	12	556	954,939	0	100.00%	99.94%	2.11%
IIVD	6	136	485633	0	100.00%	99.97%	4.22%
Enlite	6	381	434131	0	100.00%	99.91%	1.57%
EonisQ	0	38	35175	0	-	99.89%	-

NOTE: False negative SCID cases (clinically presenting) would be notified to the specialist services so the figure of 0 is validated.

4.1.10 Screening Laboratory Summary

The screen positive referral rate was lower and the PPV higher for the IIVD method during the period of the evaluation. This may be, in part, inherent to the method, but also to the setting of cut-offs.

Early problems with contamination and imprecision of the En-Lite method led to extreme caution regarding any lowering of the cut-off which might lead to cases being missed. Maintaining a higher cut-off was also consistent with the study aim to allow data to be collected that would enable modelling of different scenarios and, therefore, to recommendation a protocol which optimises clinical sensitivity and specificity.

Monthly referral data suggests that the changes to the algorithm made on 5 September 2022 resulted in a reduction in the referral rate for IIVD but had little or no impact on the referral rates for the En-Lite method. The differential outcome for the two methods was unexpected and thus far defies explanation.

There is insufficient data at present to assess the clinical performance of the EONISQ method. Optimisation and evaluation of this method is on-going.

4.2 Clinical findings

4.2.1 Assignment of benefit to outcomes

It is not possible to ascertain whether an individual baby has benefitted from TREC screening on the basis of the condition identified. A baby with SCID might have been identified on the basis of a positive family history; some cases of 22q11 deletion have no or minimal T-cell dysfunction and almost half of the babies with Down syndrome had normal T-cell subsets. Therefore, the Diagnostic Review Group (DRG) was asked to ascertain, in view of the condition and the clinical features, whether the particular baby had benefitted from screening, using the following categories. No attempt was made by DRG to describe the size of the benefit or otherwise.

Disbenefit - no benefit was gained and the family had been caused distress, by being told their baby might have a life-limiting condition, when this was not confirmed. This would apply to babies with a normal T-cell phenotype, those with a reversible T-cell lymphopenia and those who did not fully complete their investigations, because they died or were lost to follow up.

No/neutral benefit – the diagnosis had been suspected on clinical grounds or family history and the relevant investigations would have taken place, irrespective of the screening result.

Benefit – this might be the earlier diagnosis of SCID, a syndrome with T-cell lymphopenia such as 22q11 deletion or a non-syndromic T-cell lymphopenia such as FOXN1 heterozygocity or ligase 4 deficiency. If the diagnosis had not been suspected clinically, or on the basis of the family history, and management of the baby/child was changed to their benefit, they were assigned this category.

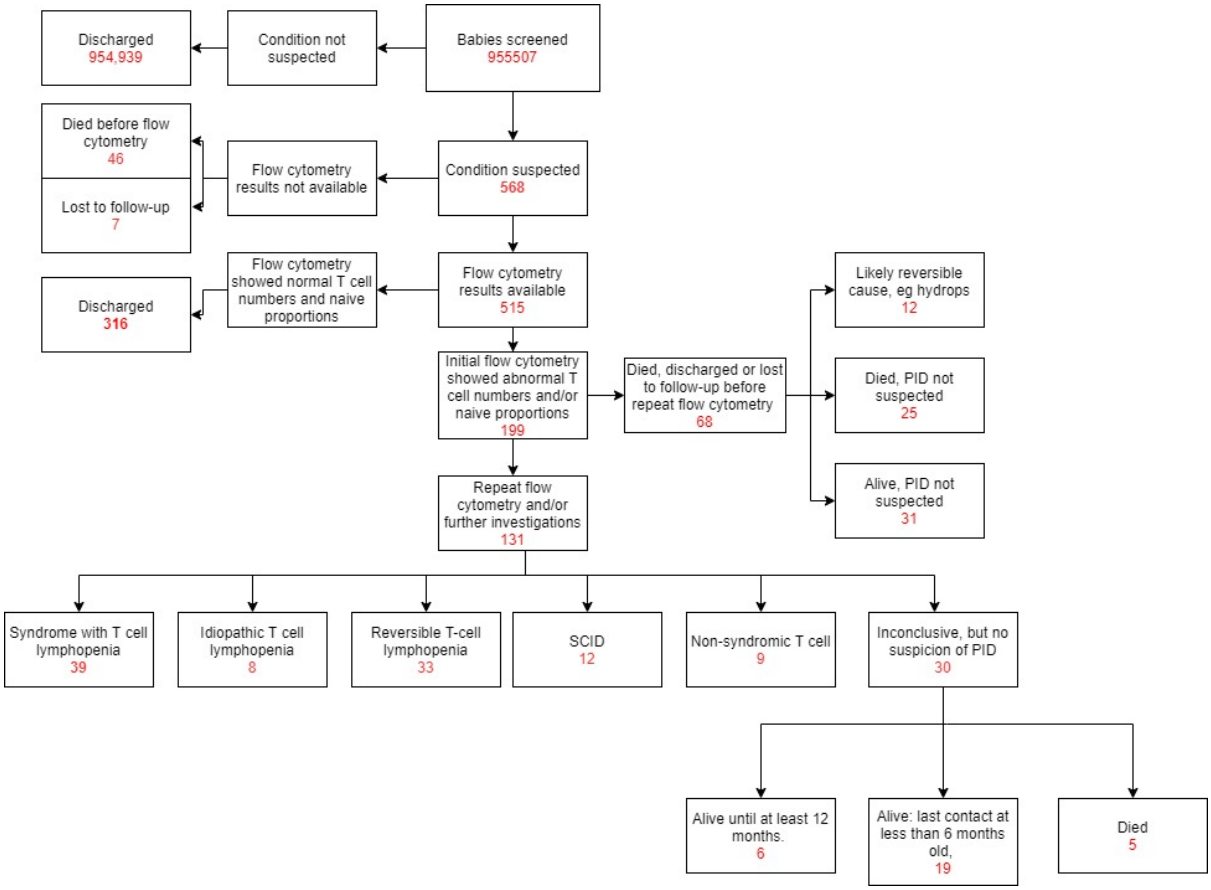
Unknown – in some cases, particularly of idiopathic T-cell lymphopenia, it was not possible to ascertain, at this stage, whether the baby/child had benefitted from screening and so they were allocated this category.

This was not an easy task and knowledge subsequently obtained from the utilities study that a screen positive result was an additional burden, even for families of sick prem babies, means that in retrospect, the benefit for some babies would probably be reclassified from ‘neutral’ to ‘dis-benefit’. The numbers that this would apply to are

small and the utility change for each is small, so it would not impact on the economic analysis.

4.2.2 Outcomes for screen positive babies

Following TREC assay, 568 children (1 in 1,680 screened) were referred to the local immunology service for further assessment. Premature babies were overrepresented in this group (23% <28 weeks gestation as opposed to 7.5% in the screen negative population), most of whom had major complications. This was reflected in the fact that 8% (46/568) died before having a satisfactory flow cytometry (FC) assessment. A total of 56% (316/568) had a normal T-cell phenotype on initial flow cytometry (Total CD3 count >1,500 and naïve T-cells ≥70%), while a further 30 died without PID being suspected. In addition, another 68 children did not complete a full diagnostic assessment, but were known to be alive without suspicion of PID, of whom 12 had a likely reversible condition causing the abnormal flow FC. In relation to this group of 68 children, many of the children had been discharged by the clinician on the basis of the flow cytometry and clinical status. They had judged that the child had no significant T-cell anomaly and that no further intervention was appropriate.



Flowchart: clinical outcomes for all babies tested.

(Appendix 11 shows the outcomes for babies screened using IIVD only.)

Classification	No.	Benefit	Disbenefit	Neutral	Unknown
SCID	12	10		2	
Syndrome with T-cell lymphopenia	39	21		14	4
Non syndromic T-cell lymphopenia	9	4	1	3	1
Idiopathic T-cell lymphopenia	8	2			6
TOTAL with confirmed non-transient TCL	68	37	1	19	11

Proven reversible T -cell lymphopenia	33		26	6	1
Normal T-cell subsets	316		316		
Inconclusive	151		137		14
GRAND TOTAL	568	37	480	25	26

Table 5 Analysis of cases of T-cell lymphopenia and assignment of benefit – all screened babies

Appendix 12 shows the same data but only for babies tested using IIVD

4.2.2.1 SCID Cases ascertained during the evaluation period

The historic or 'retrospective' cohort of babies with SCID, from 2010 to the start of the evaluation are considered in some detail in the economic report and so are not discussed further here. This section is devoted to those babies screened or presenting during the evaluation period.

The evaluation period ran from September 2021 to February 2024 and covered initially six, then seven of the 13 screening regions in England. During that period 21 patients were referred to the two supra-regional services that treat patients with SCID, at the Great North Children's Hospital, (GNCH) Newcastle upon Tyne and Great Ormond Street Hospital (GOSH), London. Of these 21 patients, 11 were treated at GNCH (5 male, 6 female) and 10 were treated at GOSH (7male, 3 female). Four patients were known to have consanguineous parents.

The following analysis is broken down into 3 sections – screened patients without a family history of SCID, unscreened patients without a family history of SCID and patients with a family history irrespective of whether screened.

Screened Patients

Of the 21 patients, 12 were detected by screening, and of these, 2 also had a family history of SCID – they were diagnosed before the screening result was available, but

both were also detected by screening. Subsequent analysis covers those without a family history.

The median age at being referred to the SCID specialist centre was 19 days (range 12-31 days). In one patient, the screening test initially had to be repeated – by the time a follow up test had been performed, the patient had been admitted to hospital with cytomegalovirus (CMV) and respiratory syncytial virus (RSV) infection – CMV was likely contracted from breast milk, as the mother was breast feeding at the time of diagnosis). None of the patients received the BCG or rotavirus vaccine. One contracted rhinovirus infection pre-treatment. In terms of molecular diagnosis, One each had *RAG1* and *RAG2* deficiency. Two patients had *IL2RG* deficiency, 2 had *RMRP* (Cartilage Hair Hypoplasia) deficiency, and one each had *ADA* (and received 138 days of PEG-ADA treatment), and *PSMB10* (recently described) deficiency. One had *FOXI3* deficiency (a thymic defect), and one was undefined. The median age at definitive treatment was 92 days (range 49-136 days). No patient in this cohort required paediatric intensive care unit (PICU) admission prior to definitive treatment. The median ward admission prior to definitive treatment was 1 day (range 0-89 days). Four patients required PICU admission post-definitive treatment and for them the median length of admission was 8 days (range 3-39 days). The median length of admission on the ward post-definitive treatment was 58 days (range 13-111 days). Overall survival was 10/10 The majority of patients have or are about to discontinue immunoglobulin treatment, and have either responded to vaccines or are about to be vaccinated. The patient with FOXI3 deficiency has poor immune reconstitution and is awaiting a second transplant.

Non-screened

Two unscreened patients presented with a family history and seven patients presented with recurrent infection.. Subsequent analysis covers those without a family history. Two patients had consanguineous parents. In one patient, consanguinity status was undocumented. The median age at being referred to the SCID centre was 148 days (range 90-173 days). Five patients received BCG vaccine and 6 received the rotavirus vaccine. All patients had infection at time of presentation. In 3, rotavirus was documented, and in 3 BCG infection was documented. In 5, *Pneumocystis jiroveci pneumonitis (PJP) was present, of whom 3 required mechanical ventilation prior to HSCT.* One patient had CMV infection, and 2 had RSV infection. Two patients had *RAG1.* There was one case each of RAG2

deficiency and *DCLRE1C* (artemis)One patient with 22q11 deletion had a thymic transplant. 2 patients were molecularly undefined – one proceeded to haematopoietic stem cell transplantation (HSCT) and one was eligible for a thymic transplant, but died before this could take place. The median age at definitive treatment in was 198 days (range 153-241 days). Four patients required PICU admission prior to definitive treatment for 7, 15, 21 and 43 days respectively. The median ward admission prior to treatment was 58 days (range 20-258 days). Twor patients required PICU admission post-definitive treatment. The median length of admission on the ward post-definitive treatment was at least 146 days (range 35->437 days). Two patients died before receiving definitive therapy – one pre-HSCT from pseudomonas sepsis and PJP, and one pre-thymic transplant from BCG pneumonitis. Two other patients died post-HSCT, one each, of fungal sepsis and veno-occlusive disease complicated by rotavirus and BCG infection and another of parainfluenzae and RSV pneumonitis. A further patient died of klebsiella sepsis post thymic transplant. Thus, overall survival was 2/7 (29%)

Of the 2 survivors in this cohort, at the time of reporting, one remains in hospital more than 1.5 years post transplant and has multiple complications, including autoimmune disease, CMV retinitis and global developmental delay.

Patients with a family history of SCID

There were four patients in this group, two of whom had also been ascertained by screening. Their characteristics were very similar to those patients ascertained by screening alone. All were treated with HSCT, one twice. One patient with NUDCD3 died and one required two transplants.

The following table summarises the results from these three cohorts.

	Screened positive	Family history of SCID	Unscreened
Total No.	10	4	7
Age at referral to SCID centre	21 (12-37)	0	148 (90-173)
Given BCG	0	0	5
BCGosis	0	0	3
Rotavirus infection	0	0	3
PICU stay prior to definitive treatment (median, range)	0	0	18 (7-43) for the 4 who needed it
Standard care prior to definitive treatment (median, range)	1 (0-89)	11.5 (0-27)	55 (20-258)
Age at definitive treatment (median, range)	79 (49-136)	73 (74-80)	189 (76-241)
PICU stay after definitive treatment (median, range)	8 (3-39) for the 4 who needed ICU	3 (0-39)	>20, 21 and 41 for those needing it
Standard care after definitive treatment (median, range)	58 (13-111)	58 (31-113)	132 (35-437*)
Survival	10/10 (100%)	3 /4 (75%)	2/7 (43%)

All measurements are in days, with median and ranges.

*This baby was still on PICU at the time of reporting.

Table 8: summary of outcomes for babies ascertained with SCID during the evaluation period

Patients ascertained in 12 months following the evaluation period, ie March 2024 to February 2025

There were at least 16 patients in this category, four of whom had a family history. It is too early to assess any outcome data.

Conclusion

In summary, overall survival in the group ascertained by screening or family history was 86% (12/14) with fewer days in PICU. None of these children had complications related to BCG or rotavirus vaccine and they had predominantly good outcomes post-definitive treatment with the majority of patients stopping long term immunoglobulin replacement and responding to vaccination. In the non-screened cohort, overall survival was poor at (3/7 (43%) with two patients dying before definitive therapy and another two after., Many experienced complications secondary to BCG and/or rotavirus infection and post transplant outcomes have been complicated necessitating prolonged hospital admission or ongoing treatment.

4.2.2.2 Non-SCID T-cell lymphopenias

The following applies to all cases arising as a result of screening, irrespective of screening modality.

Syndromes with T cell lymphopenia

The estimates of incidence and prevalence of some of these conditions in the general, as opposed to screened, population varies significantly between studies. For more details of this and other characteristics of the conditions, see Appendix 4 of the NSC report *Newborn screening for severe combined immunodeficiency (SCID) in the NHS Newborn Blood Spot (NBS) screening programme: A rapid evidence review*.

The degree of T-cell abnormality in the cases in this group varied from borderline clinical significance to profound. Amongst the conditions in this classification were the following:

22q11.2 deletion (Di George) syndrome

Deletion of 22q11.2 results in a broad range of clinical findings. The incidence at birth is of the order of 1 in 2,000 to 1 in 6,000 live births. Most cases are not detected on newborn screening for SCID. A longitudinal study based in Philadelphia revealed a multitude of features including congenital heart disease (64%), hypocalcaemia (55%),T-cell dysfunction (50%), cervical spine abnormalities and a wide range of dysmorphologies. It and CHARGE syndrome (see below) are the commonest causes of congenital athymia and may require thymic transplantation. The median age at diagnosis was 2.6 months in those with congenital heart disease and 3.1 years in those without. Limited data on morbidity and mortality suggests that early diagnosis through NBS improves outcome.

22q11.2 deletion was the commonest condition, by far, in this category, during the evaluation, with 17 cases. Nine cases were associated with congenital heart disease and a further two with neonatal hypocalcaemia. It was felt that at least nine cases had benefited from screening, as they would not otherwise have been recognised and, as well as their immunodeficiency not being recognised early, other known associated anomalies could be sought and more optimally managed.

A further case had abnormal TRECs, but normal flow cytometry.

Down Syndrome

Children with Down syndrome are known to have increased susceptibility to a number of infections for a variety of reasons, including immunological abnormalities. Of 19 babies who had abnormal TRECs, nine had normal T-cell subsets, eight had varying degrees of T-cell function, in one case an abnormality of flow cytometry reverted to normal and the findings were inconclusive in one. Although routine full blood counts are undertaken in babies with Down syndrome, not all T-cell anomalies would be identified.

Ataxia telangectasia (A-T)

A-T is an autosomal recessive disorder which manifests primarily as a neurological problem, most frequently abnormal gait. The reported incidence varies from about 1 in 40,000 to 1 in 300,000. The disorder is associated with an increased risk of infections, particularly of the respiratory tract, however severe and opportunistic infections are uncommon. Patients with the disorder have an increase in malignancy, secondary to radiosensitivity. Carrier women, and therefore the mothers of babies with A-T, are at increased risk of breast cancer. A recent systematic review found the median age of death to be 14 years with a very wide range. The most common cause of death is malignancy, followed by respiratory problems. The benefit of early detection is not primarily related to the avoidance of infection, but the theoretical reduction of the risk of malignancy, by the avoidance, where possible, of ionising radiation and reduction in exposure to ultraviolet light. There is no direct evidence for this. A recent case report discussed the benefit of early HSCT but highlighted the variable course of the lymphopenia and the lack of robust evidence for this intervention.

The indirect identification of an increased risk of breast cancer in A-T carrier women and the lack of evidence for a definitive treatment for A-T raises some ethical issues. In a survey of parents of children with A-T or healthy newborns, c. 75% of each group favoured specific investigations for A-T in a child with a positive screening result for which another explanation was not available, before they developed symptoms. When asked if they would favour the introduction of A-T in the NBS programme, 76% of parents of children with A-T said “yes” and over 90% of parents with healthy children favoured its introduction.

In the evaluation, there were four unlinked cases identified. In one, family members were being investigated for neurological abnormalities.

CHARGE syndrome

The incidence of the syndrome is c. 1 in 10,000 to 1 in 17,000 live births. It is usually due to a mutation in the CHD7 gene of which 90% occur de novo. CHARGE is an acronym for coloboma, heart disease, atresia of the choanae, retarded growth and mental development, genital anomalies, and ear malformations and hearing loss. Immunodeficiency may also be present due to athymia. The phenotype is very

variable. The immunodeficieny may be so profound as to require thymic transplantation.

In the evaluation, there were three cases, of whom two had been recognised, independent of screening.

Other

There were seven other cases, in three of whom screening led to an earlier diagnosis of the underlying problem.

Idiopathic T cell lymphopenia (ITCL)

These babies had at least two abnormal flow cytometry results, with no PID variants found on genomics and no cause found for the TCL. The degree of T-cell abnormality varied and it was difficult to assess whether the children had benefited from identification. In two cases, a lower threshold for antibiotic treatment was instigated and live vaccines were withheld.

This is a condition in which many cases with T-cell abnormalities are probably not recognised in the absence of screening and the natural histories of those that are is not well characterised. In some, the T-cell abnormality may resolve in time and never cause a problem. In these cases, identification by newborn screening would be harmful as it would cause unnecessary worry for parents and a waste of healthcare resources. All cases of ITCL will be followed up long term to follow the natural history of their T-cell abnormality and its effects on the child’s health.

Reversible T cell lymphopenia

A total of 45 babies had proven or assumed reversible cause for their T-cell lymphopenia. The single largest group of conditions in this category was that secondary to conditions such as hydrops fetalis and pleural effusions. There was other genetic and acquired conditions and one instance where a mother had taken azathioprine during pregnancy.

Non syndromic T-cell lymphopenia

The nine cases in this group were made up of two cases each of FOXN1 heterozygocity and neonatal haemochromatosis, and one each of cartilage hair hypoplasia, following surgery for transposition of the great vessels, SGPL1 and STAT2 deficiency, ligase 4 deficiency and juvenile myelomonocytic leukaemia (JMML).

Summary

Of the 568 babies who had abnormal TRECs, the majority (316) had normal flow cytometry or a proven or probably reversible condition (45). Of the remainder, a large group (76) died before investigations were completed, reflecting the high number of severely ill premature babies. Babies in these groups derived no benefit from screening. To the contrary many families were put through the stress of being told that their baby may have a life-threatening disorder, when this did not turn out to be true.

12 babies were identified with SCID, of whom, 10 in the absence of screening, would not have presented until symptomatic and therefore more difficult to treat. Although the numbers are small, the magnitude of the benefit to them probably outweighs the disbenefit to the larger number of babies who were found to have no significant T-cell anomalies.

There was a small group of babies (48) with a variety of T-cell anomalies, about half of whom benefitted from early recognition.

In addition, there were eight babies who had a persistent T-cell abnormality for which no cause has been identified. In at least two, the management of these babies was altered because the condition had been detected by screening. These babies probably benefitted from screening, but it is unclear and only long term follow up of all will help clarify whether screening has been of benefit to this group. This is similar to the uncertainty inherent in the designation of Cystic Fibrosis Screen Positive, Inconclusive Diagnosis (CFSPID) following newborn screening for CF.

4.3 Algorithm Modelling

Since it had been confirmed that Revvity would no longer be supporting the implementation of the En-Lite methodology in new laboratories and data on its replacement, the EONISQ method, was limited a decision was made to confine algorithm modelling to the ImmunoIVD method.

Based on previous studies , , , and in the interest of ascertaining whether we can improve on current performance of the IIVD method it was agreed to test the following scenarios, where A represents the Analytical Cut-off, B represents the Clinical cut-off and C represents the cut-off for immediate clinical referral of premature babies

Scenario 1 - Aims to identify all true SCIDS and as many non-SCID TCLs as possible who would benefit whilst maintaining positive referral rates within acceptable limits. A=12 B=8 C=4

Scenario 2 - Aims to reduce the false positive rate whilst detecting all true SCIDs but not aiming to capture non-SCID TCLs A=10 B=6 C=4

Scenario 3 - Focuses on minimising the number of premature referrals whilst ensuring no true SCIDs are missed. A=10 B=6 C=1.08 (=Limit of Blank)

The cut-offs in each scenario were applied to SCID algorithm version 2 (see Lab Appendix 6) and assumed a prevalence for SCID of 1 in 50,000.

Table 7: Modelling Outcome – Scenario1

Policy A = 12; B = 8, C = 4	**Gestational age at delivery (weeks)**				
	<32	**[32, 34)**	**[34, 37)**	**≥37**	**All**
Population	5,518	4,138	24,831	424,880	459,367
Composition	1.2%	0.9%	5.4%	92.5%	100.0%
Expected cases (1 in 50000)	0.11	0.08	0.50	8.50	9.19
Duplicates	168	90	228	655	1141
	3.0%	2.2%	0.9%	0.2%	0.2%
Repeats	28	18	50	143	239
	0.51%	0.43%	0.20%	0.03%	0.05%
Term repeats	18	3	4	0	25
	0.33%	0.07%	0.02%	0.00%	0.01%
Referrals	30	5	17	60	112
	0.54%	0.12%	0.07%	0.014%	0.02%
PPV (for SCID)	0.37%	1.66%	2.92%	14.16%	8.20%
	1 in 272	1 in 60	1 in 34	1 in 7	1 in 12

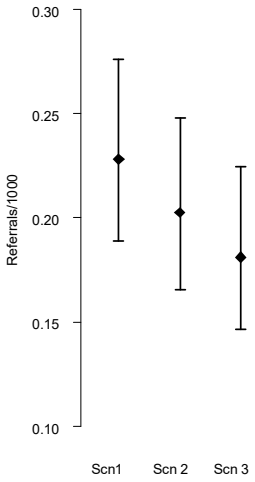
Table 8: Modelling Outcome – Scenario 2

Policy A = 10; B = 6, C = 4	**Gestational age at delivery (weeks)**				
	<32	**[32, 34)**	**[34, 37)**	**≥37**	**All**
Population	5,518	4,138	24,831	424,880	459,367
Composition	1.2%	0.9%	5.4%	92.5%	100.0%
Expected cases (1 in 50000)	0.11	0.08	0.50	8.50	9.19
Duplicates	149	87	213	605	1054
	2.7%	2.1%	0.9%	0.1%	0.2%
Repeats	27	17	50	141	235
	0.49%	0.41%	0.20%	0.03%	0.05%
Term repeats	8	3	1	0	12
	0.14%	0.07%	0.00%	0.00%	0.00%
Referrals	29	5	15	46	95
	0.55%	0.12%	0.06%	0.011%	0.02%
PPV (for SCID)	0.37%	1.66%	3.31%	18.47%	9.56%
	1 in 273	1 in 60	1 in 30	1 in 5	1 in 10

Table 9: Modelling Outcome-Scenario3

Policy A = 10; B = 6, C = 1.08	**Gestational age at delivery (weeks)**				
	<32	**[32, 34)**	**[34, 37)**	**≥37**	**All**
Population	5,518	4,138	24,831	424,880	459,367
Composition	1.2%	0.9%	5.4%	92.5%	100.0%
Expected cases (1 in 50000)	0.11	0.08	0.50	8.50	9.19
Duplicates	149	87	213	605	1054
	2.7%	2.1%	0.9%	0.1%	0.2%
Repeats	27	17	50	141	235
	0.49%	0.41%	0.20%	0.03%	0.05%
Term repeats	25	5	7	0	37
	0.45%	0.12%	0.03%	0.00%	0.01%
Referrals	22	5	12	46	85
	0.40%	0.11%	0.05%	0.011%	0.02%
PPV (for SCID)	0.50%	1.76%	4.10%	18.47%	10.83%
	1 in 199	1 in 57	1 in 24	1 in 5	1 in 9

Referral by scenario



Estimated referral rates byscenario with 95% confidence intervals.

Referrals per 1000 tests

Scenario 1 : 0.228 (95% CI: 0.189 -0.276)
Scenario 2 : 0.202 (95% CI: 0.166 -0.248)
Scenario 3 : 0.181 (95% CI: 0.146 -0.224)

Compared to the baseline policy

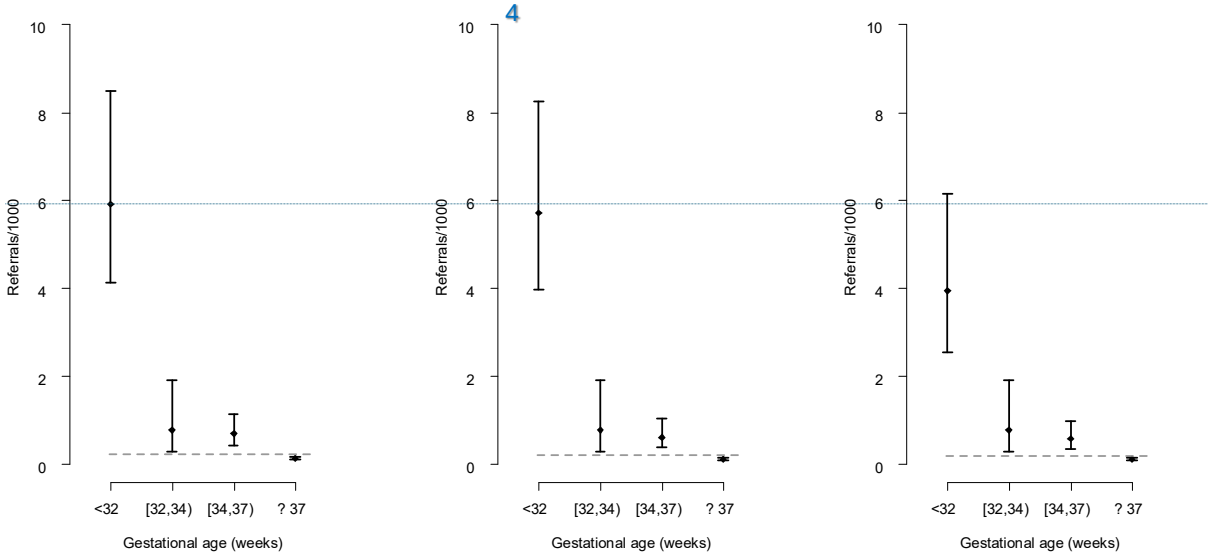
- Scenario 2 reduces the referral rate by 0.026 (95% CI: 0.014 – 0.045) per thousand tests
- Scenario 3 reduced the referral rate by 0.047 (95% CI: 0.031 – 0.072) per thousand tests

Referral rates by gestation

Scenario 1: A = 12; B = 8, C = 4

Scenario 2: A = 10; B = 6, C = 4

Scenario 3: A = 10; B = 6, C = LoB



Benefit by Scenario

All of the screen positive cases identified by the IIVD method during the course of the evaluation were reviewed and benefit assigned by the DRG group. Of 141 screen positives identified by IIVD in total - in 6 cases benefit was unknown and in 12 benefit was neutral. Of the remaining 123 cases, 14 benefited from screening and for 109 there was disbenefit. The number of babies who would have tested screen positive in the benefit and disbenefit categories for each scenario modelled are shown in the table below.

Table 10: benefit by scenarios 1 to 3

	Babies who benefit from screening (n=14)		Babies who dis-benefit from screening (n=109)	
	Number screen positive	% screen positive	Number screen positive	% screen positive
Scenario 1 Cut-offs A12/B8/C4	14	100	76	70
Scenario 2 Cut-offs A10/B6/C4	13	93	69	63
Scenario 3 Cut-offs A10/B6/C 1.08 (Limit of Blank)	13	93	58	53

Summary

PPVs for all three scenarios modelled are higher than those found for the actual in-service evaluation. This is partly because the initial algorithm used at the start of the evaluation (prior to the introduction of the geometric mean) was associated with higher referral and higher false positive rates and partly due to the modelling being based on a disease prevalence of 1 in 50, 000 whereas the prevalence during the course of the ISE was 1 in 79, 000.

Referral rates decrease and PPVs improve with reduction in cut-offs A & B (scenario 2) and cut-offs A, B & C (scenario 3)

The referral rate in babies <32 weeks gestation is disproportionately high and the PPV for this sub-group very low. Reducing cut-off C (scenario 3) leads to a small improvement to the PPV in this sub-group. Consideration should be given to the benefits versus harm for immediate referral of these babies. One option, as discussed earlier would be to dispense with immediate referral altogether and obtain a repeat sample at 37 weeks equivalent gestation in all babies in this sub-group with low initial TRECs.

Reducing the cut-offs significantly reduces the number of referrals in babies who suffer a dis-benefit from screening. By choosing scenario 3 for a trade-off of missing 1 baby with non-SCID TCL who may have benefited the referral of approximately half of the babies who were screen positive in the ISE and dis-benefited could be avoided.

4.4. Retrospective data

Data was collected on 146 cases of SCID ascertain between 1st January 2010 and 6th September 2021. This was used to inform the modelling. Information was also collected from HES, ONS mortality data sets and the National Child Mortality Database to ensure that all babies with a diagnosis of SCID were included.

4.5 Incidents related to SCID screening evaluation

Incidents in relation to screening for SCID were reported in line with national guidance on Managing safety incidents in NHS screening programmes - GOV.UK.

Incidents were reported in relation to the implementation of screening for SCID which are similar in nature to those reported with the implementation of inherited metabolic diseases. These related to declines for screening due to miscommunication to families about the nature of the ISE and consent to be part of research.

There were some incidents which related specifically to screening for SCID:

- during implementation of SCID screening methodology 2 laboratories reported high referral rates of screen positive babies due to issues with contamination -

laboratories implemented business as usual arrangements and samples were sent to other laboratories for screening for a 2 month period

- 2 laboratory freezer failures in relation to the storage of SCID screening assays resulted in the destruction of assays and a risk to continuity of the service
- 2 babies with delay in referral into clinical care due to poor communication processes in the diagnostic referral pathway
- an incident was reported in relation to incomplete wording in the SCID outcome reporting code. This resulted in confusion with reporting of SCID results and subsequent delays in babies referred for BCG vaccine

4.6 Utilities from SCID Screening

The aims of this research were to ascertain the effects of the SCID screening programme on families who experienced a range of results and service providers, including midwives, laboratory staff, immunologists and clinical nurse specialists/health visitors.

Summary

Background: This project evaluated the impact of including screening for Severe Combined Immunodeficiency (SCID) in the NHS Newborn Bloodspot Screening[8] (NBS) Programme. Parents[9] and health professionals were recruited primarily from sites (n=8) where this new form of screening is being evaluated as well as additional sites (n=4) where clinicians are involved in the care of these babies and a comparator site (n=1) to recruit parents of children who had received an inconclusive result from other areas of the screening programme e.g., CFSPID.

Work Packages: The project consisted of two work packages to explore:

1. The effects on families whose babies had a positive screening test (low TRECs). This included babies who went on to have a confirmed diagnosis of SCID (true positive for

[8] NBS and screening are used interchangeably throughout this report
[9] Throughout this work we refer to 'parents' for simplicity. However, we recognise that the family situations of the infants may be more complex, with the existence of legal guardians and non-resident parents.

SCID), babies with a normal result on flow cytometry (false positive), as well as those with a screening result suggesting they may have another disorder affecting their immune system.

2. The views and experiences of a sample of health professionals e.g., midwives, laboratory staff, health visitors, immunologists/clinical nurse specialists who were involved in the screening process.

Methods

The project consisted of 2 work packages:

Work Package 1*:* A mixed-methods study (postcards, interviews including journey mapping and questionnaires) conducted with families from the point of screening results being returned to parents (with the intention for this to continue until the child's fifth birthday). Data analysis included simple descriptive statistics and content analysis (postcards), thematic analysis (interviews) and official scoring procedures (questionnaires).

Work Package 2*:* A qualitative interview study conducted with health professionals during the clinical evaluation phase of the national pilot programme. Analysis of interview data from health professionals was guided by Normalisation Process Theory.

Results

Work Package 1: A total of 62 postcards were either returned prior to the first interview or completed as part of the first interview by parents. Of these, 46 were from families who had received a false positive NBS result, nine had received a negative NBS result, three had received a true positive for SCID result and three results were for non-SCID TCL. One family had received a positive NBS for CF and following diagnostic testing was given a CFSPID designation.

Thirty-three families were interviewed; for nine of these, both the child's mother and father were interviewed and therefore a total of 42 parents were included in the interviews; 28 parents who had received a false positive NBS result (n=6 fathers, n=22 mothers) two mothers who had received a negative NBS result, five parents who had received a true positive result for SCID and six a result of non-SCID TCL and one mother who had received a CFSPID designation.

Work Package 2*:* Interviews were undertaken with health professionals (n=45) who had been involved in the SCID screening programme. This included immunologists / consultants, (n=14), midwives (n=11), CNS/screening co-ordinators (n=10), health visitors (n=7), and laboratory staff (n=3) across all study sites.

Findings

Information provision prior to NBS and at the time of the 'heel prick' was considered suboptimal and compromised the informed consent processes. This also appeared to negatively impact parents' experiences when receiving a positive screening result from clinical teams as they reported being unaware of the conditions that had been screened for and the variable outcomes of NBS.

"I do remember her saying that…when the midwife took the bloods that…the initial heel prick test, that we could choose not to have done if we decided to… I didn't really know what I was consenting to, I knew I was consenting to some screening tests, but I didn't know exactly what they were testing for." P38 False Positive

Positive screening results for SCID are distressing for parents. This is particularly true for parents whose baby is an inpatient and is already grappling with the additional stresses associated with having a sick newborn.

"I was on my own…very scary, because you're miles away from home really [admitted to neonatal unit due to extreme prematurity] and you've got nobody to talk to and, you know, share your concerns and your worries with and that's really hard, because she was very ill anyway, so touch and go at the time. So, to have that on top was worrying…shock, I think, because we felt, you know, really unlucky because, 'How can she have that when she's already fighting with what she's got?'" P7 False positive

True positive SCID, non-SCID TCL and false positive screening results, can lead to parental concerns about their child's vulnerability and can lead to parents isolating their children to prevent them being exposed to infections. Parents also reported altering life plans in response to their child's screening result which included decision making concerned with returning to work, enrolling their children in nursery and future reproductive plans.

"And I've been sort of putting it off [returning to work] for a while. Because I was initially supposed to go back in January, and I've said I would reconsider maybe in September… I

don’t really want to put her into childcare at the minute, just because I just don’t want her to be exposed to so many different illnesses because they end up coming home with so many illnesses… I think, due to this and the way that [baby] is, I’ve been quite reluctant to put her into childcare. And obviously, like go out to work and go, and back into teaching.” P28 False Positive

However, for children with a false positive screening result for SCID, these concerns had mostly resolved by the time the child reached their first birthday as they started to be exposed to common childhood infections and their parents could see evidence of them mounting an appropriate immune response. Positive screening results for SCID had the potential to positively impact parenting relationships but could negatively impact parental mental health; the latter did not appear to resolve over time.

Midwives and health visitors in the community reported that screening for SCID had only had a minimal impact on their workload. However, laboratory and immunology teams reported SCID screening had resulted in significant implications for their workloads. Some of this was reported to be attributable to the lack of parental awareness of screening for SCID and the subsequent management of parental anxiety and distress associated with the unexpected positive screening result. These clinicians therefore felt if SCID were to be added to the national screening programme, it would need very careful consideration.

Both parents and clinicians are in favour of SCID being added to the national NBS programme and were able to provide recommendations to improve the success of this.

Conclusions

Receiving a positive screening result for SCID was distressing for parents but revisiting the way screening information is provided to parents and therefore the informed consent process may help to mitigate this. False positive screening results for SCID could cause parents to have a distorted view of their child’s vulnerability in the short term but due to the nature of SCID, exposing children to ‘normal’ infections in the first year of life provided evidence to parents that their child’s immune system was functional, and this helped to mitigate the long-term negative sequalae associated with this.

Consideration needs to be given to how NBS for SCID is implemented in preterm infants who are in the care of the NICU both in terms of decision-making around communication of the positive NBS result or the outcome of confirmatory testing as well as timing due to the impacts of prematurity and underlying illness.

Support for families following a positive screening result for SCID needs to be considered. This includes financial support for families to enable them to safely attend immunology centres at short notice for confirmatory testing as well as psychosocial support given that their experiences will not be the same as parents' experiences prior to the SCID evaluation.

If NBS for SCID is to be rolled out nationally, careful consideration needs to be given to the additional workload this triggers for laboratory and immunology teams and how this can best be managed given finite resources.

Main recommendations

Based on their experiences, parents would recommend that screening for SCID should be included in the national NBS programme.

Consideration needs to be given to the management of screening for SCID in pre-term infants due to the additional anxiety they experience and the high false positive rate.

Screening information resources provided antenatally and at the time of screening and consent processes need to be revisited so that the potential outcomes of screening and the meaning of these outcomes are clearer.

Support (financial and psychosocial) for parents following a positive screening result for SCID requires careful consideration.

Funding for laboratory and immunological team needs revisiting if screening for SCID is added to the national NBS programme.

Consideration should be given to the provision of information to parents following screening in relation to parent-initiated care that is specific to their child's designation/diagnosis.

The impact of screening on parental health-related quality of life, as evidenced by the ITQOL-47, should be recognized in any decision relating to SCID screening. Whether this could be incorporated into economic modelling is less certain, as is specific data that would be used to parameterize such an analysis.

Consideration should be given to using electronic record linkage to assess the extent and cost of patient-initiated care relating to SCID screening results. This would allow one to ascertain whether parents of children with a false positive result, seek healthcare more

often than parents of children with a condition not suspected result. Such an approach should also be considered for any future expansions of NBS to other conditions.

4.7 Health Economics

A report on assessing the cost-effectiveness of screening for SCID, conducted by SCHARR, estimated that the cost effectiveness of screening for SCID compared to not screening is above current NICE and UK Government thresholds but that SCID screening may be economically viable when combined with SMA screening. See the accompanying SCHARR cost-effectiveness report for information on the health economic modelling methods used and the full findings.

The ISE report for screening for SCID can only be properly interpreted by reading, at least, the executive summary of the SCHARR cost-effectiveness report.

4.8 Operational delivery findings

Equipment contracting

Commercial, Contracting and business arrangements were novated to NHSE following transition from Public Health England.

4.9 BCG vaccine, Tuberculosis and screening for Severe Combined Immunodeficiency (SCID)

For a full report, see Appendix 13

In view of the fact that BCG is a live attenuated vaccine and contraindicated in someone who is immunosuppressed, a new pathway was designed and disseminated, along with the IT changes already mentioned. This change was designed to reduce the risk of babies developing disseminated infection with the vaccine (‘BCGosis’), but an inevitable adverse

effect of this change was to delay the vaccine and possibly even reduce the overall uptake of the vaccine. A number of outcomes were monitored to assess the effect of the change.

4.9.1 The effect on the BCG programme, including the uptake of BCG in eligible groups of infants, by age, in a timely fashion.

Most areas of the country give the vaccine to a selected group in the population. Unfortunately, although the number of doses of vaccine given may be known the size of the targeted group was not in the past, so uptake could not be calculated. Improved data collection, as a result of the evaluation, means this data is now available, but a before and after comparison is not usually possible.

Five areas of the country have been offering the vaccine to all babies and in these areas, the uptake is as high as it was before the change. However, the vaccine is being given later than previously. Routine data in the past was collected for babies at 12 months of age. With the change in programme, it is now also collected at 3 months of age. From one area, there are detailed data on timeliness. This showed that prior to the change, the median age of those babies receiving the vaccine was 2 days. In 2022, this had risen to 32 days, but as the new system bedded in, it declined to 27 days in 2023 and 24 days in 2024. From 2016 to 2020, of those vaccinated, between 66.1% and 78.4% of babies received the vaccine by 28 days. For 2022, 2023 and 2024 the figures were 41.6%, 55.0% and 68.8% respectively.

4.9.2 Effect of change in programme on commissioners, providers and parents

Two studies were commissioned to seek views on the change in the programme – one with BCG commissioners and providers, the other with parents. The former has been published.[10] The other has been submitted for publication.

The study with commissioners and providers showed that the change was disruptive and it took time to settle in. Some felt they would have benefitted from more direction on implementation of the pathway. The main challenges were appointment non-attendance and data systems and reaching the 28 day vaccination target. This latter was exacerbated by many units not booking appointments until results were received, as opposed to booking appointments and then, in the rare instance of a 'screen suspected' result cancelling. It was acknowledged that the system now could be monitored properly whereas that was not so easy beforehand.

The study with parents whose children were eligible for the vaccine revealed that many could not remember hearing anything about BCG until receiving an appointment, confusion amongst staff as to eligibility for the vaccine, short notice of the appointment and difficulty getting to the appointment. Parents accepted the logic for the age of vaccination being delayed.

4.9.3 The incidence of TB in young children, including the number of cases of severe TB (TB meningitis, miliary or cryptic disseminateTB).

The change in the BCG programme coincided with the COVID-19 pandemic and so the interpretation of changes in the prevalence of any infectious disease is difficult. The prevalence of TB around the world, including in USA and Europe, including UK, has gone up since the COVID-19 pandemic, 'though it hasn't yet reached pre-pandemic levels. Notifications of active TB, in UK, in 0-14 year olds make up a small proportion of the total – 2.9% (140/4,850) in 2023 and 2.8% (155/5,480) in 2024. Severe TB in under 6s is very uncommon – 7 cases and 6 cases in 2023 and 2024 respectively. There is no clear indication of a rise due the change, but it is too soon to make any firm conclusions.

[10] Jones K, Chisnall, Crocker-Buque T, *et al.* A new neonatal BCG vaccination pathway in England: a mixed methods evaluation of its implementation. BMC Public Health 2024;24:1175. https://doi.org/10.1186/s12889-024-18586-8

4.9.4 The incidence of BCGosis and its association with SCID and other immunodeficiencies.

Data was sought from a number of sources to examine the incidence of BCGosis and its association with SCID. 2 of 9 children who were reported by the National Child Mortality Database (NCMD), since its inception in 2019, to have died from SCID developed BCGosis as did 2 of 7 with other immunodeficiencies. Of 51 children transplanted for SCID at one of the two national centres, prior to the evaluation, none of the 14 infants who were ascertained via a family history had received BCG. Of the 37 infants who presented clinically, 15 had received BCG. Of these, 4 had superficial infection (lymphadenitis, abscess), 4 had systemic/organ disease and 7 had no symptoms.

None of the 14 babies ascertained by screening or a family history, during the evaluation period, received BCG. Of the seven babies presenting symptomatically, five were known to have received BCG and three developed BCGosis, in one of whom BCG pneumonitis was a major factor in the child's death. The BCG status of one was unknown.

Taking the data from clinically presenting cases, in the retrospective cohort from one centre, of 45 cases, 19 (42%) had received BCG and, of these, 7 developed serious BCG disease. This confirms the overlap between eligibility for BCG and risk of SCID.

Conclusion

The BCG programme undoubtedly was a major organisational change. The lack of good data prior to the change makes generalised comments difficult. It is reassuring that uptake in the areas where the offer of BCG was universal did not fall and that the detailed data from the West Midlands have shown that the timeliness of vaccination, although affected by the change is improving as new systems settle in. However much it improves, timeliness will not revert to what it was, but it is unclear how this will affect the incidence of TB.

The numbers of cases of TB in children below 5 years old is small, those with severe TB is very small and COVID-19 has disturbed the epidemiology of the disease. The pattern of disease in young children has followed that in the total population and it will take some years to be certain whether there has been an effect on the small number of cases in young children.

The evidence is that screening for SCID will reduce the number of cases of BCGosis, but the magnitude of this change is difficult to predict. Extrapolating from the Newcastle historical data, this would be of the order of four cases for every 50 cases of SCID identified by screening. Detailed data from the evaluation suggests this may be an underestimate

An undoubtedly beneficial effect associated with the change in programme has been a much improved system of recording uptake, which is essential for any national vaccination programme.

4.9 Links to devolved nations, cross border flows

See 2.1.14

5. Summary

5.1 Answers to UKNSC questions posed

Cost of the TREC test

For the IIVD method, this amounted in total to £7.58 per baby. This includes test reagents, equipment and human resources.

Incidence of SCID in UK during the evaluation

21 cases of SCID (14 via screening or family history and seven presenting symptomatically. were ascertained during the 30 month evaluation period. This amounts to a birth prevalence of approximately 1 in 71,000. This seemed low. In the 12 months immediately following the evaluation period, 16 cases of SCID were reported in the screened and unscreened areas combined. This amounts to a birth prevalence of 1 in 35,100. If combined with the cases from the evaluation, this amounts to 1 in 55,500, approximately equal to what was expected. The birth prevalence in the period 2010-2020 was 1 in 53,000.

Post HSCT mortality rates in the early diagnosed population

The follow-up data from the evaluation is limited due both to the small numbers and the limited duration of follow-up. However, at the time of follow-up, only one of the 14 cases screened or with a positive family history had died and this was after HSCT. On the other hand, of the seven symptomatically presenting cases, two died before definitive treatment could be initiated and three died in spite of transplantation (one was a thymic transplant). All three had a history of severe infection, including one with BCGosis. Heavy use was made of the retrospective data. See the economic evaluation for a full discussion of this.

Length of stay in hospital of the early diagnosed SCID patients

The follow-up data from the evaluation is limited due both to the small numbers and the limited duration of follow-up. However, it is clear that the total length of stay and the use of Intensive Care Units (ICU) was much higher in the clinically presenting population. Heavy use was made of the retrospective data. See the economic evaluation for a full discussion of this.

Proportion of patients detected by family history in the absence of screening

This was 37/146 (25%) from the retrospective data. The numbers ascertained during the evaluation were 2/12 (17%) in those screened and 2/9 (22%) in those not screened, giving a total of 4/21 (19%) overall during the evaluation period. If this is combined with the cases reported in the 12 months after the evaluation, there was a positive family history in 7/37 (19%).

Effect of the screening programme on the participant families

A ‘SCID suspected’ result was undoubtedly stressful for families. This was compounded by the fact that by the time the result came through, they had no recollection of being told this was one of the things being screened for and so it came as a total surprise. Families of sick babies felt this even more. For those families where there was a false positive result this effect wore off. Those families where there was a significant immunodeficiency, whether SCID or non-SCID identified, were grateful to know sooner. The overwhelming view was

that screening for SCID was welcomed. These findings are seen in other newborn screening programmes.

Capacity of NHS services

The large number of 'SCID suspected' babies put an extra workload on immunology services and added to the workload of the screening labs. Unlike the screening labs, no extra resources were provided for the immunology services. (clinical and lab) services, especially the smaller ones, found this a difficult burden to carry.

5.2 Measures adopted during the evaluation to reduce unnecessary referrals

During the evaluation and as part of the modelling, ways of improving the specificity without reducing the sensitivity were examined. Early on, the TREC cut-off level was reduced. Later, using the geometric mean to calculate the cut-off reduced the referral rate. The modelling suggested further adjustments would be appropriate.

6. Questions requiring longer term follow up

6.1 Outcomes for SCID babies

Babies with SCID will continue to be followed to assess their outcome clinically. Thought should be given to also look at their and their families' quality of life.

6.2 Outcomes for Non-SCID TCLs

Some of these children, particularly those with an idiopathic T-cell lymphopenia will be followed up to monitor their progress and see whether their condition resolves or goes on to case significant problems.

6.3 Performance of laboratory tests as these develop and as greater numbers are screened

As with any screening programme, there will be continued monitoring of screening test performance. This will, in part, require monitoring of clinical data. As confirmed SCID is only one of the possible outcomes, means of continuing to collect data on other conditions will need to be put in place. As new technologies are developed and with the possibility that new screening programmes might use the same platform, laboratory parameters may need reviewing.

6.4 Long term impacts of false positives – 5 year follow up for utilities

The follow-up of children in the utilities study will continue until they are five years old. The outcomes from this will be useful in indicating whether any alteration in provision of information to parents or support provided to them should be altered.

7. Discussion

7.1 Lessons for future ISEs and for introducing new conditions to NBS

Many lessons learned from the evaluation, described below by theme. Overall lessons include:

Building awareness among those families tested is challenging. Families often struggle to recall the offer of screening, and this accentuates the surprise and shock associated with a clinical referral following a screen positive result. There is reason to believe that this is also true for other elements of the blood spot screen.

The offer of screening seems to be acceptable to the public. Newborn screening for SCID is the first condition screened for in England to use a DNA based test as the initial screen. This has not discouraged participation.

Effective and rapid communication with all health professionals involved in the screening pathway is key to success.

Transfer of information between clinical services is essential for successful operation of a screening pathway. For the evaluation, this included information between maternity

services, screening laboratory, immunology services, child health services, GPs and immunisation services. An effective and interoperable IT infrastructure is required for this.

The transition from PHE to NHSEI and now NHSE caused an additional challenge to the progress of the evaluation, requiring new data and organisational and information governance procedures, and new workflows across the two organisations to be established. An accurate population denominator is essential for outcomes of any population programme to be evaluated. For newborn babies born in England this is in place, but accurate uptake is harder to measure for the mover in population (babies aged up to 1 year who have moved to England who may or may not already have been offered newborn screening). Evaluation of the impact of SCID screening on selective BCG uptake required accurate ascertainment and recording of the eligible population, as previously, in many places, such recording was not in place. The importance of a register of eligible population should be considered for any population health programme aimed at a high-risk population.

Information governance, data protection and approvals

Organisational systems to support the development of the data processing impact assessment (DPIA) and compliance with information governance requirements within NHSE are not currently set up to facilitate an ISE. The processes for seeking approvals are unclear and not yet bedded into the new NHSE structures, leading to conflicting advice from multiple sources and significant delay in negotiating permissions for new data flows, access and linkage. Organisational systems are focused on the control of access to data and cannot advise on how to access data most effectively. It is essential that a team undertaking an ISE ensure they have a source for expert advice on datasets that are available and how these may be accessed and used, and that any applications to access data begin at the start of the ISE as they may take months to years to fulfil.

Any future evaluations need a data team to scrutinise data as it comes in. This is needed to receive and monitor the quality of the data as it's received in real time, checking all data fields and ensuring completeness and consistency. Lack of this resource has led to this work needing to be done at the end of the study by individuals of high grade, thus impinging on their time for data analysis and modelling and potentially delaying some of this crucial work.

Funding

Funding was arranged both for the evaluation and the immediate period following. It is important to agree funding streams to cover the period where the UKNSC review the evidence from the evaluation, and to consider the costs and training implications of either national roll-out of screening or ceasing screening in the evaluation areas.

7.2 Impact of changes to clinical pathways (e.g. BCG)

Health professionals need to be aware of the impact of a screening programme in accelerating diagnosis and the consequent pressure this can bring on clinical services. Screen positive babies will need rapid diagnostic testing. While the ultimate impact of a successful screening programme can reduce overall clinical workload associated with severely ill patients long term, this benefit is not immediately realised. In contrast, the clinical activity required and diagnostic testing associated with screen positive babies is a clearly identifiable immediate demand.

The clinical pathway should be adequate to support this additional workload when dealing with the early detection of true positive cases and those where other related conditions are identified, together with offering reassurance when the result is a false positive.

Ensuring that all potential outcomes of the screening programme have been considered and are being evaluated is essential. For the SCID evaluation this includes the impact on BCG and rotavirus immunisations, necessitating collaboration across NHSE and PHE (now UKHSA).

A regular dialogue between the laboratories screening for SCID, the doctors treating the patients identified and the immunology laboratories confirming the presence and type of disease has been hugely important. This would need to be consolidated into the regular work of the Blood Spot Programme team, as occurs for the other screened conditions, if screening for SCID were recommended and adopted.

The workload of data collection, reporting and analysis for the evaluation has proved challenging. For future evaluations this should be considered in greater detail.

7.3 Impact on clinical management of Neonatal Intensive Care Units (NICU) babies

A significant proportion of babies who screened positive for SCID were in NICUs and so not able to attend immunology clinics. Regional immunology teams made local arrangements to ensure timely diagnostic testing was undertaken. This was dependent on location of

neonatal services and standalone children's hospitals. On occasions immunology teams use technology to speak to parents in neonatal units with the support of the teams caring for these babies.

In addition to dialogue with those involved in the direct screening pathway, dialogue with those more peripherally impacted is also important. This includes NICU consultants who may have to explain a screen positive finding to the family of a baby in NICU, and clinicians involved in the BCG pathway.

Information to all health professionals needs to include the entire clinical screening pathway. Newborn screening programmes have complex pathways and individual health professionals are rarely involved in the entire pathway. This means that they may be unaware of the broader picture of risks and benefits. Therefore, it is important that all those involved in the programme see the complete picture. The SCID evaluation is one such programme.

7.4 Impact of related screening programmes

Rapid technological development impacts not only SCID screening but also potential other screening programmes using similar techniques. These related screening programmes may impact on the cost effectiveness of screening programmes e.g. SMA.

7.5 Evolutionary nature of technologies

Careful attention to quality control is vital to ensure that lab tests are fit for purpose. This is particularly important where evaluation requires recently developed and developing technologies, which may not have been extensively used previously.

Rapid development of technology may mean that over the timescale of an evaluation the technology evaluated will be obsolete.

The possible impact of changes to UK regulations for diagnostic testing and in-vitro diagnostic devices. We need to work alongside notified bodies in order to ensure that the regulatory changes do not limit our ability to evaluate new technologies in an opportune way.

7.6 Miscellaneous

The text description of the “not offered screening” code was a long string starting with “inconclusive”. Many IT systems displayed only this start of the string, and this caused confusion clinically with providers waiting for a repeat test result. Potentially ambiguities in IT, such as these should be anticipated and guidance provided.

8. Conclusions

The ISE tested a pathway for the screening, confirmatory testing, diagnosis and care of newborn babies with SCID. Of 955,507 babies screened, 568 had a ‘SCID suspected’ result, of whom 316 had a normal T-cell phenotype and 45 were proven (33) or assumed (12) to have a reversible abnormality. 12 babies with SCID were found, only two of whom would have been identified without screening. The PPV for SCID, for all methods combined, was 2%. Modelling showed that this could be increased to 11%, using the IIVD methodology.

56 babies were identified with non-transient non-SCID T-cell lymphopenia, several of whom would derive benefit from early detection, e.g. a lower threshold for antibiotic use and avoidance of live vaccines. 8 of these babies had idiopathic T-cell lymphopenia (unexplained abnormal T-cell profile). The management of two of these babies was influenced by this knowledge. The PPV rises to 6.7% if taking into account all cases judged to have benefitted from screening. Using the parameters set during the evaluation, the IIVD method performed better than the PE Enlite method.

Babies with non-SCID lymphopenias are not the target of the screening programme. However, their numbers are such that an attempt should be made to factor in their costs and benefits. At this stage it has only been possible to factor in the costs.

Although the programme was stressful for parents of babies with ‘SCID suspected’ results, whatever their outcome, and was an increased burden on services, all welcomed the programme as an addition to the newborn screening offer.

Screening for SCID has disrupted the BCG programme. Inevitably, babies are now older when they receive the vaccine. The limited data available on vaccination uptake indicates that the uptake by 12 months of age has increased in some areas. There is no suggestion that TB has increased because of the change in the programme.

Modelling shows that the programme has a cost per QALY gained of £80-90k, which is well above the threshold usually considered cost effective (£20-30k). It is unlikely that any adjustments to the programme would make it cost effective as a stand-alone addition to screening. This is because the burden of costs resides predominantly with the cost of carrying out the screening test – the cost of the test itself, the equipment and human resources. Screening for SCID can be multiplexed on the IIVD and EONISQ platforms used for SCID testing. If screening for SMA were to be introduced, and the cost of SCID screening was considered as an incremental cost, or other apportionment of costs of the two programmes was made, screening for SCID would become cost effective.

9. Recommendations

SCID screening cannot be recommended as a standalone programme, as it is not cost effective.

The programme should continue as it is now until a decision is made about screening for SMA.

In the meantime, work on further adjustments to the cut-offs and possible modification of the preterm pathway should proceed.

The use of reflex genomics should be considered in the work to reduce the number of false positives. Babies with absent or extremely low levels of TRECs would be referred while those with higher levels, but below the cut-off, would be referred for genomics. The panel chosen for this purpose could include just SCID genes or a broader range of actionable conditions.

Follow-up of babies with SCID and Idiopathic T cell Lymphopenia (ITCL) should continue, and consideration should be given to follow-up of some other groups of babies. Linkage to routine health and education data, enhanced by periods of rich clinical data collection, should be considered as methods for follow-up.

The content and mode of delivery of parent information should be reviewed for all NBS screening programmes, particularly as the number of conditions screened may increase substantially. Any lessons learnt from the Generation Study should be fed into this work.

The outcome of the monitoring of the BCG programme, which has substantially improved, and of the epidemiology of childhood TB should be fed into reviews of the screening programme, if continued.

Clinical protocols for the management of babies found to have non-SCID T-cell lymphopenia should be reviewed by relevant professional bodies to ensure there is consistency in management.

Appendix 1: Project board terms of reference

Terms of Reference

1. Purpose
The Project Board exists to provide direction to the Evaluation of Severe Combined Immune Deficiency (SCID). It monitors and records progress against the business case and plan, identifies and escalates risks to the evaluation, supports the resolution of risks and issues, and reviews and approves requests for change.
The Project Board oversees the SCID screening evaluation and provides overall direction and management of the evaluation. The board reports in to both Office for Health Improvement and Disparities (OHID) and NHSE, who each have ultimate accountability for different aspects of the success of the evaluation.

The evaluation was requested by the UKNSC to answer the following questions: Document to be embedded.

OHID are responsible for ensuring that these questions are on track to being answered. They report into UKNSC on the evaluation's progress.

NHSE are responsible under the S7A arrangements for the operational aspects of the evaluation.

These terms of reference have been developed to align with the agreed principles for In-Service Evaluations (ISEs) between OHID and NHSE. To minimise disruption to the evaluation, some of the previously existing well-established arrangements will continue, e.g. one Project Board will oversee the evaluation rather than disbanding the board and establishing two new boards. These Terms of Reference were revised following the formal end of the evaluation, to cover the interim period while the evaluation report is written and until a final ministerial decision on SCID screening is made.

During the transition from PHE to NHSE, the contracts for the academic research elements of the evaluation were novated to NHSE. To minimise disruption to the

evaluation, these contracts remain with NHSE and so NHSE has accountability for monitoring these contracts.

The Project Board supports the senior responsible officer (SRO) who has ultimate accountability for the success of the evaluation and provides the overall direction and management.

The Project Board will be required to make key decisions to support the overall success of the evaluation and to deliver the required business outcomes. NHSE will agree the commitment of resources as previously identified in the 'Tablet of Stone', with agreement from OHID for any change to the evaluation methodology.

2. Duties and Responsibilities

The Board will provide strategic overview and approval across the SCID Evaluation. The role of the Board is to:

- ensure the delivery of the benefits of the SCID Evaluation
- ensure the SCID Evaluation delivers within its agreed parameters (e.g. time, cost, organisational impact and expected benefits realisation etc.)
- resolve strategic and directional issues between projects, which need the input and agreement of senior stakeholders or other NHSE directorates to ensure the progress of the work
- support the escalation and resolution of risks and issues within NHSE and OHID governance arrangements as relevant
- oversee any external dependencies of the SCID Evaluation
- provide formal approval in relation to deliverables and services produced by the SCID Evaluation
- to monitor and maintain oversight of service delivery in the SCID screening areas while this is not a commissioned service and until the UKNSC make a recommendation on screening for SCID in England
- oversee the production of a final report on the evaluation to inform the UKNSC of the evaluation findings.
- oversee the production of any other reports or journal articles on the evaluation findings and learning from the evaluation.
- consider ongoing data collection, storage and analysis following the end of the evaluation, both if a decision is made to include SCID screening in the Newborn Bloodspot Programme or if a decision is made to not include SCID screening and to stop screening in the evaluation sites.
- Following a recommendation from the UKNSC, support NHSE with transition to either end screening for SCID in England or plan for the roll out of screening for SCID across the rest of England

The Project Board supports NHSE and OHID to:

- Specify the scope and design of any amendments to the evaluation which may be required, as well as the implications of these decisions (resource, effort, timescales, risk etc.) in the form of an amended evaluation protocol
- Monitor the agreed outcome measures and expected effect sizes for the evaluation to ensure that these are on track to answer the UKNS questions.

- Generate suitable task and finish groups to address specific work-streams identified by the group
- Ensure that any findings are fed back into the existing evidence base, and that interim findings are fed in to NHSE and to the UKNSC via OHID.
- Ensure SCID screening interim arrangements continue until a decision is made on the future arrangements for SCID screening.

Senior Responsible Owner (SRO)

The SRO has ultimate responsibility for the evaluation: They should perform the following key functions:

- Approving the PID and evaluation plans (Road Map and Procurement Plan and timeline) (NB: already completed for SCID Evaluation) Ensuring that the evaluation is subject to review and delivering to outputs to meet the Procurement Plan
- Making certain that any action points from reviews are met keeping track of the business case and ensuring it remains viable
- Ensuring that benefits are realised during and after the evaluation
- Ensuring that risks to the project are considered, and escalated as appropriate
- Final decision-maker on changes
- Ensuring adequate funding is available
- Ensuring sign off and commitment from all key stakeholders, both internal and external
- Approving costs at key milestones
- Committing resources as agreed in the plans
- Deciding what type of evaluation assurance is required
- Taking ultimate responsibility for the evaluation

Project Coordinator

Responsible for the day-to-day management of the evaluation, reporting to the SRO, supported by PHE Project Team. The Project Coordinator's responsibilities include:

- Agreeing with the SRO what the evaluation is hoping to achieve, the evaluation outputs and deliverables, scope and necessary resources
- Following corporate evaluation management guidelines and producing the agreed documentation for review by the SRO/Project Board and senior management
- Planning and delivering all elements of the evaluation to budget and agreed timescales
- Organising and directing the evaluation team
- Ensuring the external suppliers (if used) deliver the agreed solutions.
- Monitoring, controlling and reporting progress/costs to all interested parties
- Ensuring business expectations are managed so no surprises on completion
- Building in quality checks so that the final solution is fit for purpose
- Controlling any risks, issues and changes that may arise during the evaluation
- Resolving problems and conflicts that arise
- Ensuring that the evaluation is closed and lessons learned are captured
- Ensuring screening interim arrangements continue until a decision is made on the future arrangements
- Monitoring and reporting incidents in the SCID screening pathway inline with SQAS guidance

Project Board Representatives

Project Board representatives have the same overall set of responsibilities as the SRO (refer to above list). The Project includes key stakeholders that have an interest in the business need and evaluation deliverables.

Project Board

- Undertakes an assurance role monitoring and assessing delivery to the evaluation objectives, plan, timescales, quality, risk identification and mitigation
- Reviews, considers and confirms recommendations arising as outputs from the work streams and tasks the work streams to undertake further work to support the decision making of the board
- Establishes work streams to take forward agreed tasks/outputs to meet the overarching evaluation objectives, reflecting the evaluation objectives, evidence base, and quality; and delivered in a timely way. Work streams will make recommendations to the Project Board.

3. Membership
The Board should be made up of the Senior Responsible Owner (SRO), and key stakeholders including end user, supplier and finance representatives.

The members of the Programme Board are:

- Senior Responsible Owner (SRO) Andrew Rostron Deputy Director antenatal and newborn and CHIS
- Programme / Project Manager Liz Robinson (Senior Newborn Pathway Development Manager)
- Workstream or Project Leads as appropriate
- Representative from the business area impacted by the SCID Evaluation
- Specialists as required e.g. finance or commercial

4. Confidentiality and information sharing

All materials and information shared with the Board are assumed to be confidential, unless otherwise stated. However, members can discuss broad, non-attributable meeting outcomes, once minutes have been shared.
Members will not disclose information or written material (such as agendas, minutes, discussion papers or other documents) to other parties, unless otherwise directed by the Chair.
Members will not share data on the evaluation without prior agreement from the chair, SRO and project manager.

5. Declaration of interest

A conflict of interest is a set of circumstances by which a reasonable person would consider that an individual's ability to apply judgement or act, in the context of delivering, commissioning, or assuring tax payer funded health and care services is, or could be, impaired or influenced by another interest they hold.

All Board members should ensure that they are not placed in a position that risks, or appears to risk, compromising their role or the NHS public and statutory duties or reputation. Members must also not accept gifts or hospitality by virtue of their role on the Board.

Board members are required to declare conflicts of interest and the receipt of gifts, hospitality and/or sponsorship, in line with the national guidance to the NHS. Conflicts of interest should be declared in writing to the Board secretariat and specific conflicts should be raised at the start of any agenda item or discussion for which that conflict arises. A conflict of interest and a hospitality register will be maintained by the secretariat.

6. Meetings

- The Board will meet every 2 months. The Chair of the meeting may convene additional meetings, as necessary
- A minimum of four members of the Board will be present for the meeting to be deemed quorate
- The Chair may ask any other officials of the organisation to attend to assist it with its discussions on any particular matter

7. Quorum

It is advised to considered quorum criteria for the respective Board meeting i.e. the minimum requirements deemed necessary for the board to be effective; e.g. numbers of attendees, individually required representatives (SRO, programme manager, etc) physical location, required agenda items, etc. Meetings that are not quorate may take place but are not authorised to make any decisions impacting the project or programme.

8. Agenda

The agenda will be set by the Chair ahead of each meeting, but the standard agenda is expected to include as a minimum:

- Introduction
- Review of minutes and actions arising from last meeting
- Programme progress summary (this reporting period)
- Financial report (actual cost vs. forecast cost at current period) and update on third party assurance
- Benefit report
- Key risks / issues including mitigating actions
- Any escalated actions
- Change request approvals
- AOB
- Date of next meeting

9. Reporting

The SCID Project Board will report to both NHSE and OHID. Information and interim data reports to the board will be summarised using an agreed format and circulated with the meeting papers.

Reporting to NHSE will be to the Blood Spot Advisory Group, and on to the ANNB Programme Board as well as to the ISE Operational Steering Group.

Reporting to OHID will be through an update report sent following each board meeting to the OHID ISE Board. The format of this report will be an agreed subset of the Project Board interim data report.

The Chair and the NBS Screening Programme can escalate concerns to NHSE and via OHID to the UK UKNSC Director of Programmes.

Should any off line decisions be required, an email will be sent to the Chair, SRO, Project Co-ordinator and Bloodspot Programme Lead, and to any other key Project Board members to make this decision or a call will be set up to discuss it.

10. Secretariat

The secretariat will be provided by the programme in the *[name]* Directorate of NHS England and will liaise with other boards to avoid clashes of dates.

11. Membership

Project Board Representatives

Role	Name	Job Title	Employing Organisation
Chair	Dr Jane Scarlett	Clinical Adviser (SCID Screening Evaluation)	NHSE
Laboratory Representative	Stuart Adams	Principal Clinical Scientist	GOSH
Chair of Laboratory Workstream	Lesley Tetlow	Consultant Clinical Biochemist and Clinical Director for Laboratory Medicine	Manchester University Foundation NHS Trust
Clinical Lead NBS Programme	Dr David Elliman	Consultant Community Pediatrician	NHSE
Laboratory Lead NBS Programme	Prof Jim Bonham	Laboratory lead for Newborn Blood Spot Screening	NHSE

Health Economics Input	Jim Chilcott	Professor of Healthcare Decision Modelling	ScHARR
Health Economics Input	Alice Bessey	Health Economic Modeler	ScHARR
SCID Service Specialised Commissioner	Joan Ward	Specialised Service Commissioning Manager	NHS-E Highly Specialised Services
Third Sector/Patient Representation	Susan Walsh	CEO	Immunodeficiency UK
Clinical Representation and Chair of the Patient, Information Communication and Training workstream	Andrew Gennery	Honorary Consultant in Pediatric Immunology and Hematopoietic Stem Cell Transplantation	Newcastle Hospitals NHS Foundation Trust
Clinical Representation	Austin Worth	Consultant Paediatric Immunologist/Honorary Senior Lecturer	GOSH
Representing the Utilities work stream	Jane Chudleigh		
Immunology Lab Rep	Kimberly Gilmour	Consultant Clinical Scientist, Clinical Lead Immunology and Director of Cell Therapy	GOSH
Representative for the BCG Board	Vanessa Saliba	Consultant Epidemiologist	PHE, Immunisation and Countermeasures Division
Chair of Data Monitoring Group	Dr Rachel Knowles	Advisor to the newborn Blood spot screening programme/ Clinical Research Fellow	NHSE and UCL

UKNSC screening policy lead and Deputy Director of Prevention Services, DHSC	Dr Anne Mackie		OHID - DHSC

Devolved Nations Representatives			
Wales	Heather Payne		
Wales	Helen Tutt		
Laboratories Representation (plus devolved nations)	Stuart Moat	Director of the Wales Newborn Screening Laboratory	
Scotland	Tasmin Sommerfield	Consultant in Public Health	
Scotland	Sheila Devlin	Screening Team Leader	
Northern Ireland	Dr Carol Beattie	Senior Medical Officer DH	

Programme Officers

Name	Job Title
Patricia Connell	Finance and Business Manager
Andrew Rostron (SRO)	Head of Public Health Commissioning and Operations Directorate of the Chief Operating Officer
Liz Robinson	Senior Newborn Pathway Development Manager

Appendix 2: Screening laboratory terms of reference

In Service Evaluation of screening for Severe Combined Immune Deficiency (SCID)

1. **Purpose**

The SCID screening laboratory working group will assess and feedback the requirements for laboratories undertaking SCID screening to the SCID Evaluation Oversight Board. This group will monitor the laboratories ongoing performance in relation to SCID screening

2. **Duties and Responsibilities**

- Oversee the implementation of SCID methodology into the screening laboratories
- Oversee and monitor the SCID screening laboratories, feedback any potential risks or issues to the SCID Oversight Board
- Interface with relevant internal and external stakeholders as required including suppliers of SCID technologies
- Seek and offer technical expertise and advice where required
- Oversight of evaluation progress and appropriate escalation to the SCID Oversight board.
- On-going review of test performance and review of screening algorithm as required
- Oversight & review of data collection and liaison with statistician regarding data analysis and modelling.

3. **Accountability**

The SCID Screening Laboratory Group will report to the SCID Project Board.
Members are drawn from key stakeholders. Review of current membership and new members should be staggered to ensure continuity. If a member's job changes or they wish to retire then they are to approach the Chairperson and a suitable replacement is to be sought.
The Chairperson and the NBS Screening Programme can escalate concerns to the SCID Screening Project Board
Responsibility for escalating issues regarding the quality of the bloodspot lab data produced will be the responsibility of the Laboratory Workstream in the first instance (as agreed 23-Nov- 2020 at the Laboratory workstream meeting). This will be outlined in detail in the project analysis plan.
Actions and Decisions will be recorded.

4. **Governance**

Items for action or decisions will be made within the group and where appropriate taken to the SCID Board

5. Membership

Members should be taken on for the duration of the evaluation. In exceptional circumstances, duration of membership may be decided on an individual basis.

The members of the Laboratory group include:

- Chair

- Deputy Chair
- Senior Newborn Pathway Development manager
- Project Lead SCID In Service Evaluation Manager for the Newborn Blood Spot Programme
- Newborn Blood Spot Programme Clinical & Laboratory Advisors
- Data and Analytics team (transformation directorate)
- Representatives from each of the SCID screening laboratories (or deputies)
- Specialist statistician when needed
- Internal NHSE teams / specialists as required

Termination of membership
Absence from two consecutive meetings without sending a deputy may result in the termination of membership status. If a member is unable to fulfil their commitments for any reason they should inform the Programme Secretariat at the earliest opportunity.

Terms of reference and membership are to be reviewed at least every six months to ensure fitness for purpose.

6. Confidentiality and information sharing

All materials and information shared with the Laboratory group are assumed to be confidential, unless otherwise stated. However, members can discuss broad, non-attributable meeting outcomes, once minutes have been shared.

Members will not disclose information or written material (such as agendas, minutes, discussion papers or other documents) to other parties, unless otherwise directed by the Chair.

Members will not share data on the evaluation without prior agreement from the chair, SRO and project manager.

7. Declaration of interest

A conflict of interest is a set of circumstances by which a reasonable person would consider that an individual's ability to apply judgement or act, in the context of delivering, commissioning, or assuring tax payer funded health and care services is, or could be, impaired or influenced by another interest they hold.
All members should ensure that they are not placed in a position that risks, or appears to risk, compromising their role or the NHS public and statutory duties or reputation. Members must also not accept gifts or hospitality by virtue of their role on the Board.

Members are required to declare conflicts of interest and the receipt of gifts, hospitality and/or sponsorship, in line with the national guidance to the NHS. Conflicts of interest should be declared in writing to the Board secretariat and specific conflicts should be raised at the start of any agenda item or discussion for which that conflict arises. A conflict of interest and a hospitality register will be maintained by the secretariat.

8. Meetings

- The Laboratory group will meet every 2 months during the course of the formal evaluation period. The Chair of the meeting may convene additional meetings, as necessary or choose to reduce the frequency od meetings as needed

- The Chair may ask any other officials of the organisation to attend to assist it with its discussions on any particular matter

9. Quorum

A minimum of four members of the group will be present for the meeting to be deemed quorate.
Meetings that are not quorate may take place but are not authorised to make any decisions impacting the project or programme.

Members (and their deputies) are to inform the group as soon as possible if they are unable to attend for any reason to ensure a quorum is achieved.

Nominated deputies can represent on the Board. The named individual must be well briefed and only attend in exceptional circumstances (not in alternation).

10. Agenda
The agenda will be set by the Chair ahead of each meeting, but the standard agenda is expected to include as a minimum:

- Welcome and introductions

- Review of actions arising from last meeting

- Review of key risks & issues including mitigating actions to agree any escalations

- Agree issues and items for escalation

- Items for agreement and sign off

- Agree decisions

- Date of next meeting

11. Reporting

The SCID Project Board reports into the Newborn Screening Delivery Group. Any items for escalation will be presented to the V&SDTB which is the gatekeeper for decision making for the vaccination and screening directorate.

The Director of Screening will be responsible for providing assurance reports to the VSDTB in the format and frequency required.
The Director of Screening will be responsible for cascade of Screening programme communications as agreed by the group this may include any decisions and recommendations made.
The SCID Project Board will report to both NHSE and OHID. Information and interim data reports to the Board will be summarised using an agreed format and circulated with the meeting papers.

The Chair and the NBS Screening Programme can escalate concerns to NHSE and via OHID to the UK UKNSC Director of Programmes.

Should any off line decisions be required, an email will be sent to the Chair, SRO, Project Co-ordinator and Bloodspot Programme Lead, and to any other key Project Board members to make this decision or a call will be set up to discuss it.

12. Meeting management and co-ordination

This will be provided by NHSE SCID Project team

Action and decisions logs only will be maintained. Full minutes will not be provided due to issues around resource

Appendix 3: Data Monitoring Group terms of reference

1. **Purpose**
   To investigate the design and implementation of data collection, analysis and reporting for the Inservice Evaluation (ISE) of screening for Severe Combined Immune Deficiency (SCID).

2. **Duties and Responsibilities**

- Oversee the collection and analysis of data and the reporting of results
- Oversight of evaluation progress and appropriate escalation to the SCID Oversight board.
- Monitor the analysis plan as the evaluation evolves to make sure it is fit for purpose for the ScHARR economic model
- To continue to review the Information Governance requirements for the evaluation in light of any organisational or evaluation changes
- To identify outcome measures and describe the uncertainty around these to address the evaluation questions

3. **Accountability**
   The SCID Screening Data Monitoring Group will report to the SCID Project Board. Members are drawn from key stakeholders. Review of current membership and new members should be staggered to ensure continuity. If a member's job changes or they wish to retire then they are to approach the Chairperson and a suitable replacement is to be sought.
   The Chairperson and the NBS Screening Programme can escalate concerns to the SCID Screening Project Board
   Responsibility for escalating issues regarding the quality of the bloodspot lab data produced will be the responsibility of the Laboratory Workstream in the first instance (as agreed 23-Nov- 2020 at the Laboratory workstream meeting). This will be outlined in detail in the project analysis plan.
   Actions and Decisions will be recorded.

Governance
Items for action or decisions will be made within the group and where appropriate taken to the SCID Board

5. Membership
Members should be taken on for the duration of the evaluation. In exceptional circumstances, duration of membership may be decided on an individual basis.

The members of the data monitoring group include:

- Chair
- Deputy Chair
- Senior Newborn Pathway Development manager
- Project Lead SCID In Service Evaluation Manager for the Newborn Blood Spot Programme
- Newborn Blood Spot Programme Clinical & Laboratory Advisors

- Data and Analytics team (transformation directorate)
- Representatives from the economic modelers team at School of Health and Related research
- Specialist statistician
- Clinical Immunologist
- Consultant SCID specialist team
- Internal NHSE teams / specialists as required

Termination of membership

Absence from two consecutive meetings without sending a deputy may result in the termination of membership status. If a member is unable to fulfil their commitments for any reason they should inform the Programme Secretariat at the earliest opportunity.

Terms of reference and membership are to be reviewed at least every six months to ensure fitness for purpose.

6. Confidentiality and information sharing

All materials and information shared with the DMG are assumed to be confidential, unless otherwise stated. However, members can discuss broad, non-attributable meeting outcomes, once minutes have been shared.

Members will not disclose information or written material (such as agendas, minutes, discussion papers or other documents) to other parties, unless otherwise directed by the Chair.

Members will not share data on the evaluation without prior agreement from the chair, SRO and project manager.

7. Declaration of interest

A conflict of interest is a set of circumstances by which a reasonable person would consider that an individual's ability to apply judgement or act, in the context of delivering, commissioning, or assuring tax payer funded health and care services is, or could be, impaired or influenced by another interest they hold.

All members should ensure that they are not placed in a position that risks, or appears to risk, compromising their role or the NHS public and statutory duties or reputation. Members must also not accept gifts or hospitality by virtue of their role on the Board.

Members are required to declare conflicts of interest and the receipt of gifts, hospitality and/or sponsorship, in line with the national guidance to the NHS. Conflicts of interest should be declared in writing to the Board secretariat and specific conflicts should be raised at the start of any agenda item or discussion for which that conflict arises. A conflict of interest and a hospitality register will be maintained by the secretariat.

8. Meetings

- The DMG will meet every 2 months. The Chair of the meeting may convene additional meetings, as necessary
- The Chair may ask any other officials of the organisation to attend to assist it with its discussions on any particular matter

9. Quorum

A minimum of four members of the group will be present for the meeting to be deemed quorate.
Meetings that are not quorate may take place but are not authorised to make any decisions impacting the project or programme.

Members (and their deputies) are to inform the group as soon as possible if they are unable to attend for any reason to ensure a quorum is achieved.

Nominated deputies can represent on the Board. The named individual must be well briefed and only attend in exceptional circumstances (not in alternation).

10. Agenda

The agenda will be set by the Chair ahead of each meeting, but the standard agenda is expected to include as a minimum:

- Welcome and introductions
- Review of actions arising from last meeting
- Review of key risks & issues including mitigating actions to agree any escalations
- Agree issues and items for escalation
- Items for agreement and sign off
- Agree decisions
- Date of next meeting

11. Reporting

The SCID Project Board reports into the Newborn Screening Delivery Group. Any items for escalation will be presented to the V&SDTB which is the gatekeeper for decision making for the vaccination and screening directorate.

The Director of Screening will be responsible for providing assurance reports to the VSDTB in the format and frequency required.
The Director of Screening will be responsible for cascade of Screening programme communications as agreed by the group this may include any decisions and recommendations made.
The SCID Project Board will report to both NHSE and OHID. Information and interim data reports to the Board will be summarised using an agreed format and circulated with the meeting papers.

The Chair and the NBS Screening Programme can escalate concerns to NHSE and via OHID to the UK UKNSC Director of Programmes.

Should any off line decisions be required, an email will be sent to the Chair, SRO, Project Co-ordinator and Bloodspot Programme Lead, and to any other key Project Board members to make this decision or a call will be set up to discuss it.

12. Meeting management and co-ordination

This will be provided by NHSE SCID Project team

.

Appendix 4: Diagnostic review group classification process

Diagnostic Review Group: Diagnostic Information

Every child should be categorised at Level 2 if information is available and only categorised at Level 1 if lacking data for Level 2.
Outline of Diagnostic Categories and Sub-Categories
Level 1 categories = I, II, III
Level 2 categories = 1, 2, 3 & bullets

I. **Normal T-cell subsets**
- 'true' False Positives

II. **SCID**
- Typical/classical SCID
- Leaky SCID
- Omenn Syndrome
- Atypical SCID
- Radiosensitive SCID

III. **Non-SCID T Cell Lymphopenia**

a. Syndromes with T-cell impairment
BMT considered
- Dock8 - BMT
- Rac2 defect - BMT
- Cartilage hair hypoplasia - BMT
- Nijmegen breakage syndrome – BMT

Thymic transplant
- DiGeorge - Thymic transplant
- CHARGE - Thymic transplant

Transplant unlikely
- Noonan
- Schimke immuno-osseous dysplasia
- Down syndrome (trisomy 21)
- CLOVES
- Ataxia telangiectasia
- Jacobsen
- Tar
- Cytogenetic abnormality
- ECC
- Kabuki
- Fryns syndrome
- Renpenning
- Other

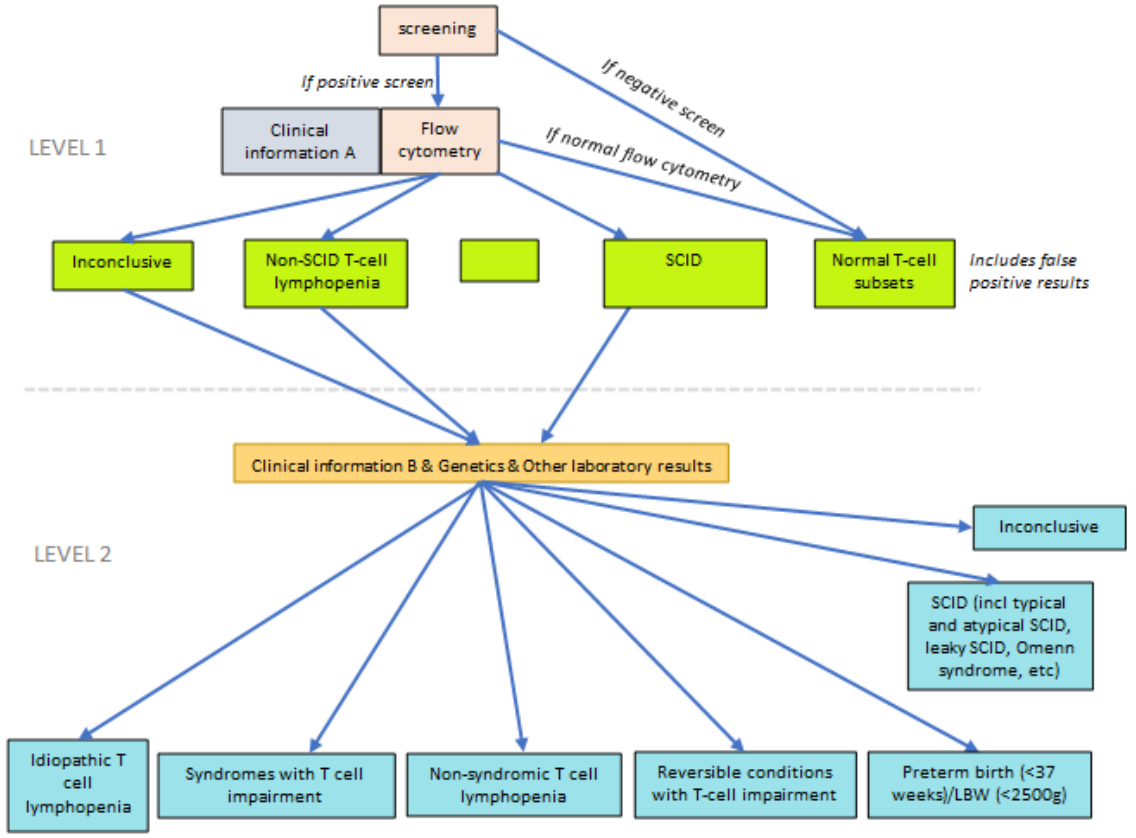
b. Secondary causes of TCL
- Congenital heart disease (apart from DiGeorge syndrome);
- Gastrointestinal malformations such as intestinal lymphangiectasia and hydrops;
- Neonatal leukaemia
- HIV
- Other

c. Idiopathic TCL
d. Non-syndromic T Cell Lymphopenia (non-SCID)

IV. **Preterm birth (<37 weeks) and/or low birth weight (<2500g) alone**

V. **Inconclusive**

Revised Diagnostic Process Flowchart



LEVEL 1: Green = Categories based on **Screening**, **Flow Cytometry** and **Clinical Information A** (e.g. perinatal, cardiac, etc)
LEVEL 2: Blue = Final categories based on **Screening**, **Flow Cytometry** and **Clinical Information B** (e.g. infection history) and **Genetic test results** (available at 3 months in most cases)

Information to be provided to DRG to enable categorisation
LEVEL 1: Information available around time of flow cytometry
Screen results:

- Test Used
- TREC_test_1_reason
- TREC_test_1_result_singlicate
- TREC_test_1_result_duplicate
- TREC_test_1_result_B_actin
- TREC_test_2_reason
- TREC_test_2_result_ singlicate
- TREC_test_2_result_duplicate
- TREC_test_2_result_B_actin
- TREC_test_threshold_used

Flow Cytometry Results:

- Hb
- WBC
- neuts
- platelets
- CD3
- CD4
- CD8
- CD19
- CD16
- CD56
- DR
- CD4CD45RACD27
- CD8CD45RACD27

Clinical details

- Gestational age at birth: in weeks (for comparisons; low TREC may be assoc with preterm)
- Maternal immunosuppression during pregnancy: YES/NO
- Family history of SCID: YES/NO
- Dysmorphic features: YES/NO
- Congenital Heart Disease: YES/NO
- Other congenital disease: YES/NO
- Is the child well: YES - well/NO – unwell
- Any other clinical details of note: YES/NO
  If any answers are YES, provide details

LEVEL 2: Information to be collected 3 months after flow cytometry:

- History of bacterial, fungal or viral infections in first 3 months
- Results of CGH array/Genetics/PID exome panel
- Repeat flow cytometry results
- TREC results if analysis repeated after screening
- Details of functional assays (YES/NO; results normal/abnormal; other details) – drop down
  - Genetics (R15)
  - IgM/IgA
  - T proliferative assays (PHA, CD3, other)
  - STAT5 phosphorylation
  - cGC expression
  - Spectratyping / Vbeta array
  - HLA-DR expression
  - MHC class 1 expression
  - Fibroblast radiosensitivity
  - DEB testing / centromeric instability
  - DOCK8 expression
  - ATM functional assays
  - OP9-DL1 assay
  - Other

Appendix 5: SCID Screening Evaluation Diagnostic Review Panel

Terms of reference

1. **Purpose**

To agree diagnoses and case definitions for children with SCID screen positive results, in line with the agreed DRG case definitions.

To assign benefits, if any, of screening to children with screen positive results

2. **Duties and Responsibilities**

1. The remit covers babies screened in England between 6th September 2021 and 1st March 2024 and babies identified with SCID during this period, clinically or by family history, in the rest of UK.
2. To agree case definitions utilising clinical, and laboratory data, as appropriate, that will allow classification of the following children into diagnostic categories/groups:

    1. children with positive screening results
    2. symptomatically diagnosed children
    3. affected siblings of symptomatically diagnosed cases

3. To agree definitions of the diagnostic categories/groups.
4. To assign each child to a single diagnostic category/group for subsequent analyses.
5. To review all screen positive cases and assign diagnoses.
6. To review all screen positive cases and assign benefit
7. To review all cases of SCID from non-screening areas, whether diagnosed on the basis of a symptomatic presentation or family history, and assign to a diagnostic group based on the agreed algorithm.

Accountability and reporting arrangements

1. The SCID Screening Diagnostic Review Group (DRG) will report to the SCID Board (Oversight Group) which in turn reports to the Blood Spot Advisory Group, as well as to the Strategy and Management Group (SMG) of the screening section.

2. Members are drawn from key fields. If a member's job changes or they wish to retire then they are to approach the Chairperson and a suitable replacement is to be sought.

3. The Chairperson and the NBS Screening Programme Manager can escalate concerns to the SCID Board (Oversight group)

Quorum arrangements
Quorate arrangements state that the meeting must not proceed without 50% of members present (including deputies, not including Programme Officers). Members (and their deputies) are to inform the group as soon as possible if they are unable to attend for any reason to ensure a quorum is achieved.

Deputy arrangements
Nominated deputies can substitute for their colleague on the Board. The named individual must be well briefed and only attend in exceptional circumstances (not in alternation).

Frequency and management of meetings
Once the evaluation has started, the group will meet every two months initially, with this subject to change based on the views of the members. Meetings will be face-to-face or virtual, as appropriate. Members will be invited to contribute to the agenda. The agenda and meeting papers will be distributed 10 days before the meeting. Draft minutes to be available promptly (four weeks) for members to comment.

Unless agreed with the programme manager, papers and other documents should be considered as internal working documents and should not be shared outside of the advisory group.

Declaration of interests
Members and officers should declare conflicts of interests annually; however significant conflicts should be made known, to the Chairman, as they arrive prior to meetings.

Membership
Members should be taken on for the duration of the evaluation. In exceptional circumstances, duration of membership may be decided on an individual basis.

Termination of membership

Absence from two consecutive meetings without sending a deputy may result in the termination of membership status. If a member is unable to fulfil their commitments for any reason they should inform the Programme Secretariat at the earliest opportunity.

Terms of reference and membership are to be reviewed at least every six months to ensure fitness for purpose.

Membership

Role	Name	Job Title	Employing Organisation
Chair – International Expertise	Mirjam van der Burg	Associate Professor Primary Immunodeficiencies	Leiden University Medical Centre, Netherlands
Programme Centre Representation	Jim Bonham	Laboratory Lead for Newborn Blood Spot Screening	Sheffield Children's NHS Foundation Trust
Programme Centre	David Elliman	Consultant Community Paediatrician	UK National Screening Committee
Health Economics Input	Jim Chilcott	Health Economic Modeler	ScHARR
Laboratory Representation	Lesley Tetlow	Consultant Clinical Biochemist	Manchester University NHS Foundation Trust
Screening Academic	Rachel Knowles	Senior Clinical Research Fellow	UCL Great Ormond Street Institute of Child Health
Clinical Scientist	Elizabeth Bateman	Consultant Clinical Scientist	Oxford University Hospitals
Immunologist	Siobhan Burns	Reader in Immunology	UCL Institute of Immunity and Transplantation
Immunologist	Austen Worth	Consultant Immunologist	GOSH
Immunologist	Peter Arkwright	Consultant Immunologist	Manchester Hospitals NHS FT
International Expertise	Robbert Bredius	Paediatrician	Leiden University Medical Centre, Netherlands
Immunology Laboratory	Kimberly Gilmour	Laboratory Immunology consultant	GOSH
Genetic advisor	Oliver Murch	Clinical Geneticist	

Programme Officers

Name	Job Title	Employing Organisation
Christine Cavanagh	Programme Manager - NHS Newborn Blood Spot Screening Programme	NHSEI
Simon Hailstone	Head of QA / Consultant in Public Health	NHSEI
Liz Robinson	Clinical Project Lead - SCID project - NHS Newborn Blood Spot Screening Programme	NHSEI
John Kirwan	Data Manager SCID	NHSEI
Lauren Cooper	Project Lead NBS	NHSEI

Appendix 6: Definition of SCID

To meet the definition of SCID, a potential case would have to meet two or more of the following criteria:-

a) Absolute T cell (CD3) count of <0.05 x 109

b) Naïve CD4 count <20%

c) Oligoclonal T cell expansion

d) Clinical Omenn syndrome

e) Pathogenic or likely pathogenic variant(s) in a SCID related gene.

Appendix 7: Definition of Idiopathic T-cell Lymphopenia

To meet the definition of Idiopathic T-cell lymphopenia one of the following criteria need to be fulfilled;

a) A total CD3 count of <1500 or
b) A proportion of naïve CD4 cells of <40% total CD4 T cells or
c) An absolute CD8 T cells count of <0.05 x 10^9

AND

The exclusion of all other cause, including, but not confined to, genetic conditions that may result in lymphopenia

Appendix 8: Diagnostic Classification of SCID suspected cases

Normal T-cell subsets. Defined as ≥1,500 CD3/µl and naïve cells ≥70%

- 'true' or 'false' Positives

SCID - See separate appendix for definition

- Includes typical and atypical SCID, leaky SCID, Ommen syndrome, etc.

Non-SCID T Cell Lymphopenia

- Includes conditions such as CHARGE, 22q11.2 deletion (Di George) syndrome, Down syndrome, etc.

Non-syndromic T Cell Lymphopenia (non-SCID)

- Includes conditions such as FOXN1 heterozygote, juvenile myelomonocytic leukaemia (JMML), etc.

Idiopathic T-cell lymphopenia

- Persistent T-cell lymphopenia with no cause found after investigation, including genomics.

Reversible conditions with T-cell impairment

- Initial T-cell lymphopenia which resolves. Includes hydrops, chylothorax, etc.

Inconclusive

- Died or lost to follow-up without full investigation

Appendix 9: Scenarios for Screening Babies for SCID depending on where the screening sample(s) is taken

Currently, some babies are given BCG, soon after birth, if at increased risk of developing TB. With the introduction of screening for SCID, it has been agreed that BCG vaccination will be delayed until the result of the screening test for SCID becomes available on CHIS. This is because if a baby has SCID, their treatment is made even more complex if they are given BCG, as it is a live vaccine. However, it is important not to delay the results longer than is absolutely necessary because one does not want to delay BCG administration in those babies who need it, more than is absolutely necessary.

It has been agreed that a 'not offered' code will be added to all LIMS systems, prior to the evaluation starting and will be switched on in advance. When the evaluation commences, this code will remain switched on for those labs not screening for SCID, and the result of SCID screening will be recorded on LIMS for those labs who are screening.

As the evaluation is not involving all of England and will not include the devolved nations, and babies sometimes move in their first weeks of life, we have set out some scenarios and, where there is a potential problem, the possible solution. Most of the scenarios are fairly straightforward, however for numbers 3 and 6, there are potential problems. The success of the suggested solutions relies on the midwives/HCP being assiduous in recording that a sample is a repeat. This will be emphasised in the training materials/events.

At the time the blood spot sample is taken, as well as consent for screening, consent for research contact will also be sought and dissent should be recorded on the card

1. First blood spot taken[11] in a non-screening area[12] and the baby either stays put or moves to another non-screening area.

The baby will not be screened for SCID.

[11] This could be in the normal place of residence or an inpatient facility. In the latter scenario, especially if a tertiary unit, it is possible that the screening lab to which the sample is sent and the lab covering the normal place of residence would be different and one might screen for SCID and the other not. The sample will be sent to the lab whose catchment includes the locality where the sample is taken, as is usual practice.
[12] 'non-screening' in the scenarios means not screening for SCID

A ‘not offered SCID’ code will be assigned to the screening results.

There is nothing additional for the Midwife/HCP to do in this scenario

2. Baby born in a non-screening area but the baby moves to a screening area before the initial sample is taken.

The baby will be screened for SCID.

All the tests will be performed by a screening lab and the appropriate screening outcome will be assigned to the results

The midwife/HCP in the screening area will get consent for screening and take the sample. Permission for research contact should be sought at the same time.

3. First blood spot taken in a non-screening area, but requires a repeat, by which time the baby is in a screening area.

The lab will not usually know from the card, where the first sample was taken. On noting that the sample is a repeat, the lab will check on their system to see if it is one of their own. If not, they will then check the NBSFS (failsafe). This will tell them where the first sample was tested and whether it was tested for SCID. On finding that the first sample was tested in a non-SCID screening lab, the second lab will not screen for SCID. This is not 100% perfect but minimises risk to a very low level.

This will mean extra work for the lab. There is nothing extra for the Midwife/HCP to do in this scenario, but it is even more important that the fact that it is a repeat is recorded on the card.

4. First blood spot taken **in a screening area and the baby doesn’t move.**

The baby will be screened for SCID.

All the tests will be performed by a SCID screening lab and the appropriate screening outcome will be assigned to the results.

The midwife makes the offer of screening for SCID and takes the sample

5. *Baby born* in a screening area and the baby moves, before any samples have been taken, to a non-screening area.

A ‘not offered SCID’ code will be assigned to the screening results.

Midwife in the non-screening area may need to explain that the baby is no longer eligible for SCID screening.

6.*Blood spot taken* in a screening area. The baby will be screened for SCID. The baby then move to a non-screening area and a repeat is required.

The repeat sample should be sent to the laboratory in the area where the repeat sample is taken. If the repeat is for SCID only then the entire card should be sent immediately to the original (SCID screening) laboratory. If the repeat is for all tests (e.g insufficient, multi-spotted, missing date of birth etc) then the receiving (non-SCID screening lab) will complete the analysis for all tests except SCID and send a single spot to the original (SCID screening) laboratory for the SCID re-test. Samples can be sent in accordance with the normal route used by the laboratory for referring samples. In this scenario it is important that the second (non SCID screening lab) issue a report for the 9 conditions included in the current screening programme and a 'repeat required' code against SCID. The initial (SCID screening) lab should also check for completeness of results and follow up if they do not receive a repeat, as is normal practice. If no repeat has been received, they should check the failsafe to ascertain if the repeat has been taken elsewhere and contact the relevant NBS laboratory who will have received the repeat and explain what is required.,

As before, the Midwife or HCP will need to record if the sample is a repeat.

The HCP will send the sample to their local lab as usual. It is not the HCP's responsibility to get the sample to the 'original 'lab.

7. First blood spot taken out of England and the baby moves into a screening area. If any screening is required, i.e. the baby has not already been screened for the basic nine non-SCID conditions, screening will include SCID. Screening for SCID only will not be offered.

The appropriate screening outcome will be assigned to the results.

If no screening is required, no SCID outcome will be assigned, by the lab, but the baby will be assigned a "not offered" code by CHIS.

Midwife/HCP will make the offer as usual.

8.*Baby born out of England and moves into a non-screening area.*

If any screening is required**, i.e. the baby has not already been screened for all nine non-SCID conditions**, screening for the nine conditions will take place and will not include SCID. The 'not offered' code will be assigned to the results.

Midwife/HCP will offer NBS screening but exclude SCID

9.Baby born out of England and requires screening. The sample is taken, but a repeat sample is required. In the interim the baby moves from a screening area to a non-screening area or vice-versa.

The same principles would apply as to a baby born in England. (scenarios 3 and 6)

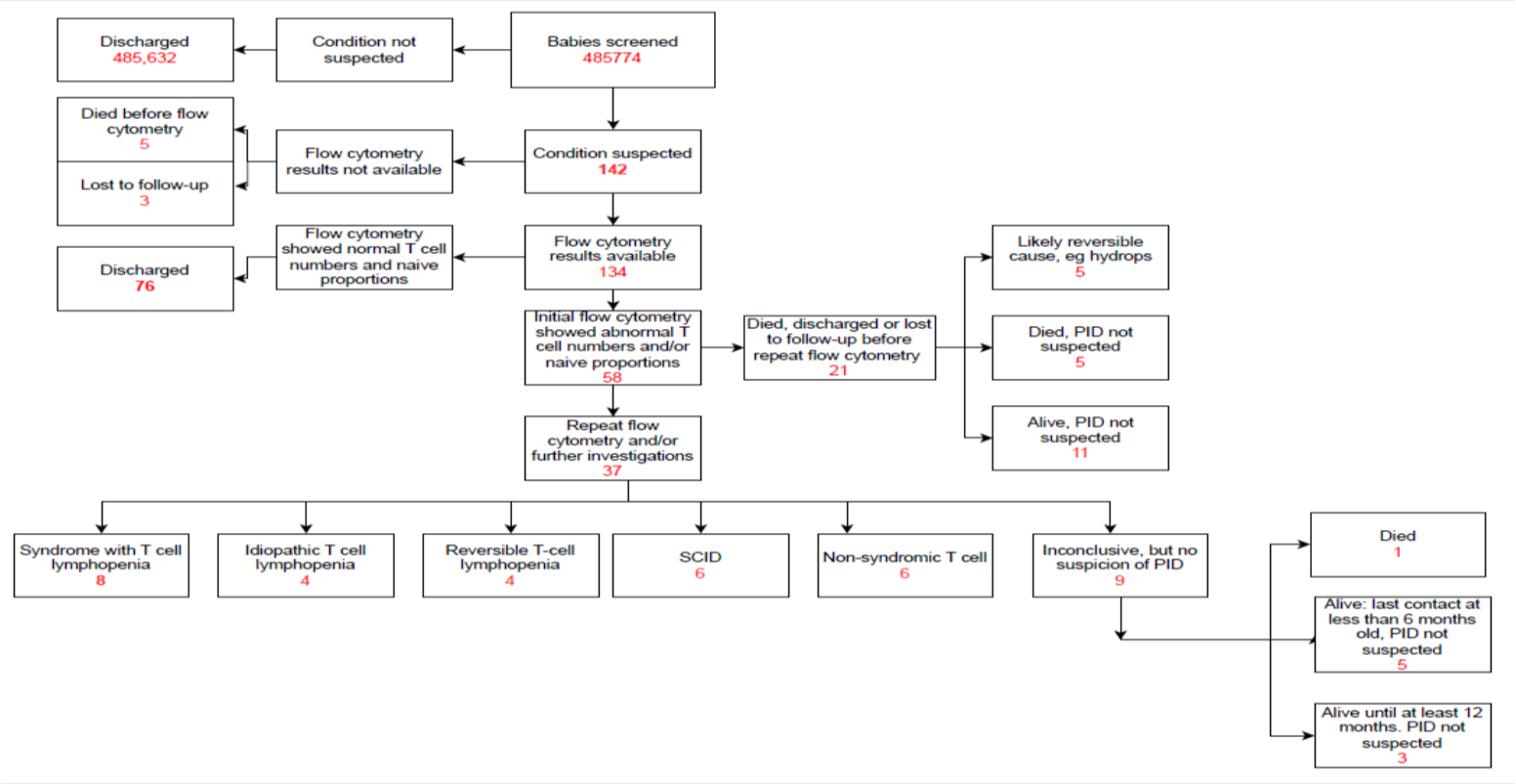
10. Movement between countries within United Kingdom

Management of babies who move across UK borders, before screening has been completed, will be managed in accordance with the principles outlined above.

The Welsh screening laboratory has access to the English newborn blood spot failsafe solution and will be able to check this for details of repeats required. In all other instances, the laboratory would need to communicate with the relevant screening laboratory in England to ascertain details about a repeat on a baby whose original sample was taken in another nation.

29-04-2021

Appendix 10: Flowchart showing outcomes, by category, for all babies screened using IIVD only



Appendix 11: Categorisation of outcomes and benefits of babies with T-cell lymphopenia – IIVD only

Classification	No.	Assessment of benefit from screening benefit from screening			
		Benefit	Disbenefit	Neutral	Unknown
Syndrome with T cell lymphopenia	8	6		2	
Idiopathic T cell lymphopenia	4	2			2
Proven reversible T cell lymphopenia	4	1	3		
SCID	6	4		2	
Non syndromic T-cell lymphopenia	6	2	1	3	
Total	28	15	4	7	2

Appendix 12: BCG vaccine, Tuberculosis and screening for Severe Combined Immunodeficiency (SCID)

Bacillus Calmette–Guérin (BCG) vaccine is a live attenuated bacterial vaccine which protects against tuberculosis (TB). It is now given mainly to neonates whose parents or grandparents come from a country with a high incidence of TB. It is also given to babies in a very small number of localities where there is a high incidence of TB in the general population. It was usually given soon after birth, before the baby leaves hospital. Severe TB is commoner in children than in adults, particularly in children under 5 years old. BCG protects better against severe disease than against pulmonary disease.

Children who have SCID are more likely to be eligible for BCG. This is because some ethnic groups have a higher prevalence of some rare diseases. The ethnic origin of these groups means they are more likely to be eligible for BCG.

One of the contraindications to BCG is the presence of significant immunosuppression If BCG is administered to someone who is immunosuppressed, rather than just resulting in a local reaction, the BCG organism may spread within the body to multiple organs ('BCGosis'), occasionally resulting in death. SCID is a form of severe immunosuppression.

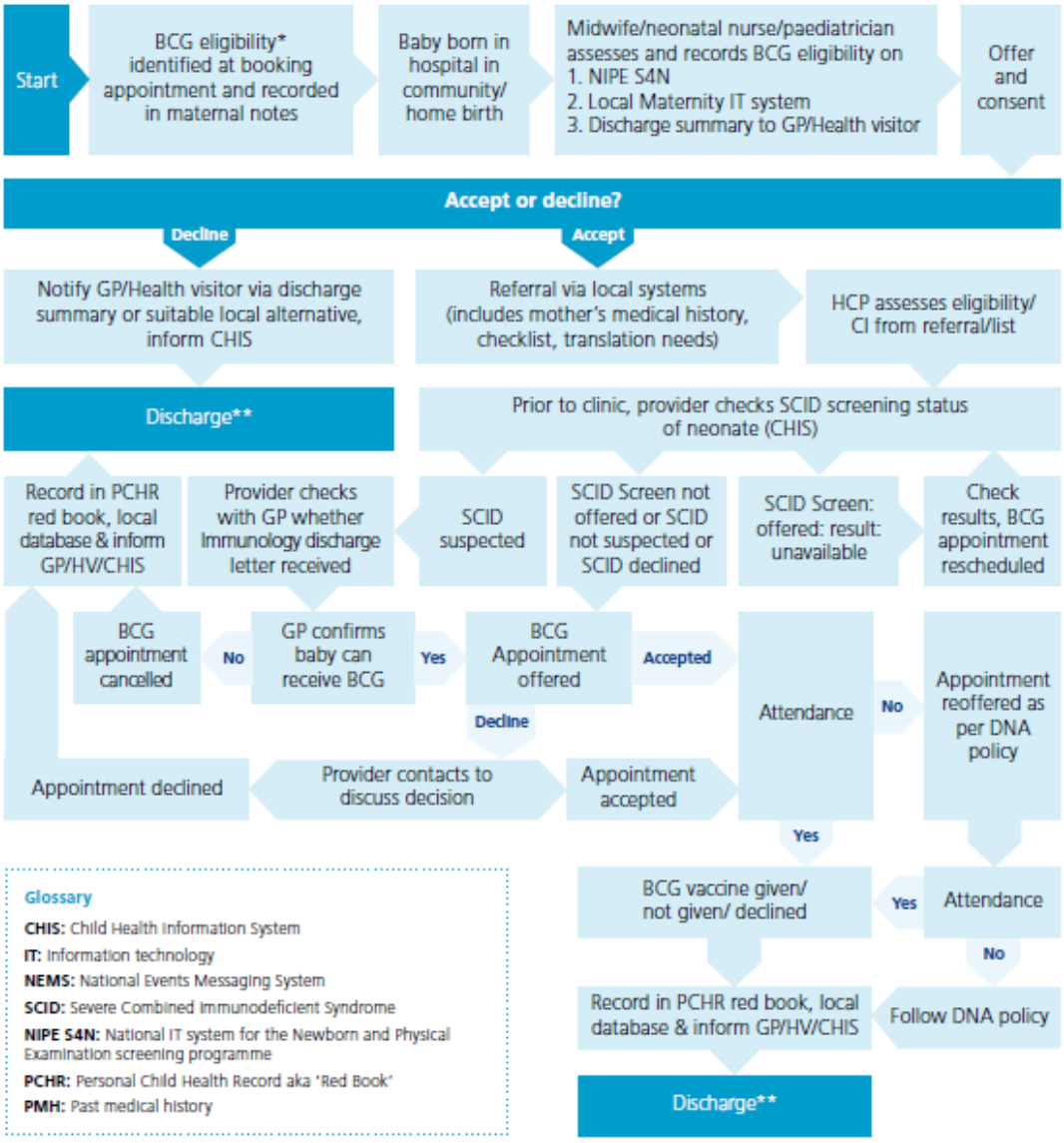
Based on these considerations, the JCVI decided that, with the initiation of the evaluation of screening for SCID, at the beginning of September 2021, BCG should be postponed until the result of the screen was available. As children move around the country, a pragmatic decision was taken that this should apply across the country whether or not the baby was offered screening for SCID. (Babies who fall outside the evaluation area are given a 'not offered' result and can be given BCG if eligible.)
Whereas BCG had been given in the first few days of life, the target was now set that it should be given as soon as the screening result was available and certainly within the first 28 days, unless there was very good reason to delay. It was felt that the downside of this change and therefore a delay in administering BCG, was outweighed by the benefits of not complicating the treatment of any child with SCID, but that this should be monitored.

The new pathway is shown below:

UK Health Security Agency

NHS

BCG patient flowchart



*BCG eligibility is described in chapter 32 of the Green Book www.gov.uk/government/publications/tuberculosis-the-green-book-chapter-32
**Follow local discharge policies

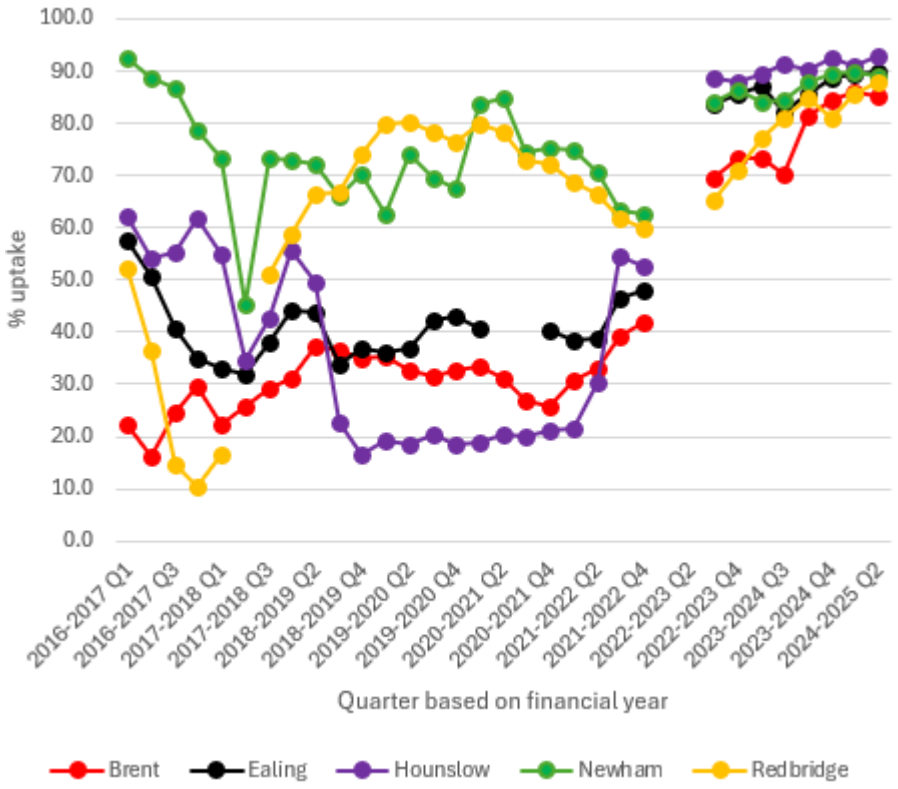
Figure 1. The new BCG pathway from September 2021

The effect of the change in the pathway can be monitored in a number of ways

1. The effect on the BCG programme, including the uptake of BCG in eligible groups of infants, by age, in a timely fashion.

A change in the BCG programme could affect both the timeliness of the vaccination and the overall coverage. However, granularity of timing of the vaccination is not available at a national level and so only coverage is considered below, except for one area.

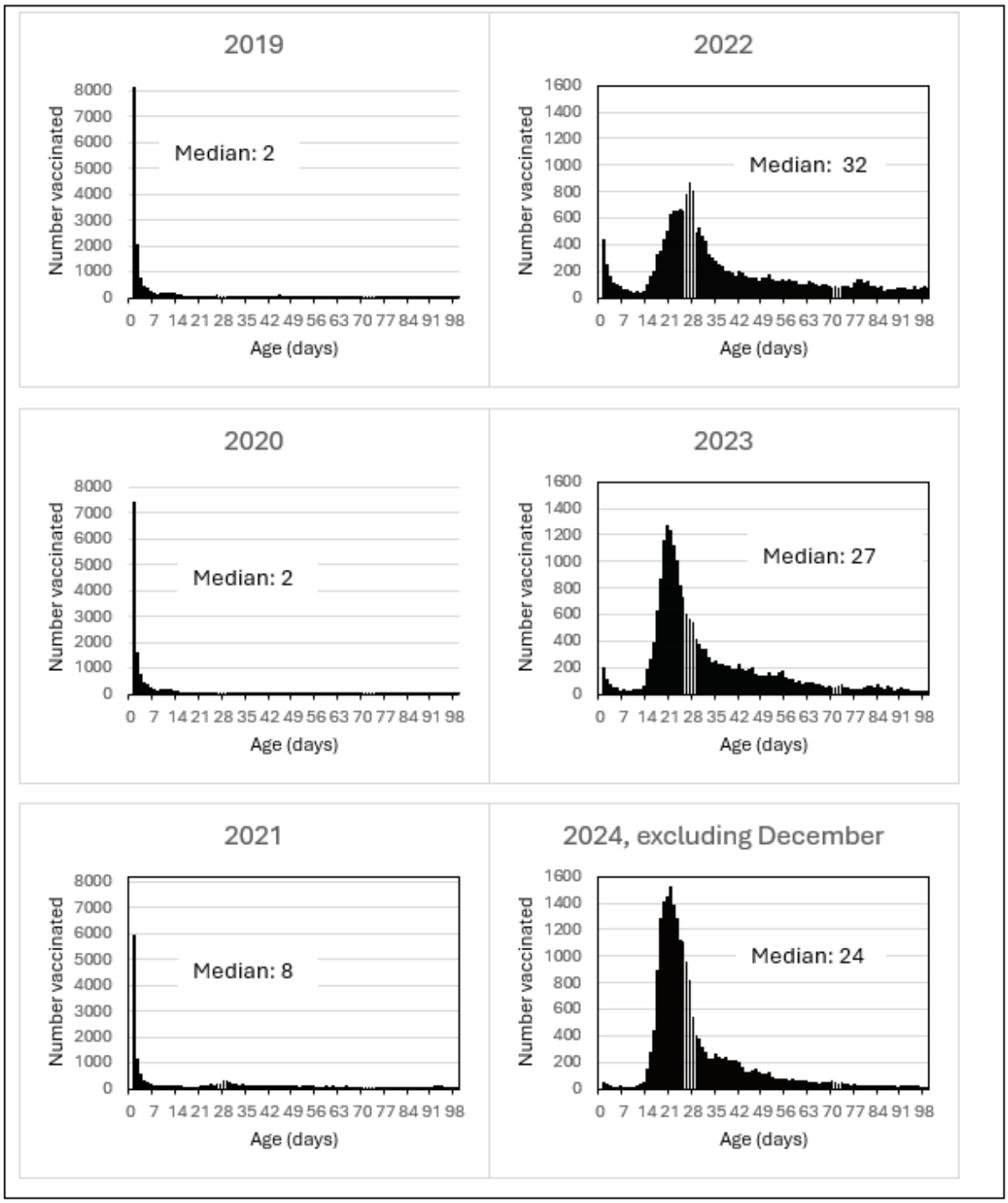
Figure 2 Uptake of BCG vaccine, by 12 months old, in the targeted population



In the 5 areas that have offered BCG universally until very recently, it is apparent that the uptake following the change is better than before the change. This may in part be due to better data collection.

Data on timing of BCG vaccination is rarely available, however there are data from West Midlands covering November 2005 to November 2024. The series of graphs below show this data for 2019 to 2024 with vaccination at ages >100 days excluded.

Figure 3: Number of BCG vaccines given by age, in West Midlands, from 2019 to November 2024
(Note change in vertical axis from 2022. Median is for those children vaccinated by 180 days.)



From 2016 to 2020, of those vaccinated, between 66.1% and 78.4% of babies received the vaccine by 28 days. For 2022, 2023 and 2024 the figures were 41.6%, 55.0% and 68.8% respectively.
As was expected, after the implementation of newborn screening for SCID BCG vaccine is being given when the baby is older. However, timeliness seems to be improving. This is only one area and one must be careful about drawing general conclusions, but it is likely the same shift has taken place in other areas. It is good to

see that most babies had received the vaccine by the target age of 28 days, but there is still a large group being vaccinated later.

Effect of change in programme on commissioners, providers and parents
Two studies were commissioned to seek views on the change in the programme – one with BCG commissioners and providers, the other with parents. The former has been published.[13] The abstract is shown.

Abstract

Introduction The introduction of a national evaluation of newborn screening for Severe Combined Immunodeficiency (SCID) in England triggered a change to the selective Bacillus Calmette-Guerin (BCG) vaccination programme delivery pathway, as this live attenuated vaccine is contraindicated in infants with SCID. The neonatal BCG vaccination programme is a targeted programme for infants at increased risk of tuberculosis and used to be offered shortly after birth. Since September 2021 the BCG vaccine is given to eligible infants within 28 days of birth, when the SCID screening outcome is available. We explore the experiences of those implementing the new pathway, and how they made sense of, engaged with, and appraised the change.

Methods A mixed-methods evaluation was conducted between October 2022 and February 2023. This involved national online surveys with BCG commissioners and providers and qualitative semi-structured interviews with commissioners, providers, and Child Health Information System stakeholders in two urban areas. Survey data was analysed using descriptive statistics and interview data was analysed thematically. The data was triangulated using Normalization Process Theory as a guiding framework.

Results Survey respondents ($n=65$) and qualitative interviewees ($n=16$) revealed that making sense of the new pathway was an iterative process. Some expressed a desire for more direction on how to implement the new pathway. The perceived value of the change varied from positive, ambivalent, to concerned. Some felt well-prepared and that improvements to data capture, eligibility screening, and accountably brought by the change were valuable. Others were concerned about the feasibility of the 28-day target, reductions in vaccination coverage, increased resource burden, and the outcome of the SCID evaluation. New collaborations and communities of practice were required to facilitate the change. Three main challenges in implementing the pathway and meeting the 28-day vaccination target were identified: appointment non-attendance; appointment and data systems; and staffing and resourcing. Feedback mechanisms were informal and took place in tandem with implementation.

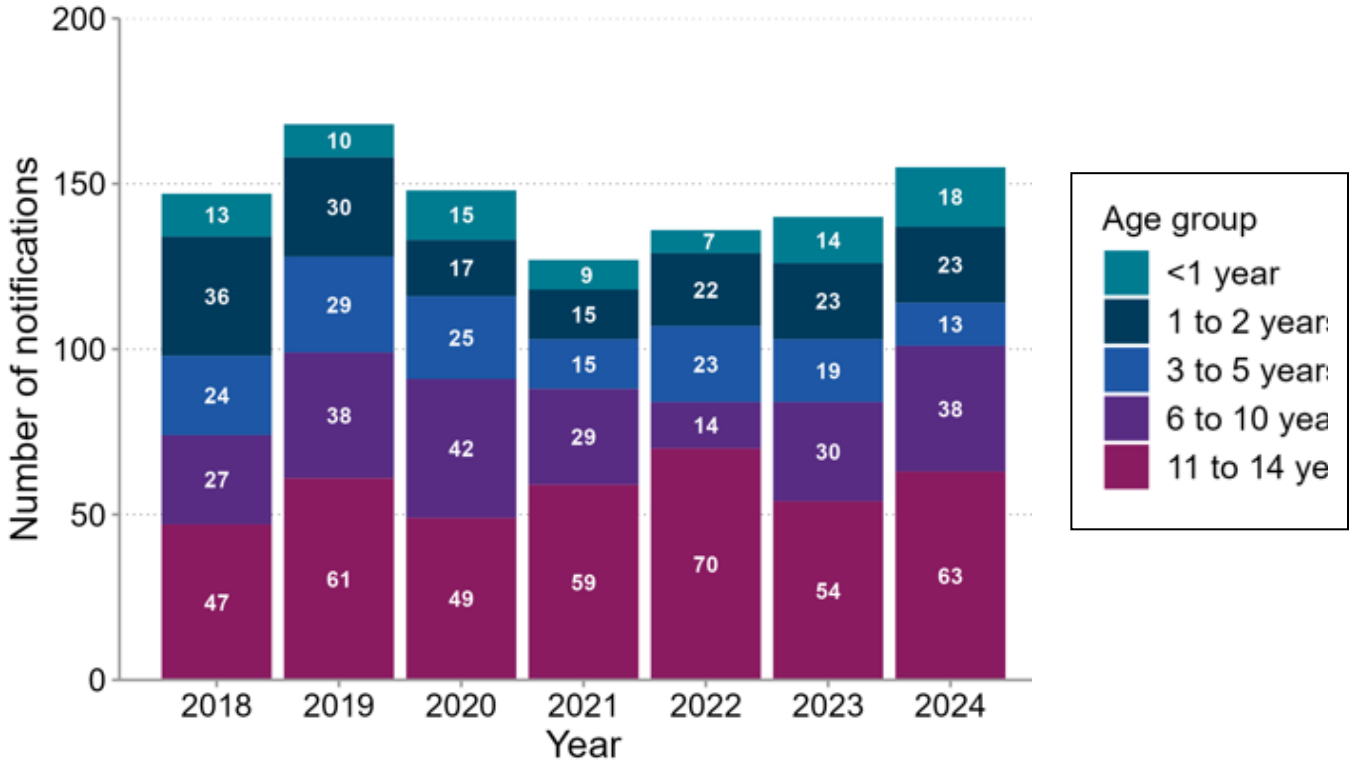
A complementary qualitative study in the same two areas sought the views of parents who were deemed eligible for BCG. Parents were accepting of the SCID-related delay, but encountered multiple problems.

> **"**errors led to confusion regarding eligibility, with some ineligible infants receiving invitations and others who were eligible having trouble accessing an appointment. Many parents first learned about BCG vaccination postnatally, describing it as a "surprise vaccine," with limited antenatal discussions affecting informed decision-making. Appointment notification systems were inconsistent, with some parents receiving short-notice invitations or no notification at all. Physical access barriers included unfamiliar and distant clinic locations, difficulties with transport, and the challenges of traveling soon after birth, with a newborn. Parents with limited social support or financial constraints faced additional difficulties."

2. The incidence of TB in young children, including the number of cases of severe TB (TB meningitis, miliary or cryptic disseminateTB).
The number of children less than 15 years old who develop TB in England has declined over the last 5 years, from 147 in 2019 to 105 in 2023. Figure 2 shows a breakdown, by age, for the period 2018 to 2024.

[13] Jones K, Chisnall, Crocker-Buque T, *et al.* A new neonatal BCG vaccination pathway in England: a mixed methods evaluation of its implementation. BMC Public Health 2024;24:1175. https://doi.org/10.1186/s12889-024-18586-8

Figure 5 Children notified with active TB, by age, England 2018-2024 (Provisional data for 2024)

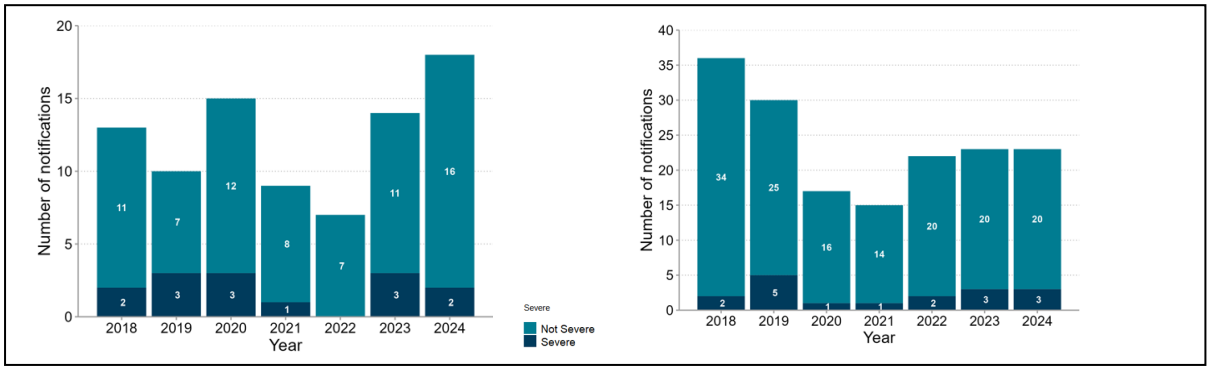


Of particular concern are the children who develop severe disease, as these are the children likely to suffer long term consequences.

Figure 6. Severe TB (meningitis, cryptic or miliary) in children aged 5 years or less, England 2018 to 2024 (Provisional date for 2024)

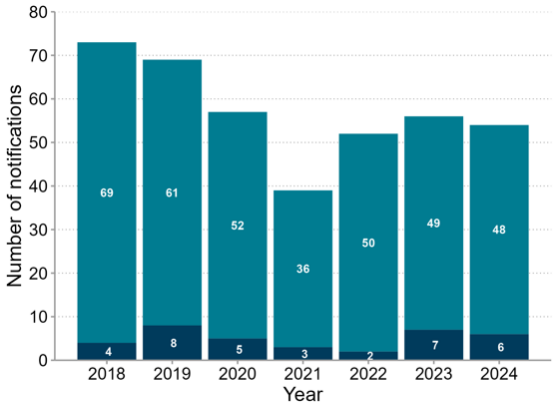
Figure 6a. Number of notifications <1 year old.

Figure 6b. Number of notifications 1 and 2 years old



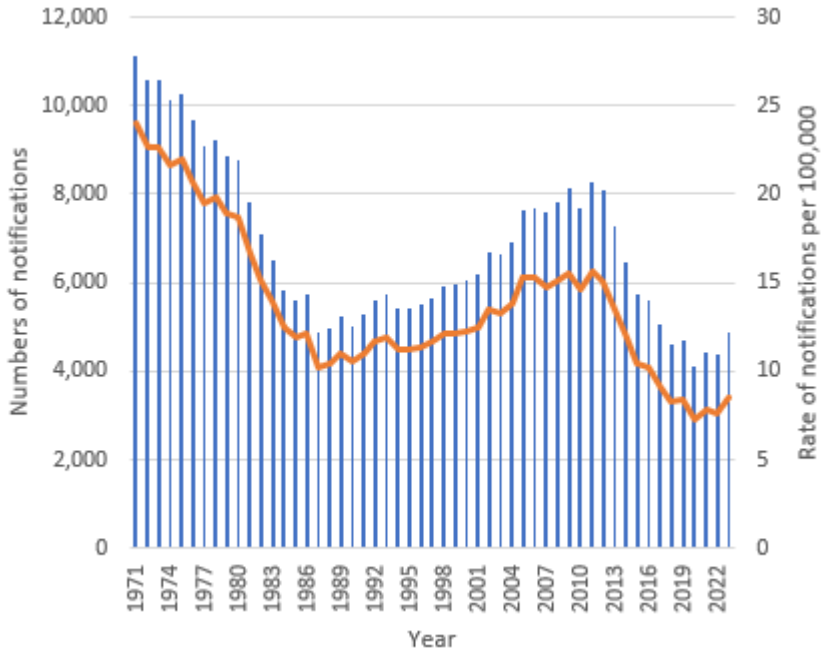
The numbers of cases are small and it would be unwise to draw any conclusions.

Figure 6c. Number of notifications ≤5 years old



Notifications of TB in the population as a whole, have changed over time, with a total of 5480 cases reported in 2024, in England, an increase of 13% on 2023.

Figure 7 Numbers and rates of TB notifications in England 1971 to 2023. (2024 is provisional)



TB cases have risen around the world since 2020. Figure x shows the recent increase of cases of TB in children and adolescents in Europe. The figures are not as high as pre-pandemic.

The numbers of notifications of TB in children are too small to draw any firm conclusions. The trend has been complicated by the intervention of COVID-19 and follows that seen with adult TB. Suffice to say there has been no significant increase compared with the immediate period before the change.

3. The incidence of BCGosis and its association with SCID and other immunodeficiencies.

Data sought collected from the National Child Mortality Database (NCMD), Hospital Episode Statistics., ONS mortality statistics, the UK Medicines and Healthcare products Regulatory Agency (**MHRA**) and specialist SCID centres in England. The available data from these sources revealed no cases of BCGosis amongst babies with SCID who had been ascertained by screening, but cases amongst those presenting clinically. 2 of 9 children who were reported by NCMD to have died from SCID developed BCGosis as did 2 of 7 with other immunodeficiencies.

Data for 51 children transplanted for SCID at one of the two national centres, prior to the evaluation, showed that none of the 14 infants who were ascertained via a family history had received BCG. Of the 37 infants who presented clinically, 15 received BCG. Of these, 4 had superficial infection (lymphadenitis, abscess), 4 had systemic/organ disease and 7 had no symptoms.

None of the 12 babies ascertained by screening received BCG. Of the eight babies presenting symptomatically during the evaluation period, five were known to have received BCG and three developed BCGosis, in one of whom, BCG pneumonitis was a major factor in the child's death. The BCG status of one was unknown.

A search of the MHRA database up to and including 10/03/2025 was made for adverse reactions to BCG vaccine. Of the 2,471 reports of reactions in 1,159 patients, there were 14 reports of 'disseminated Bacille Calmette-Guerin Infection" and 2 deaths. From the data available, it was not possible to say how many were related to an underlying immunodeficiency.

Taking the data from clinically presenting cases to the SCID centre and during the evaluation, of 45 cases, 20 (44%) had received BCG and, of these, 7 developed serious BCG disease. This confirms the overlap between eligibility for BCG and risk of SCID.

Conclusion

The BCG programme undoubtedly was a major organisational change. The lack of good data prior to the change makes generalised comments difficult. It is reassuring that uptake in the areas where the offer of BCG was universal did not fall and that the detailed data from the West Midlands have shown that the timeliness of vaccination, although affected by the change is improving as new systems settle in. However much it improves, it will not revert to what it was, but it is unclear how this will affect the incidence of TB.

The numbers of cases of TB in children below 5 years old is small, those with severe TB is very small and COVID-19 has disturbed the epidemiology of the disease. The pattern of disease in young children has followed that in the total population and it will take some years to be certain whether there has been an effect on the small number of cases in young children.

The evidence is that screening for SCID will reduce the number of cases of BCGosis, but the magnitude of this change is difficult to predict. Extrapolating from the Newcastle historical data, this would be of the order of four cases for every 50 cases of SCID identified by screening. Detailed data from the evaluation suggests this may be an underestimate

An undoubtedly beneficial effect associated with the change in programme has been a much improved system of recording uptake, which is essential for any national vaccination programme.

Acknowledgements

Colleagues from UKHSA have provided data and advice on BCG coverage and data and some figures for the epidemiology of TB.

Laboratory Appendix 1: Options for delivery of laboratory services for SCID screening

Report to:	SCID Evaluation Oversight Group
Brief prepared by:	SCID Evaluation Laboratory Group
Date of Brief:	19/06/2018
Subject:	**Options for delivery of Laboratory Services for SCID screening**
Purpose:	To provide options and a recommendation for newborn screening laboratory services for SCID both in relation to the practical evaluation and subsequent inclusion in the newborn screening panel (if approved by the UKNSC)

Background

The UKNSC has recommended that a practical evaluation of newborn screening for SCID should be undertaken NHS England. The aim of this would be to generate sufficient information on key issues to inform a future recommendation on whether SCID should be added to the newborn screening panel.

Newborn Screening for SCID relies on the accurate assessment of the numbers of T cell receptor excision circles (TRECs). Very low or absent TRECs is a screening indicator for potential SCID positive babies. Cut-off values will vary according to methodology chosen and need to be carefully assessed in order that no SCID babies are missed by screening whilst minimising the number of false positives. All of the potential methods for assessing TRECs levels rely on the polymerase chain reaction (PCR) to amplify the TRECs and a control gene marker whilst using fluorescent dyes to label these amplified pieces of DNA. The fluorescence levels are measured to quantify the levels of both TRECs and the control gene.

The technology required to perform SCID screening differs from that currently employed for any of the current disorders which comprise the newborn blood spot screening programme. This has implications for the resources required to set up and deliver the service both in terms of the equipment and staff time and expertise required. Additionally the technology requires provision of a clean room or UV hood/cabinet in order to minimise the risk of contamination and for some laboratories this may necessitate some estates work in order to deliver this. Consideration needs to be given to the resources needed both for the pilot and for the inclusion of SCID in the newborn blood spot screening programme should a decision be made to do so following completion of the evaluation.

The laboratory working group was asked to consider options for both the pilot and potential “roll out” on completion of the pilot and specifically the benefits and risks of providing the service in a restricted number of screening laboratories versus its inclusion in the repertoire for all laboratories. A comparison of the options is presented in the following table.

Option Appraisal for Newborn Screening Laboratory Services

Option	Description	Advantages	Disadvantages/Risks
1	**Pilot in a restricted number of screening laboratories/ Roll-out in a restricted number of screening laboratories.**	***For the pilot:*** Provision of equipment for fewer laboratories. Estates work minimised or possibly circumvented altogether depending on the laboratories selected. Staff costs reduced. This model used successfully in previous pilot studies for new disorders. ***For roll-out:*** Provision of equipment for fewer laboratories. Estates work minimised or possibly circumvented altogether depending on the laboratories selected. Reduction in technical/scientific staff required.	***For the pilot:*** Fewer number of babies will be screened overall than if pilot were to be run in all laboratories. Population in geographical area covered by the laboratories chosen may not be representative of the population in the country as a whole. It may be necessary to run the pilot for an extended period of time to deliver all of the objectives encompassed within the project scope. ***For roll-out:*** Currently there is a single screening card which would need to be split. This would involve separating the spot from the patient ID and reattaching and relabelling it. The risks associated with errors in doing this and potential patient mix-ups is significant. Splitting the cards and organising packaging and transportation would be time consuming and any staff saving in technical/scientific staff may be negated by the increase in support staff required. Audit checks would be required at each stage to ensure that all cards had been sent and received. An alternative to splitting the card would be to wait until the primary lab had completed all of the other screening analyses (including repeats) and then send the whole card – this would impose considerable delays to SCID screening and create additional governance issues associated with possible loss of the entire card. Another option would involve preparation of the samples in the receiving laboratory with the prepared plates being sent to the designated SCID laboratory for analysis. This mirrors the common NBS lab contingency plan (designed to cover equipment/assay failures etc). Whilst this is difficult but feasible in an emergency it would be a huge logistical challenge for a SCID

			laboratory to receive plates from multiple primary labs as part of the regular service provision. Results would need to be returned to the primary receiving lab for reporting to CHRD. It may be possible for electronic transmission to occur between labs using the same screening information system but for labs with different information systems it is likely that results would need to be inputted manually with all the associated risks this entails. Labs receiving and analysing samples for SCID would need space to accommodate additional equipment and staff – space is already restricted in many screening labs and this may not be possible. Modifications to reporting to CHRD and the failsafe would be required. Separate timeframes for reporting SCID would be required. Quality of the current programme relies on the close links between the laboratory and the other health professionals involved delivering the programme – midwives, health visitors, key clinicians etc. SCID screening would lose the benefit derived from these close working relationships.
2	**Pilot in a restricted number of screening laboratories/ Roll-out in all screening laboratories**	***For the pilot:*** Provision of equipment for fewer laboratories. Estates work minimised or possibly circumvented altogether depending on the laboratories selected. Staff costs reduced. This model used successfully in previous pilot studies for new disorders. ***For roll-out:*** Preserves the integrity and continuity of the blood spot programmes. Avoids risks and costs associated with splitting samples and fragmenting results reporting.	***For the pilot:*** Fewer number of babies will be screened overall than if pilot were to be run in all laboratories. Population in geographical area covered by the laboratories chosen may not be representative of the population in the country as a whole. It may be necessary to run the pilot for an extended period of time to deliver all of the objectives encompassed within the project scope. ***For roll-out:*** Higher start-up costs associated with need to equip all laboratories.

		Maximises number of labs available to provide back-up in the event of assay or equipment failure in any laboratory. Provides a national pool of staff with expertise in the application of PCR techniques to newborn screening.	Potential requirement for estates work in some laboratories in order to provide a clean area. Additional technical/scientific staffing resource required for each laboratory.
3	**Pilot in all screening laboratories/ Roll-out in all screening laboratories**	***For the pilot:*** Will provide an accurate reflection of true positives and false positives within the total population who would be offered screening. Ensures that all laboratories have the expertise, infrastructure and staffing to enable implementation with no delay if roll out approved. Equity of screening provision for the whole population during the pilot. ***For roll-out:*** Preserves the integrity and continuity of the blood spot programmes. Avoids risks and costs associated with splitting samples and fragmenting results reporting. Maximises number of labs available to provide back-up in the event of assay or equipment failure in any laboratory. Provides a national pool of staff with expertise in the application of PCR techniques to newborn screening.	***For the pilot:*** Potential investment in equipment that would be redundant if roll-out was not approved at the end of the evaluation period. Requirement for additional scientific staff who may not be required permanently if roll-out not approved. Potential requirement for estates work which would have been unnecessary if roll-out not approved. ***For roll-out:*** Higher start-up costs associated with need to equip all laboratories. Potential requirement for estates work in some laboratories in order to provide a clean area. Additional technical/scientific staffing resource required for each laboratory.

Conclusions

- The benefits of carrying out the pilot in every laboratory are small relative to the significant additional costs and cannot be justified.
- By selecting, for the pilot, the largest screening laboratories and/or those laboratories in centres with strong clinical immunology links and where there are currently clinical services for SCID will maximise the information which can be gathered on both the performance of the screening programme and treatment pathways at minimal cost.
- By running the pilot over 2 years the total number of babies screened will be equivalent to running the pilot in all laboratories over 1 year.
- The risks of rolling out the programme to only a selected number of screening laboratories creates huge logistical problems and risks that cannot be justified by the financial gain.
- If the recommendation at the end of the evaluation period is to add SCID to the newborn screening panel then SCID screening should be rolled out to all of the existing screening laboratories.

Recommendations

- Option 2 is recommended by the SCID laboratory working group.
- It is proposed that the following screening laboratories should participate in the pilot: GOSH, Viapath, Birmingham, Newcastle, Manchester, Sheffield.
- GOSH, Viapath, Sheffield, Birmingham and Manchester are the five largest screening labs in England and all screen more than 50,000 babies/annum. Four of these labs (GOSH, Sheffield, Birmingham and Manchester) also have strong links with paediatric immunology. Newcastle has been selected in addition because of its expertise in paediatric immunology and SCID in particular being one of only two national treatment centres for this disorder.
- It is considered that by selecting the above centres maximum information can be gathered in the most cost-effective way regarding the performance of the screening programme and the effectiveness of the diagnostic protocols.

Laboratory Appendix 2: Protocol for the Validation of a Kit-Based Method of Measurement of T-cell Receptor Excision Circles (TRECs) for Newborn Bloodspot Screening for Severe Combined Immune Deficiency (SCID).

Revision 3: (July 2023)

1. Background

Severe combined immune deficiency (SCID) is a rare congenital disorder that is not evident at birth. It is characterized by the disturbed development of functional T cells and B cells caused by numerous genetic mutations that result in differing clinical presentations. SCID patients are usually affected by severe bacterial, viral, or fungal infections early in life and often present with interstitial lung disease, chronic diarrhoea, and failure to thrive. These babies, if untreated, usually die within one year due to severe, recurrent infections unless they have undergone successful haematopoietic stem cell transplantation (HSCT), gene therapy or have been placed on enzyme replacement therapy (ADA SCID subtype). Early identification of SCID through newborn screening would allow prompt intervention before infections occur.

In September 2021, 6 laboratories in England commenced a 2-year evaluation of SCID screening. The screening test for SCID involves measurement of T-cell receptor excision circles (TRECs). TRECs are stable circular DNA fragments generated during T-cell receptor rearrangement. In healthy newborns, TRECs are made in large numbers, whilst in newborns with SCID they are barely detectable. Two IVD approved commercial kits were available for SCID screening at the start of the evaluation, both of which utilise the measurement of TRECs – the Perkin Elmer Enlite™ Neonatal TREC kit and the Immuno IVD Spot-it™ kit. A third kit, the Perkin Elmer EONISQ kit has now been launched.

The EnLite™ kit provides the semi-quantitative determination of TREC by polymerase chain (PCR) based nucleic acid amplification and time resolved fluorescence resonance energy transfer based detection. The assay detects TREC and β–actin. TREC is the marker of SCID and β–actin is used as a control for monitoring DNA amplification. The Spot-it™ kit uses real time PCR. The kit is based on DNA elution followed by real-time quantitative polymerase chain reaction (qPCR) for TREC and β-actin. The EONISQ kit uses automated dry real time PCR technology for TREC and RPP30, the latter being the control for monitoring DNA amplification.

Three laboratories (Manchester, Sheffield and Birmingham) are currently evaluating the En-Lite™ kit and three laboratories (SE Thames, GOSH and Newcastle) are evaluating the Spot-it™ kit. The plan was for Manchester & Sheffield laboratories to switch to EONISQ at the beginning of April 2023 and for SW Thames to also commence SCID screening using EONISQ at the same time. SE Thames, GOSH & Newcastle laboratories would continue to run the Spot-it™ kit. Birmingham laboratory would continue to run the En-Lite™ kit but with the intention that this would be reviewed. However the validation of the EONISQ kit revealed a high

number of samples with zero TREC levels which would have resulted in an unacceptable false positive rate. The problem was discussed at length with Perkin Elmer (now REVVITY) who have now made modifications to the kit, preliminary studies suggest a reduction in the frequency of zero TREC results in the reformulated kit. A revised plan was agreed – the new kits will be distributed to the three laboratories at the beginning of August 2023 and the method will be validated and data analysed ready to go live at the beginning of November 2023 provided performance of the revised method is found to be satisfactory. A 6-month extension to the evaluation study period for SCID screening has been agreed – the study will now run until the beginning of March 2024 which will enable 4 months EONISQ data to be obtained. Whilst this is less data than originally planned it should provide a valuable insight into the performance of the method relative to En-Lite™ and Spot-it™.

The method validation work will:

- Enable the programme to decide whether the performance of the method is adequate to progress with the plan to incorporate EONIS Q into the evaluation for the remainder of the study period. If progress is agreed, it will allow the laboratories to apply to UKAS for an extension to scope to incorporate TREC analysis by EONISQ within their portfolio of accredited assays.

- Establish population centiles for TREC levels by EONISQ - this will inform the choice of initial cut-off to be used for this method.

This protocol describes the work to be completed during the pre-evaluation (validation) and evaluation phases of the study.

1.1 Testing Pathway Risk Assessment

Errors in producing an analytical result can occur within the pre-analytical, analytical or post-analytical phase. The Testing Pathway Risk Assessment is designed to:

- document the processes involved in the whole testing pathway
- identify the risks associated with each step of the process
- document the control measures to remove or minimise the risks
- consider quality improvements to further reduce the likelihood of risks occurring

A risk assessment for the TREC analysis testing pathway for SCID screening will be included with the method validation.

2. Assessment of En-Lite™ , Spot-it™ & EONISQ Kits for TREC Analysis

2.1 Validation vs Verification

In the majority of cases when the laboratory purchases analytical methods from diagnostic companies or kit manufacturers the methods are used in line with the manufacturer's instructions and have therefore been fully validated. In this case only a verification is required. In the case of the TREC methods there is a requirement to set cut-offs based on both assessed kit performance and population data. These cut-offs may differ from those defined by the manufacturers in which case use of the kit would be defined as being outside its intended scope. Since this is the case a full validation is required for UKAS purposes. Specific guidance on the validation of TREC methods can be found in the Clinical and Laboratory Standards Institute (CLSI) Guideline NBSo6-A[1].

2.2 Performance Characteristics to be Assessed

2.2.1 Accuracy

The following experiments will be performed to assess the accuracy of the methods:

2.2.1.1 Comparison with Kit Controls

The following controls are supplied with each kit:

Perkin Elmer Enlite™	Immuno IVD Spot-it™	EONISQ
C1 – low control with low level of both TREC and beta-actin* C2 – no TREC control with no TREC and high levels of beta-actin* C3 – high control with high level of both TREC and beta-actin*	TP (TREC Positive) control card – expected TREC copies >20, expected ACTB copies >1000 TN (TREC Negative) control card – expected TREC copies ≤6, expected ACTB copies >1000	C1 no TREC, normal RRP30 C2 low TREC, normal RRP30 C3 high TREC, normal RRP30 NTC – no TREC, no RRP30

* The exact TREC and beta-actin concentrations are given on the lot-specific quality control certificate included in the kit.

Values obtained will be compared with the values assigned by the relevant kit manufacturer and will be acceptable if they fall within the assigned range. Several different analytical runs on different days (minimum n=5) will be included in the comparison.

2.2.1.2 Comparison With An Established Method

The laboratories who are currently running the En-Lite method (Sheffield & Manchester) can compare the EONISQ result for samples used for the population data (see section 3) with the EnLite result. However it would be important to also obtain comparative data with IIVD method.

Screen positive samples (including some true SCID positives and some T-cell lymphopenias) will already have been sent to the partner IIVD labs and the IIVD results are available. However in view of the reported instability of TRECs and the age of some of the samples it would be desirable if there is sufficient material to reanalyse by both Enlite and IIVD in order to obtain contemporaneous results. Additionally 20-30 samples with normal TREC results will be sent for analysis by IIVD. Blood spots will be anonymised prior to submission to the partner laboratory.

The categorical classification of samples based on the EONISQ kit results will be compared with the classification using the En-Lite and Spot-it^{TM} kits once EONISQ cut-offs have been agreed.

2.2.1.3 Analysis of External Quality Assurance Samples

A UKNEQAS scheme is in place for TREC analysis. Samples include the following:

- Samples with TREC content within the expected range for newborns prepared from normal paediatric blood samples. The use of different Dried Blood Spot (DBS) reference material prepared from paediatric blood samples that have varying levels of TREC content provide assessment of the assay performance within the expected range.

- Samples with TREC content below the expected range for newborns simulating SCID. These samples are prepared from blood of older adults (generally over the age of 50) who have recently undergone HSCT and are profoundly lymphopaenic.

- Samples that are inconclusive as evidenced by failure to amplify genomic DNA. These samples will have both TREC and reference gene content below the expected newborn range and are prepared from leukocyte –reduced adult blood.

Individual laboratories report both TREC concentration for each sample and also classify each as screen positive, screen negative or inconclusive based on agreed initial cut-offs for each method. Good agreement between laboratories performing the same method and equivalent classification across all 7 laboratories will be deemed acceptable.

The EQA samples provide a valuable additional source of material for the EONISQ validation and will be utilised :

1. To compare PCR machines – 6 EQA samples will be run in triplicate on each EONSQ machine, the mean of the triplicates calculated and categorical classification compared with the EQA assigned classification once EONISQ cut-off have been agreed.

2. As an additional measure of accuracy – 12 EQA samples from 4 rounds (and a range of TREC values) will be analysed. 6 of these can be taken from the PCR machine comparison.

2.3 Precision

2.3.1 Repeatability

This will be determined by calculating the variability from a minimum of 20 results obtained from repeat analysis of samples with “normal” and “low” TREC levels on the same plate. This will be performed using:

- Kit IQC – C1 and C3 for the Perkin Elmer Enlite™ , C2 (low) and C3 (normal) for the PE EONISQ kit and TP and TN for the Immuno IVD Spot-it™.

Because TRECs are not normally distributed data must be log transformed and results should be reported as Mean TREC Ln (copies/μL), SDs in the logarithmic (Ln) scale and as % CVs in lognormal scale.

Repeatability will be deemed acceptable if it is consistent with the SDs and CVs defined by the kit manufacturer. See Appendix 1 for the manufacturers’ precision data. For the EONISQ method the Enlite precision will be used as the target for repeatability since there is no manufacturers’ data as yet for this new method.

2.3.2 Intermediate Precision

This will be determined by calculating the variability from results (ideally a minimum of n=20) obtained from repeat analysis of samples with “normal” and “low” TREC

levels on different plates spread across all EONISQ machines. This will be performed using:

- Kit IQC – C1 and C3 for the Perkin Elmer Enlite™ kit, C2 and C3 for the PE EONISQ kit and TP and TN for the Immuno IVD Spot-it™.
- Additionally for the EONIS Q validation the sample with low TREC levels provided by Stuart Adams. Material is limited (only 4 spots) so it will be important to punch carefully.

Data should be log transformed as for the repeatability studies.

Intermediate precision will be deemed acceptable if it is consistent with the SDs and CVs defined by the kit manufacturer. See Appendix 1 for the manufacturers' precision data. For the EONISQ method the Enlite precision will be used as the target for repeatability since there is no manufacturers' data as yet for this new method.

Whilst it is desirable that intermediate precision measurements should include different calibrations, calibrators, operators and reagent lots this will not be possible to achieve this during the pre-evaluation phase. Intermediate precision will need to be reviewed and refined throughout the evaluation period.

The initial intermediate precision data (SD) determined during the pre-evaluation phase for the low and high IQC will be used to set the initial acceptance limits for the IQC at the start of the evaluation phase. These limits will be checked and modified as required.

2.4 Measurement Uncertainty

This will be assessed initially using IQC intermediate precision data. This assumes that all the uncertainties in the individual steps of the analytical process are included in the SD associated with the measurement of the IQC. Given the limitations of what we can achieve in the pre-evaluation phase in terms of number of batches, kit lot numbers, operators etc the MoU will be monitored and regularly reviewed as part of the test quality management systems. MoU may need to be revised as these additional factors come into play.

Assuming a lognormal distribution, the standard deviation (s) of the natural logarithms will be used for assessment of measurement uncertainty. The 95% limits for the relative error are then given by

Lower 95% probability limit $\exp(-1.96s)$

Upper 95% probability limit $\exp(+1.96s)$.

For example, if the standard deviation of the logarithm of were 0.1, the relative error limits are given below.

sd (log_e TREC)	Probability limit (95%)		Error Limits (95%)	
	Lower	Upper	Lower	Upper
0.1	0.82	1.22	-18%	22%

The probability limits relate to the multiplicative effect on the median so that with 95% probability measurements range from 0.82×median to 1.22×median. Corresponding to percentage errors ranging from -18% to +22%.

2.5 Analytical Specificity

PCR specificity for sequences unique to TREC is confirmed by showing no amplification of human genomic DNA obtained from any source other than T-cells. According to CLSI Guideline NBSo6-A1[1] all the TREC assay probes and primers currently used for SCID NBS have well-documented specificity, and use of established reagents provides *a priori* assurance of PCR specificity. It is not therefore proposed to investigate PCR specificity as part of this evaluation.

2.6 Analytical Sensitivity

2.6.1 Limit of Blank (LoB)

The LoB is determined by repeated analysis of a blank reference material. For practical evaluation of the real time qPCR TREC assay, LoB can be estimated by repeated analysis of DBS that contain no TREC but have genomic DNA at a concentration typical for a newborn.[1] These DBS may be made by using T-cell-depleted peripheral blood from older adults. It may be possible to use the C2 control from the Perkin Elmer Enlite™ kit, the C1 control from the EONISQ kit and the TN control from Immuno IVD Spot-it™ for LoB studies. However Immuno IVD state that TRECs in the TN control are $\leq$6 rather than zero and so it may be better to use a sample prepared by Stuart Adams which is known to contain zero TRECs.

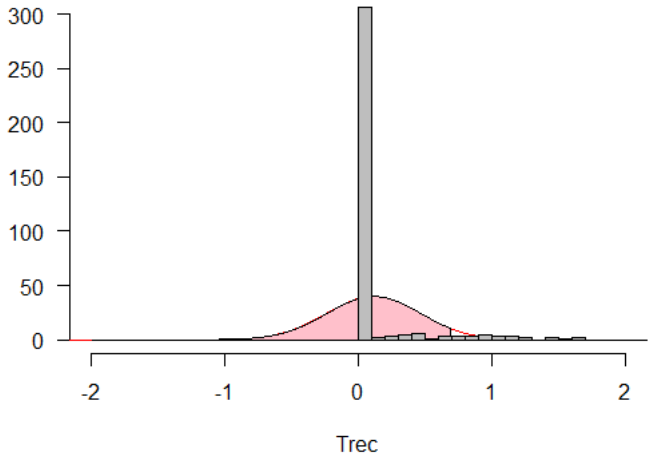
To estimate LoB, the above DBS (minimum n=20) will be analysed in multiple runs over a reasonable time period (e.g. intermediate precision conditions). Calibrators will be included in each run. Failure to amplify is counted as zero TREC. Any signal is converted to a TREC value interpolated from the standard curve or extrapolated by extending it below the lowest standard.

Statistical Analysis

A simple nonparametric method is to use the 95th percentile of the data. Using bootstrapping 95% confidence intervals for the true LOB can be obtained.

Problems with the Gaussian approach

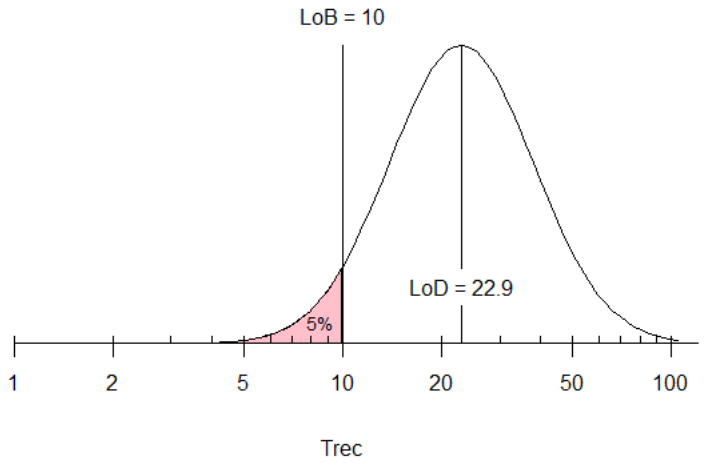
The parametric methodology assumes that the blank TREC values follow a Gaussian distribution and uses the 95th percentile of the fitted Gaussian distribution as the LoB. The histogram (grey) shows some blank TREC measurements with 305 (87%) zeros. The red distribution curve is the fitted Gaussian distribution which clearly does not reflect the distribution of the data. The assumption that the blank TREC counts follow a Gaussian distribution is inappropriate and the method should not be used.



2.6.2 Limit of Detection (LoD)

The LoD is the lowest analyte concentration likely to be reliably distinguished from the LoB. Evaluation of the LoD requires analysis of DBS materials (minimum n=20) with independently determined TREC levels near the LoB. Samples with low level TRECS prepared by the participating laboratories and/or provided by Stuart Adams will be used for this evaluation and will be analysed in multiple runs over a reasonable time period (e.g. intermediate precision conditions). Differences in the technology and chemistries of the Immuno IVD Spot-it^{TM} kit and the Perkin Elmer Enlite^{TM} and EONIS Q kits mean it is likely that separate samples will need to be utilised to assess the LoD for each kit. For the EONISQ validation the data obtained from the intermediate precision studies for the low TREC material provided by Stuart Adams can be used.

The TREC data generally follows a log Gaussian distribution as illustrated below. The example below shows the distribution measured TREC concentrations for a sample with a true concentration of 22.9. For this concentration, just 5% of measurements fall below the LoD.



The LoD can be obtained by estimating the standard deviation of the log transformed measurement close to the LoD and adding 1.645 standard deviations to the log transformed LoD. The antilogarithm of the result is the LoD.

For the example shown above, using natural logarithms (LN in Excel)

LoB = 10

SD of log TREC (low TREC sample) = 0.5042

log (LoD) = log(LoB) +1.645*SD of log TREC (low TREC sample) = log(10) + 1.645*0.5042 = 3.1320

LoD = exp(3.1320) = 22.9

As illustrated in the Figure, just 5% of measurements of the LoD concentration will fall below the LoB.

2.6.3 Limit of Quantitation (LoQ)

LoQ can be defined as the lowest concentration with a total SD <0.90 on logarithmic (Ln) scale. This concentration can be assessed from the LOQ and accuracy studies using samples with known TREC concentrations. However from experience with the En-Lite kit we know that this is likely to generate an analytical cut-off which will lead to a high rate of false positives. LoQ is better defined in terms categorical classification as the lowest concentration which detects 100% of true positives whilst minimising false positives. A provisional

LoQ can be determined using population centiles and EQA samples which can be refined as further data is acquired during the evaluation period.

Values obtained for LoB, LoD and LoQ obtained will be compared with those quoted by each manufacturer (see Appendix 2). Currently no manufacturer data is available for EONISQ.

2.7 Contamination and Carry Over

Because SCID NBS depends on identifying samples with absent or very low TREC content, contamination by even the smallest amount of amplified material could lead to a missed case. To monitor for contamination and carry-over each assay or plate will contain no target controls (NTCs) scattered across the plate. CLSI guidelines propose that NTCs are prepared by punching from blank filter paper cards into wells which are then subject to the entire testing procedure. Specific NTCs prepared using pigs blood which more closely matrix match the neonatal bloodspots have been provided by PE for use with the En-Lite kit and the EONISQ kit includes NTC material.

Suggested plate map to assess carry over.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Zero	C1	C2	C3	P1	P2	NTC	NTC	P3	P4	NTC	NTC
B	P5	P6	NTC	NTC	P7	P8	NTC	NTC	P9	P10	NTC	NTC
C	P11	P12	NTC	NTC	P13	P14	NTC	NTC	P15	P16	NTC	NTC
D	P17	P18	NTC	NTC	P19	P20	NTC	NTC	P21	P22	NTC	NTC
E	P23	P24	NTC	NTC	P25	P26	NTC	NTC	P27	P28	NTC	NTC
F	P29	P30	NTC	NTC	P31	P32	NTC	NTC	P33	P34	NTC	NTC
G	P35	P36	NTC	NTC	P37	P38	NTC	NTC	P39	P40	NTC	NTC
H	P41	P42	NTC	NTC	P43	P44	NTC	NTC	Zero	C1	C2	C3

P1 -P44: Patient samples that can be used to collect population data

3. Assigning Cut-offs

In order to help assign initial cut-off values to be used in the evaluation, retrospective analysis of TRECs in residual DBS specimens will be undertaken. 10,000 - 15,000 specimens will be analysed using each of the three alternative methodologies - Perkin Elmer Enlite™ ,Immuno IVD Spot-it™ and EONIS Q. The number of samples to be analysed by each individual laboratory will be allocated in proportion to their usual workload.

Data will be analysed to establish population distribution for TREC results based on each kit. These results will be considered alongside international experience using each method (where this data is available) and consideration of the kit manufacturer's recommendations to help assign a suitable TREC cut-off value to indicate the need for clinical referral.

The aim will be to balance the need to avoid generating a large number of false positive results while minimising the risk of missing a SCID case. Evidence will be provided to the multidisciplinary SCID Board made up of Laboratory Scientists, Clinical

Immunologists and Public Health experts who together will agree an appropriate Cut Off Value for each method based upon an analysis of the data and published international experience with these assays. The analysis of the data and the recommendations made to the SCID Board for the Perkin Elmer Enlite™ and Immuno IVD Spot-it™ kits is attached as Appendix 3.

Once in use, the method related COV will remain under regular review as part of the evaluation. This review will be based upon the observed positive predictive value of the assays and in the unlikely event that a case is missed, the sensitivity of the assays.

For EONIS Q Sheffield, Manchester and SW Thames will each analyse 5000 samples. Gestational age will be recorded with a view to calculating term centiles and pre-term centiles separately to allow different cut-offs to be assigned.

Additional sample analysis to inform COV:

- SCID true positives. 10 of these have been identified so far as part of the evaluation. Where there is sufficient material remaining these samples will be shared between the three EONISQ laboratories.
- 20-30 non-SCID T cell lymphopenias per laboratory.
- Volunteer bloodspots with zero TRECs prepared in-house.
- TREC zero samples provided by Stuart Adams.

4. Additional studies to be Completed During Evaluation Phase

4.1 Sample Stability

Stability will be assessed by repeat analysis of IQC samples with low medium and high levels of TRECs after days, weeks, months and 1 & 2 years of storage at room temperature. 4^0C and -20^0C. More limited stability studies will be performed on DBS samples submitted to the programme, being mindful of the need to leave sufficient material for 2 or more 3.2mm sub-punches for clinical purposes after sampling for this study.

4.2 Impact of Sample Quality

The impact of sample spot size, of punching from centre and edge of the spot and of sample quality (multi-layered, multi-spotted and compressed DBS) has been found to have varying effects on the analytes measured as part of the current blood spot programme.[2,3] It will be important to assess the impact on TREC analysis. Currently multi-layered samples are accepted for screening. Although SCID screening involves looking for low values in screen positive babies, so multi-layered spots could theoretically generate false negative results, studies conducted by Prof Stuart Moat in Cardiff NBS laboratory showed this not to be the case in practice.

4.3 Direct Comparison Between Methods

On a monthly basis screen positive samples will be exchanged between laboratories as follows:

PE		Immuno IVD
Manchester	⟷	Newcastle
Sheffield	⟷	GOSH
Birmingham		SE Thames
SW Thames	⟷	SE Thames

Categorical classification will be compared to identify samples which would have been differently classified as (i.e. screen positive by one method and screen negative by the alternative method).

4.4 Assessment of Clinical Sensitivity, Specificity, PPV and NPV

The performance of both TREC assays will be assessed at regular intervals in order to fine tune and determine an optimal cut-off. By starting with a higher cut-off and obtaining outcomes on all babies referred with values below this cut-off it will be possible to calculate positive predictive value (PPV) and negative predictive value (NPV) for a range of values for the three alternative methods and to select the optimal cut-off to ensure that SCID is always detected while minimizing false-positive results. It will also be possible to directly compare the performance of the three methods at their optimal cut-off.

4.5 Evaluation of SCID Levels in Samples from Premature Neonates

It was agreed to incorporate a separate pathway within the testing algorithm for premature neonates with a lower cut-off for immediate referral. Premature babies (<37 weeks' gestation and in hospital at the time of sample collection) with TREC concentrations between this lower cut-off and the cut-off used for referral of term babies have a repeat sample taken at 37 weeks' gestation or discharge (whichever is sooner). The agreed term baby cut-offs are used to determine the action to be taken following analysis of the repeat sample. Cut-offs are in place for the Perkin Elmer Enlite™ and Immuno IVD Spot-it™ methods and will need to be agreed as part of the validation of the EONISQ method. It will be important to assess the impact of this separate pathway on the false positive rate of the programme as a whole and the false positive rate in the premature cohort specifically. Alternative pathways for premature neonates can be modelled from the data gathered in the evaluation phase.

5. References

1. Clinical and Laboratories Standards Institute: Guideline NBSo6-A Newborn Blood Spot Screening for Severe Combined Immunodeficiency by Measurement of T-cell Receptor Excision Circles; Approved Guideline (April 2013)
2. Clinical and Laboratories Standards Institute: Guideline EP17-A2 – Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures; Approved Guideline – Second Edition (June 2017)
3. Stuart J Moat, Catherine Dibden, Lesley Tetlow, Caroline Griffith, Jim Chilcott, Roanna George, Laura Hamilton, Teresa HY Wu, Finlay Mackenzie & Susan Kate Hall Effect of Blood Volume on Analytical Bias in Dried blood Spots Prepared for Newborn screening External Quality Assurance Bioanalysis 2020 Jan 19;12(2):99-109
4. George RS & Moat SJ Effect of Dried blood Spot Quality on Newborn Screening Analyte Concentrations and Recommendations for Minimum Acceptance Criteria for Sample Analysis Clin Chem 2016 62; 466-475

Laboratory Appendix 3: Considerations relating to establishing the Cut-off Value to be used when screening for SCID

The level at which we set the Cut of Value (CoV) when screening for SCID will be pivotal in balancing the harms vs benefits for families when accepting the offer of screening.

If we set the CoV too high we might miss cases, too low and we seriously worry many families unnecessarily with potentially lasting damage for the child. The CoV really is where the rubber hits the road for the families involved and the clinicians who must deliver clinical care.

There are three ways in which we might approach this:

- From an analysis of practice elsewhere, using their CoV to guide our selection – it was largely this approach that was adopted when screening for additional IMDs was adopted and we based our CoVs on those in use in the Netherlands.
- From an analysis of the distribution of our own data by applying statistical methods to define, say the bottom 0.05th centile or similar.
- Simply use the manufacturers recommended CoVs.

Sadly, none of these are straightforward for us for a variety of reasons:

- The data is not normally distributed but despite this at least one of the manufacturers (IIVD) appear to have assumed this in their treatment of the data and therefore their proposed CoV would be in doubt. The other manufacturer (PE) have assumed non parametric data but may have made an error in the way that the statistics have been used.
- The International comparisons all use a separate route for prems so we would need to consider whether to adopt this approach or to apply a universal CoV.
- The statistical approach can only be used as a guide as, in the anonymised population we do not have clinical outcomes and the low number of observations 14.6k (PE) and 19.7k (IIVD) are too few to provide certain information on rare events.

Nevertheless, taken together with our understanding of the technical performance of the assay, they can guide our thinking and will allow us to make recommendations to use at the outset of the evaluation and these can be kept under review. We can look at these separately as PE and IIVD groups.

PERKIN ELMER ENLITE
International comparisons

Four countries using PE-enlite presented their findings during two day virtual SCID meeting in January 2021.

They are summarised below. Notably all use a separate protocol for prems/NICU babies and term babies and all claimed no missed cases (100% sensitivity) at their CoVs used.

Country	Method	n	CoV	Flow cytometry/referral	T-cell lympho-penia	SCID	PPV % Lymphopenia SCID
USA	PE enlite	3.25 m	18 Separate NICU protocol	562	213	50	37.9 8.9
Spain	PE enlite	223k	Less than equal to 10 refer. 11 – 20 request repeat sample – less than equal to 20 refer. Different route for pre terms, <5 refer, 6-20 rept at 37 weeks	48	17	3	35.4 6.3
France	PE enlite	200k	Began with CoV 20, after 100k judged recall rate too high, switched to: Less than equal to 10 refer, 11-20 repeat less than equal to 20 refer. Different route for pre terms, <5 refer, 6-20 rept at 37 weeks	165	62	3 SCID + 3 leaky SCID	37.6 3.6
New Zealand	PE enlite	166k	18 (urgent 5) Separate NICU protocol	65	17	2	29.6 3.1

Taken together these results suggest that a CoV in the range of 18-20, with a separate treatment of premature babies, would produce results broadly in line with our initial expectations published by ScHARR in terms of false positive cases:

The starting assumption published by ScHARR in 2017 for the UK population assumed:

- 310 screen positive cases pa
- 26 T-cell lymphopaenia
- 17 SCID cases

Statistical analysis of the possible outcome in our own population for PE Enlite

As part of the validation and verification work, laboratories were each asked to analyse around 5,000 anonymised patient samples from residual blood spot cards. The data was not normally distributed but on log transformation assumed a normal Gaussian distribution. The results indicate:

Combining the data, (exc beta-actin <55), n = 14,621			
Screen positive at varying CoV	(CoV = 18) - 10	(CoV = 19) - 17	(CoV = 20) - 23
Extrapolated to UK pop (750k births pa)	512 screen positive	872 screen positive	1179 screen positive

While if we were to use a CoV of say, 20, this might suggest more false positive results than desirable, these data do not take in account the effect of a separate treatment for premature babies which initial evidence suggests would contribute almost half of the screen positive results.

IMMUNO-IVD
International comparisons

Country	**Method**	**n**	**CoV**	**Flow cytometry/referral**	**T-cell lympho-penia**	**SCID**	**PPV % Lymphopenia SCID**
Netherlands	IIVD	192k	Less than or equal to 10 Switching to repeat sample for TRECs 2-10 wef 1.1.21 Separate protocol for prems	62	18 (exc secondary causes)	1	29.00 1.6
Sweden	IIVD	116k	Less than equal to 6	73 (27 of these prems)	18	3	25.0 4.1

Taken together these results suggest that a CoV in the range of 6-10, with a separate treatment of premature babies, would produce results broadly in line with initial expectations.

Statistical analysis of the possible outcome in our own population for IIVD

Again as part of the validation and verification work, laboratories were each asked to analyse around 5,000 anonymised patient samples from residual blood spot cards. The data was not normally distributed but on log transformation assumed a normal Gaussian distribution The results indicate:

Combining the data (exc beta-actin <1000), n = 19,654				
Screen positive at varying CoV	(CoV = 6) - 22	(CoV = 8) - 33	(CoV = 10) - 42	CoV = 12) 47
Extrapolated to UK pop (750k births pa)	839 screen positive	1259 screen positive	1602 screen positive	1793 screen positive

If we were to use a CoV of say, 8, this might suggest more false positive results than is ideal but it again seems likely that a separate treatment for premature babies might ameliorate these effects.

Further Considerations

While the predictions concerning the likely impact of say, a CoV of 20 for PE – 1179 screen positives pa and a CoV of 8 for IIVD – 1875 screen positives pa, seem alarmist, they can be ameliorated in two ways:

- Firstly, in line with international practice, we could adopt separate treatment within the algorithm for premature babies by adopting a differential CoV. Data suggests that this may approximately half the number of false positive results.

- Secondly, the algorithm dictates a repeat sample in duplicate when the first result is close to the CoV before a decision to classify this as screen positive is made. As these results are by definition outliers from the main population we would expect to see regression to the mean if determinations are repeated. This would have the effect of making truly 'low' results lower and truly 'normal' results higher, increasing both sensitivity (the detection rate at a given CoV) and specificity (avoiding false positives at a given CoV). Without being certain of the magnitude of this effect, it would be reasonably cautious to assume that this may further reduce false positive results by at least 20%.

#

- If these predictions were true, with these two measures in place, we may, without significantly reducing sensitivity reduce false positives to:
    - In the PE group to 472 pa full year effect in a 750k population
    - In the IIVD group to 750 pa full year effect in a 750k population

- While this is more than originally predicted we can keep the numbers under regular review.

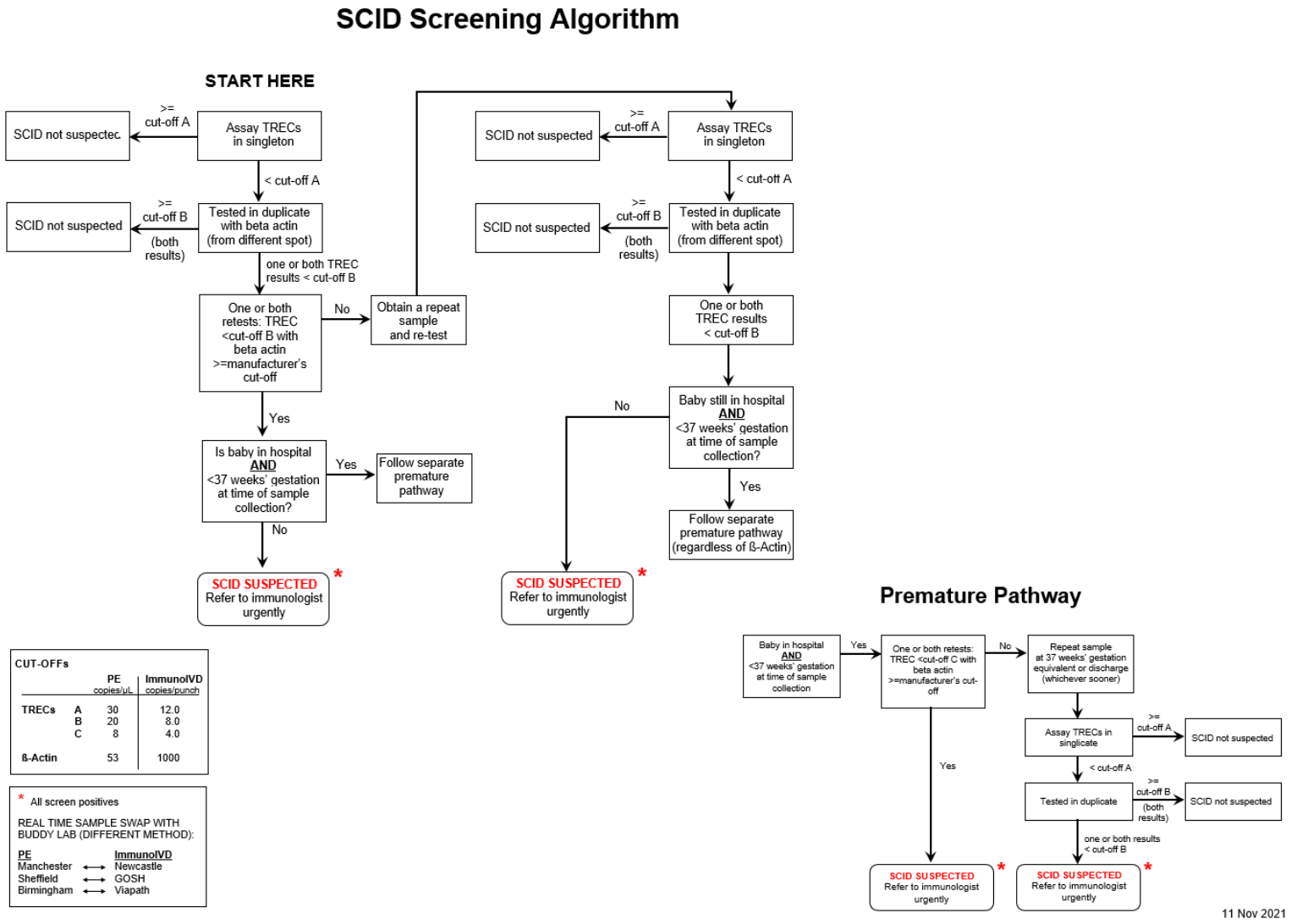
Recommendations

On this basis and synthesising: International experience, the technical performance of the assay and the limited analysis of single determinations in anonymised samples, we propose:

- That we adopt a 'clinical CoV' for referral for term babies
  - For PE = 20
  - For IIVD = 8
- That we adopt a separate 'clinical CoV' for referral for premature babies under 37weeks:
  - For PE = 8
  - For IIVD = 4
- That we adopt an 'analytic cut off' to indicate the need for a re-test in duplicate on the same card
  - In term babies, for PE = 30 for all babies (50% above the clinical CoV)
  - In term babies, for IIVD = 12 for all babies (50% above the clinical CoV)
  - In preterm babies, for PE = 12 for all babies (50% above the clinical CoV)
  - In preterm babies, for IIVD = 6 for all babies (50% above the clinical CoV)
- All results below the 'analytic CoV' would be repeated in duplicate using the same blood spot card. The initial result would not be taken into account and the lower of these two duplicates would be taken as the final result to determine 'SCID not suspected' or 'SCID suspected' using the appropriate 'clinical CoV' for the baby.
- In pre-term babies <37 weeks gestation, if the results, using the appropriate **pre-term** 'clinical CoV' indicated SCID suspected then that would be reported and the baby would be referred.
- In pre-term babies <37 weeks gestation, if the results, using the **term** baby 'clinical CoV' indicated 'SCID not suspected' then that would be reported.
- In pre-term babies <37 weeks gestation, if the results fell between the appropriate **pre-term and term** baby 'clinical CoV', then a request would be made for a repeat sample.
- The repeat sample would be taken at the equivalent of 37 weeks gestation or discharge, whichever is earlier.
- If the result is above the appropriate 'clinical CoV' for a **term** baby, 'SCID not suspected' would be reported.
- If the result is below or equal to the appropriate clinical CoV for a **term** baby the 'SCID suspected' would be reported and the baby would be referred.

These recommendations were approved by the SCID Board for use on Friday 23rd July 2021.

Laboratory Appendix 4: SCID screening algorithm



Laboratory Appendix 5: Suggested amendment to the TREC Cut-off value for Sheffield

6.9.21 – 30.9.21

The assignment of the CoV for referral seeks to balance the risk of missing a SCID case, indicated by a low TREC value, against the risk of over referral. In the pre-evaluation analysis it was estimated that this would be achieved, in the PE Laboratory Group as a whole (Manchester, Sheffield and Birmingham), by assigning a CoV of <20 TRECs for term babies, resulting in the referral of approximately 1:1,500 babies tested.

In practice, during the first month of testing, 6.9.21 – 30.9.21, 14 referrals were made by the Sheffield Laboratory from an estimated 4,070 babies tested, a referral rate of 1: 291 babies tested.

The Clinical Immunology services in the region receiving these referrals from the Sheffield Lab indicated that this increased frequency of clinical referrals could not be sustained and this prompted a re-evaluation of the CoV used to indicate referral.

It seemed from the pre-evaluation work attached that the Sheffield Lab had a significant negative bias when compared with Manchester and Birmingham, the other two labs in the PE group. The respective 'median and 1st centile' TREC values based upon the pre-evaluation analysis of 4,000 to 6,000 samples in each lab were:

- Manchester Median = 117 1st centile = 31
- Birmingham Median = 185 1st centile = 41
- **Sheffield Median = 79 1st centile = 22***

If we concentrate on the 1st centile in each case when the Sheffield results are compared with the mean of the Manchester and Birmingham, this suggests a negative bias close to the CoV of 38%. From a retrospective analysis of cases referred by Sheffield from 6th September to 30th September 2021, reducing the CoV to <15 TRECs, based upon the assessment of the lowest of the two repeat results, would reduce the number of referrals from 14 to 5. See below:

Initial TREC	Repeat 1	Repeat 2
1	9	**5***
29	19	20
29	**14***	27
11	19	24
21	**9***	17
23	19	50
19	17	12
22	17	17
20	**13***	51
23	17	19
23	**8***	49
27	19	21
17	17	49
28	18	19

5 babies would have been referred – each marked*

This would achieve a referral rate of 1:815 babies tested, equivalent to 6 referrals per month from the Sheffield Laboratory and considered acceptable by the Immunology Clinical service.

An alternative way to corroborate this would be to calculate at the 0.1 centile (equivalent to 1:1,000 babies referred) as 3.09 SDs below the mean. For the Sheffield data the mean = Ln 4.305 and the SD is 0.5055, hence the 0.1 centile is 4.305 – 3.09 x (0.5055) = Ln 2.708. This is = 15.00

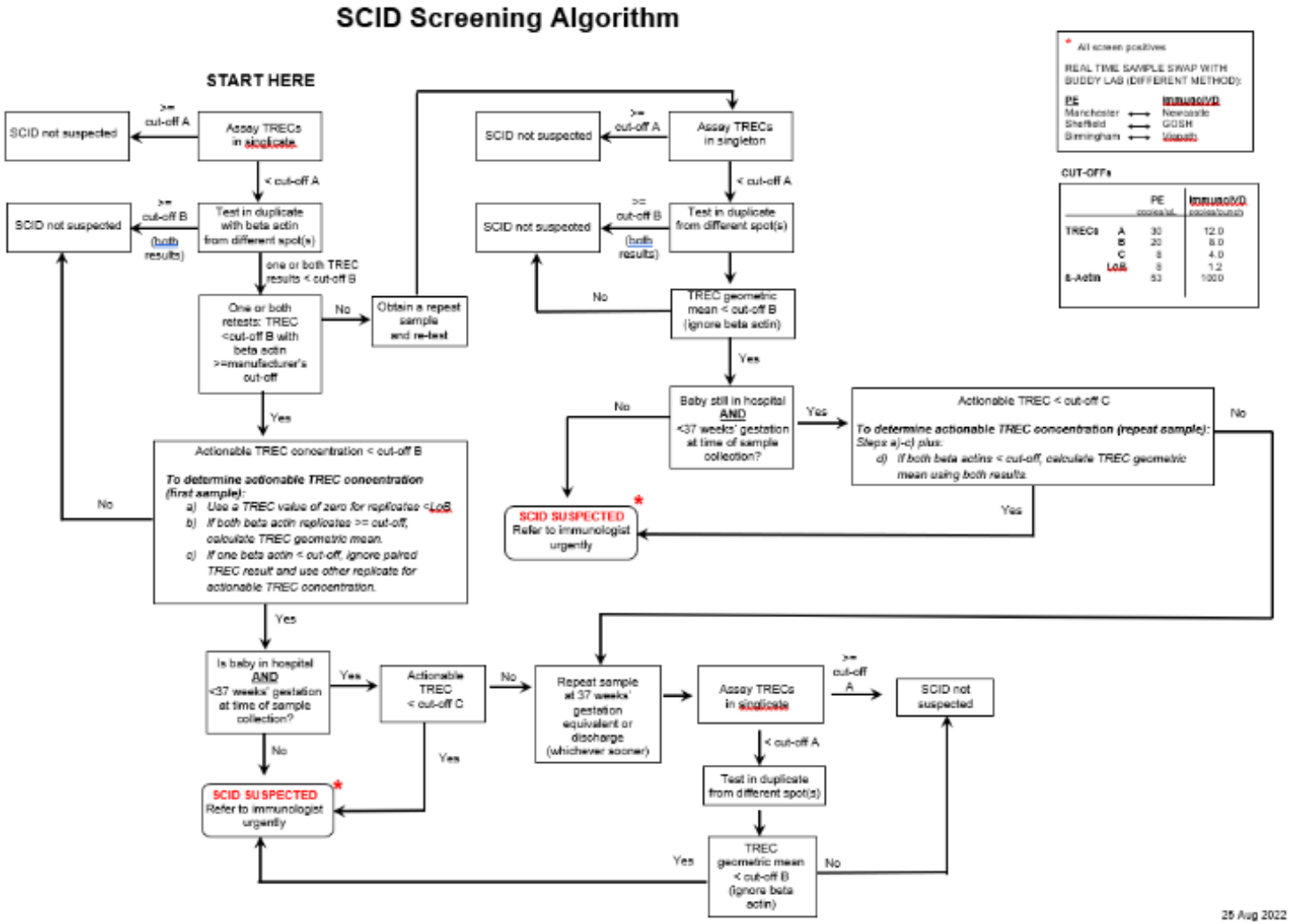
As a result, and following agreement from the SCID Evaluation Board, the CoV for the Sheffield Laboratory was modified to <15 TRECs for term babies with effect from 4th October 2021 and it was agreed that the impact of this change on clinical referrals would be assessed early in December 2021.

This subsequent monitoring data for the Sheffield Laboratory the period 4th October 2021 to 9th December 2021 when scrutinised, revealed 8 clinical referrals from 11,400 babies tested resulting in a referral rate of 1:1,422.

This was considered to be consistent with the original expectations and it was agreed that the CoV of <15 TRECs for Sheffield would be maintained for term babies by the Sheffield Laboratory.

20.12.21

Laboratory Appendix 6: retest scenarios



SCID scenarios

Retest Results		Action
TREC <cut-off B with valid β-actin	TREC <cut-off B with valid β-actin	Refer or prem pathway*
TREC <cut-off B with valid β-actin	TREC <cut-off B with low β-actin	Refer or prem pathway*
TREC <cut-off B with valid β-actin	TREC ≥cut-off B with valid β-actin	If TREC geometric mean <cut-off B: refer or prem pathway*; if ≥cut-off B: not suspected
TREC <cut-off B with valid β-actin	TREC ≥cut-off B with low β-actin	Refer or prem pathway* (use TREC replicate with valid β-actin)
TREC <cut-off B with low β-actin	TREC <cut-off B with low β-actin	Repeat (0322)
TREC <cut-off B with low β-actin	TREC ≥cut-off B with valid β-actin	Repeat (0322)
TREC <cut-off B with low β-actin	TREC ≥cut-off B with low β-actin	Repeat (0322)
TREC ≥cut-off B with valid β-actin	TREC ≥cut-off B with valid β-actin	Not suspected
TREC ≥cut-off B with valid β-actin	TREC ≥cut-off B with low β-actin	Not suspected
TREC ≥cut-off B with low β-actin	TREC ≥cut-off B with low β-actin	Not suspected

* Go to Prem Pathway table if in hospital AND <37 weeks gestation at time of sample collection

Repeat sample following 0322 result		Action
TREC <cut-off B with valid β-actin	TREC <cut-off B with valid β-actin	Refer or if prem, request 0301 repeat unless TREC geometric mean <cut-off C (refer).
TREC <cut-off B with valid β-actin	TREC <cut-off B with low β-actin	Refer or if prem, request 0301 repeat unless TREC with valid β-actin <cut-off C (refer).
TREC <cut-off B with valid β-actin	TREC ≥cut-off B with valid β-actin	If TREC geometric mean <cut-off B: refer or if prem, request 0301 repeat unless TREC geometric mean <cut-off C (refer); if ≥cut-off B: not suspected
TREC <cut-off B with valid β-actin	TREC ≥cut-off B with low β-actin	If TREC with valid β-actin <cut-off B: refer or if prem, request 0301 repeat unless TREC geometric mean <cut-off C (refer); if ≥cut-off B: not suspected
TREC <cut-off B with low β-actin	TREC <cut-off B with low β-actin	Refer or if prem, request 0301 repeat unless TREC geometric mean <cut-off C (refer).
TREC <cut-off B with low β-actin	TREC ≥cut-off B with valid β-actin	If TREC geometric mean <cut-off B: refer or if prem, request 0301 repeat; if ≥cut-off B: not suspected
TREC <cut-off B with low β-actin	TREC ≥cut-off B with low β-actin	If TREC geometric mean <cut-off B: refer or if prem, request 0301 repeat unless TREC geometric mean <cut-off C (refer); if ≥cut-off B: not suspected
TREC ≥cut-off B with valid β-actin	TREC ≥cut-off B with valid β-actin	Not suspected
TREC ≥cut-off B with valid β-actin	TREC ≥cut-off B with low β-actin	Not suspected
TREC ≥cut-off B with low β-actin	TREC ≥cut-off B with low β-actin	Not suspected

Prem Pathway (In hospital AND <37 weeks gestation at time of sample collectio		Action
TREC <cut off C & valid β-actin	TREC <cut off C & valid β-actin	Refer
TREC <cut off C & valid β-actin	TREC <cut off C & low β-actin	Refer (use TREC replicate with valid β-actin)
TREC <cut off C & valid β-actin	TREC <cut-off B but >=cut-off C & low β-actin	Refer (use TREC replicate with valid β-actin)
TREC <cut off C & valid β-actin	TREC ≥cut-off B with low β-actin	Refer (use TREC replicate with valid β-actin)
TREC <cut off C & valid β-actin	TREC ≥cut-off B with valid β-actin	If TREC geometric mean <cut-off C: refer; if ≥cut-off C: prem repeat (0301)
TREC <cut off C & valid β-actin	TREC <cut-off B but >=cut-off C & valid β-actin	If TREC geometric mean <cut-off C: refer; if ≥cut-off C: prem repeat (0301)
TREC <cut-off B but >=cut-off C & v	TREC ≥cut-off B with valid β-actin	Repeat - 0301
TREC <cut-off B but >=cut-off C & v	TREC ≥cut-off B with low β-actin	Repeat - 0301 (use TREC replicate with valid β-actin)
TREC <cut-off B but >=cut-off C & v	TREC <cut-off B but >=cut-off C & valid β-actin	Repeat - 0301
TREC <cut-off B but >=cut-off C & v	TREC <cut-off B but >=cut-off C & low β-actin	Repeat - 0301 (use TREC replicate with valid β-actin)
TREC <cut-off B but >=cut-off C & v	TREC <cut off C & low β-actin	Repeat - 0301 (use TREC replicate with valid β-actin)

Prem repeat sample (collected 37 weeks gestation equivalent or discharge)		Action
TREC <cut-off B with valid β-actin	TREC <cut-off B with valid β-actin	Refer
TREC <cut-off B with valid β-actin	TREC <cut-off B with low β-actin	Refer
TREC <cut-off B with valid β-actin	TREC ≥cut-off B with valid β-actin	If TREC geometric mean <cut-off B: refer; if ≥cut-off B: not suspected
TREC <cut-off B with valid β-actin	TREC ≥cut-off B with low β-actin	If TREC geometric mean <cut-off B: refer; if ≥cut-off B: not suspected
TREC <cut-off B with low β-actin	TREC <cut-off B with low β-actin	Refer
TREC <cut-off B with low β-actin	TREC ≥cut-off B with valid β-actin	If TREC geometric mean <cut-off B: refer; if ≥cut-off B: not suspected
TREC <cut-off B with low β-actin	TREC ≥cut-off B with low β-actin	If TREC geometric mean <cut-off B: refer; if ≥cut-off B: not suspected
TREC ≥cut-off B with valid β-actin	TREC ≥cut-off B with valid β-actin	Not suspected
TREC ≥cut-off B with valid β-actin	TREC ≥cut-off B with low β-actin	Not suspected
TREC ≥cut-off B with low β-actin	TREC ≥cut-off B with low β-actin	Not suspected

Laboratory Appendix 7: Modification in the way that duplicates are used following and initial TREC result below the analytic cut-off value when screening for SCID

Nine months after beginning to screen for SCID in September 2021, taken together, the six centres have screened approximately 300,000 babies and of these approximately 200 have been referred for further investigation. A referral rate of approximately 1:1,500.

This, while in line with initial predictions, has proved stressful for families and demanding for the clinical services.

Without changing methodology or the cut-off values in use the options to address this are limited although the way in which duplicate measurements following an initial result < the analytic CoV provides a way of reducing this.

Currently the assay is performed in duplicate on the original card and the lower of the two duplicates is used to determine any further action. Taking the mean of these duplicates, as it is a subset of the lowest estimate, will reduce the number of babies in whom further action is required.

In practice because the data follows a Gaussian distribution when log transformed, the geometric mean was explored. The also serves to reduce the impact of a single elevated result. The potential to miss a case of SCID can be further reduced by defining any value <'Limit of Blank' for each method (assumed as 8 for PE and 1.2 for IVD) as zero. This ensures that if either of the duplicates is below that value, the result will automatically be acted upon.

The following paper describes the arguments and potential impact on the referral rate for each method. On that basis this change in the algorithm was approved by the SCID Board on Friday 29th July and as a result:

The Labs are asked to adopt this modified approach for handling results generated on and after Monday 5th September 2022

The geometric mean of the duplicates can be estimated by calculating the product of the two duplicate assays, when the ß-actin result is satisfactory for each, and taking the square root of this product. If only one of the results has a satisfactory ß-actin then this should be used in isolation and the other duplicate ignored. As in current practice, the initial TREC result is not taken into account when making these calculations.

So for instance, using the PE method, if the initial TREC = 25 in a term baby, and the duplicates with satisfactory ß-actin, of 16 and 28. Under the current arrangements this baby would be referred while in the new arrangements, 16 x 28 = 448; √448 = 21.2, and this would be reported as 'SCID not suspected'.

An example where the LoB would be applied could be: initial TREC = 12, duplicates with satisfactory ß-actin of 7 and 60. As 7 is <LoB and assumed to be = zero, the product of 0 x 60 = 0 and the baby would be referred. This together with the use of

the geometric rather than arithmetic mean helps safeguard against 'fliers' preventing referral.

As in current practice, if only one of the duplicates has an acceptable ß-actin result, then a repeat sample should be requested to guide further action.

The same rules, using the appropriate CoV, would apply for the referral of premature babies and entry into the premature repeat pathway.

Please implement these changes with effect from 5th September 2022 and confirm by email that you have received this notification and again once the new rules are in force

Treatment of the duplicates that form part of the SCID newborn screening algorithm

During the period 6th September 2021 – 17th June 2022 approximately 305,000 babies were tested for SCID in the six regional centres participating in the newborn screening SCID evaluation.

The results show that from those tested, 205 babies were referred for assessment and confirmatory testing, an overall referral rate of 1:1,488.

The results also show a disparity between the two different testing methods used with an overall referral rate of 1: 2,260 in the group using the IIVD technology vs 1:1,060 in the Perkin Elmer group. There is also significant overrepresentation in clinical referral among premature babies with a combined referral rate of approximately 1:230 in those of <37 weeks gestation.

This referral rate is greater than that experienced in most national programmes and this was anticipated as part of the evaluation as the cut-off value to prompt referral and the management of the premature pathway are conservative to reduce the risk of missed cases while the programme is being optimised. It is anticipated when the data is reviewed at the end of the evaluation that the referral rate can be significantly reduced by careful consideration of:

- The method used to test babies
- The treatment of the premature pathway
- The cut-off value used to indicate the need for referral

In the meantime, there is one area in which we can implement a minor, but effective change, in the current pathway, without significant risk in detecting those affected. This relates to the way that duplicate testing is handled when the initial TREC results fall-below the 'analytical cut-off' used to prompt repeat analysis. The current practice dictates that when these repeat analyses, on the same sample card, are performed, the lowest value of the two is used to determine whether to refer the baby.

This conservative approach contrasts with the more usual and somewhat more intuitive approach of taking a mean of these two repeat results to guide subsequent action.

It is possible by retrospective analysis of those on which duplicates have been performed to calculate the impact of using the geometric mean (square root of the

product of the measurements) chosen as the results do not follow a normal/Gaussian distribution) versus choosing the lowest result of the two measurements.

When using this approach of taking the geometric mean of the duplicates, rather than using only the lowest of the two duplicates, a careful analysis of term babies referred in the two method groups indicate a consistent effect to reduce those classified as positive and as a result, in term babies at least, to reduce the number of consequent clinical referrals. While the extent of this effect will have to be determined in practice and the confidence intervals of these estimates, shown below are broad, the anticipated overall reduction is likely to be significant, see **Fig 1.**

A graphical display of the effect of this approach when applied to retrospective data is shown for term babies – see figure 2 below.

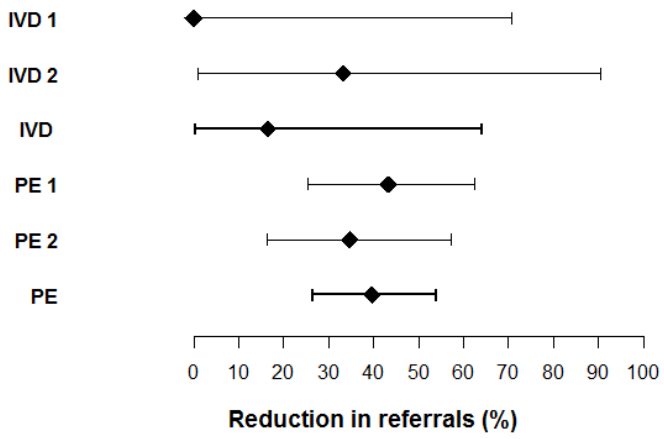


Fig 1: The figure shows the 95% confidence interval of the anticipated reduction in clinical referrals of term babies in the two method groups. IVD 1 & 2 represent the effect in the IVD group for the two time periods analysed: Sept – Dec 2021 and Jan – Mar 2022 respectively. The combined figures show a mean reduction in referrals of 17% for the IIVD group and 40% in the Perkin Elmer group.

In these data, when calculating the geometric mean we have attempted to mitigate the effects of contamination leading to a single and aberrant result falsely weighting the mean by classifying a value less than or equal to the limit of the blank (LoB)[14] as zero. This in effect guarantees that all such low values receive automatic referral independent of their paired duplicate – this acts as a safety net to help avoid missed cases arising from contamination.

The impact on the eventual referral of premature babies is difficult to estimate because this pathway is more complex with a two-step process in place for some babies reliant upon two distinct cut off values. Nevertheless, as those babies in whom the geometric mean of the results falls below the cut-off is a subset set of those in whom the lowest of the duplicates falls below the cut-off, we expect that there will also be a reduction in this group. Given the undoubted impact of clinical referral on the families

[14] LoB as the highest apparent analyte concentration expected to be found when replicates of a sample containing no analyte are tested

and the additional workload for the clinical services, this would seem a reasonable amendment to the current protocol to make at the half way point in the evaluation.

Recommendation

It is therefore recommended that with effect from Monday 5th September, when the initial TREC result falls below the method related 'analytic' cut-off, the sample will be tested in duplicate as in current practice. However, in place of the current practice of taking the lowest of these two values to determine further action, the geometric mean of the two results, provided that these are valid, will be used to guide subsequent action.

This geometric mean of the duplicate analyses will be calculated by estimating the square root of the product of the two results. When either of the individual results, provided the beta-actin is acceptable, falls below the method related limit of blank, that result and consequently the geometric mean will be reported as zero and appropriate action will be taken on that basis.

As in current practice, if only one of the duplicates has an acceptable ß-actin result, then a repeat sample should be requested to guide further action**.**

The two graphs below show the results for **term babies only**.

Perkin Elmer method **IIVD method**

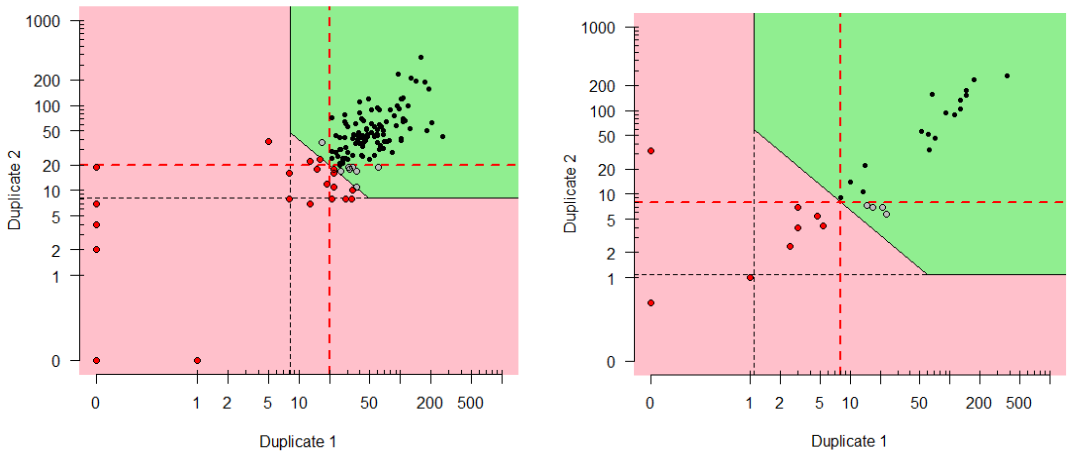
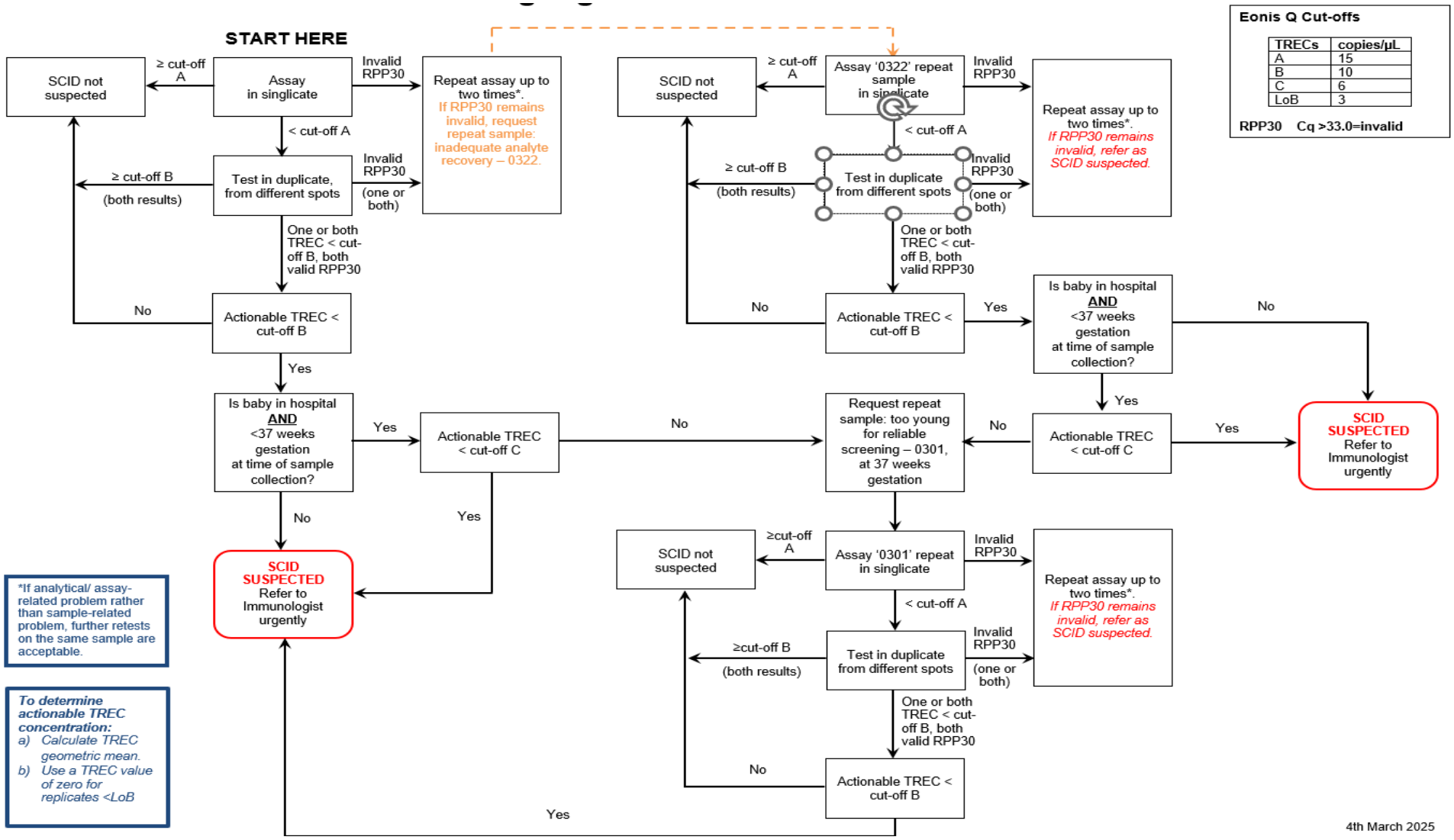


Figure 2: In the illustrations above the three quadrants to the left of and below the dashed red lines contain cases that would all be classified as screen positive using the current approach. The top right hand area bounded by a continuous black line demarcates those that would not be referred when using the geometric mean. The dashed black line marks the Limit of Blank for each method. Importantly the open circles represents those cases who would avoid clinical referral when using the geometric mean but would be referred when using the lowest of the duplicates to guide referral.

6th August 2022

Laboratory Appendix 8: SCID screening algorithm – Eonis Q



Laboratory Appendix 9: Summary Tables of Responses to Laboratory Questionnaire

Table 1: Analytical Performance - Summary Data

	ImmunoIVD	EnLite	EONISQ
Sensitivity **LOB (range)** **LOD** **LOQ**	 0.7 – 2 copies/punch 1.4 – 3.7 copies/punch 2.2 – 7.5 copies/punch	 6-9 copies/mL 16 – 21 copies/mL 46-47 copies/mL	 3 copies/mL 7 copies/mL
Intermediate Imprecision Ln CV%	TREC positive 15-33% TREC absent 60 -100% ? is this ln CV%	C1 (Mean 154 - 399) 35-70% C2 (Mean 424 – 821) 30-81%	C2 (Mean 78 -96) 5-25% C3 (Mean 395 – 528) 3-19%
Lot to Lot Variation	Significant lot to lot variation which is an issue when shelf life is short and new lots are received every few months. The company have worked on increasing stability and discussions are on-going regarding kit lot acceptance criteria.	Not formally assessed but IQC showed no obvious shift between lot numbers.	Variability between lab in addition to negative bias with some lot numbers has resulted in high referral rates with some kit lots. It may be possible to mitigate this by lowering the cut-off.
Failed Plates	0.4 – 5%	6% Manchester, 17% Birmingham, 19% Sheffield	6.5% (Manchester) 9.5% (SW Thames) ?Sheffield
Contamination Issues	Not really. Slightly more plate failures on one site since introduction of a second blank at the end of the plate – review underway to determine reason and evaluate if acceptance criteria are appropriate.	All labs experience significant problems with contamination which required reconfiguration of the laboratory space.	Occasional issues on one site only, highlighted by blank/low level QCs failing due to high results.
IQC Performance	No issues with IQC performance but kit ranges are very wide. Third party/in-house QCs required.	Generally acceptable. Higher failure rates in Sheffield due to elevated QC2/NTC or low QC3.	Kit QC analysed on every plate no significant problems noted although ranges are wide. Third party IQC required.

EQA Performance	Satisfactory	Satisfactory	Satisfactory
Analysis time	Approx 4.5 hours	Approx 3.5 – 7 hours depending on workload.	2-3 hours
Turnaround time (receipt to reporting)	1-3 days reported by labs - ?dependent on how analysis is incorporated into general NBS workflow.	1-3 days reported by labs - ?dependent on how analysis is incorporated into general NBS workflow.	1-3 days reported by labs - ?dependent on how analysis is incorporated into general NBS workflow.
Ability to multiplex	Yes but the combined SMA/SCID kit is a different kit albeit based on the same technology.	No	Yes – kits test for SCID & SMA
	ImmunoIVD	**EnLite**	**EONISQ**
General Comments	At the outset the method appeared to be quite complex with numerous steps but has proved reliable. Analysis can be undertaken by Band 4/5 staff. Method is susceptible to Lithium Heparin interference which caused a high number of repeats requiring liaison with and education of staff within the neonatal units.	None of the 3 labs would recommend this method – problems cited include variability in performance, too many plate failures, susceptibility to contamination despite workflow being configured to meet initial PE recommendations.	Method and workflow are good. Assay was marketed as fully developed – in fact labs supported Revvity in required further development work in order to make the method fit for purpose. Refinement of cut-offs is on-going but the assay has potential.

Table 2: Operational and Supplier Aspects

General Operational Issues and Experience with Suppliers

	ImmunoIVD	EnLite	EONISQ
Instrument / Method Commissioning	Instrumentation needs to be purchased separately from reagent contract. Service contracts set up initially did not include breakdown cover and TAT for breakdown calls and response from the instrument company was poor. Commissioning and training were significantly impacted by the COVID pandemic.	Instrumentation provided as part of reagent contract. Problems in commissioning on one site – detrimentally affected by the COVID pandemic.	Instrumentation provided as part of reagent contract. No problems – instrument implementation & training good
Workflow/ configuration issues	Designated areas required which allow unidirectional workflow. No issues encountered once laboratories set up according to requirements.	3 designated areas required to avoid contamination issues. All labs required changes to configuration of laboratory area post go-live.	No issues
Episodes of downtime	None since go-live	2 of the 3 laboratories had major episodes of downtimes (8 weeks & 10 weeks) due to contamination issues.	None
Instrumentation Problems	A few problems with both the PCR machines and the thermal cyclers. Generally all dealt with promptly with little or no impact on service delivery. Also problem with swelling of laptop batteries	1 lab had problems with thermal cyclers – intervention required from NHSE clinical leads to negotiate replacement. 1 lab had problems with PCR incubator block lids – replacements issued within 1 week.	1 site had an issue with one of the analysers (identified by the supplier) leading to unstable curves. Problems with the analyser PC slowing down over a period of several weeks. Resolved by rebooting periodically and/or reimaging .
Kit delivery	Initially this was a significant problem. Kits held at customs due to VAT non-payments, instances of labs receiving threatening e-mails from DHL.	No significant problems	Problems with delayed kits with kits needing to be provided by one of the other labs. Mostly this was arranged promptly but on one occasion

	Some kits which should have remained frozen were defrosted on arrival. Supplier worked pro-actively with programme and lab leads to manage the issues which have now been resolved.		intervention was required by the programme leads to avoid service downtime. On one occasion controls packed separately (on dry ice) held back at customs, kits unusable until they arrived, company arranged promptly for back-up kits from another lab.
	ImmunoIVD	**EnLite**	**EONISQ**
Kit storage, shelf life and frequency of kit lot change	qPCR plates require storage at -20^{0}C, initially elution solution needed to be refrigerated but since Jan 2024 all components other then plates can be stored at room temp. Shelf life initially 3 months but increased to 6 months from Jan 2024. Kit lot change initially every 3 months (with each delivery) which was a problem especially given the significant lot to lot variation. Problem mitigated by improvement on kit stability. Kit storage can be an issue especially for GOSH who have a very high workload.	Kit storage -20^{0}C, plates room temp. No issues with storage, shelf life or frequency of kit lot change	Elution and PCR plates stored at 4^{0}C. IQC material stored at -20^{0}C. Shelf life 8-11months. Kit lot change currently every 2-3 months, Revvity gathering data on stability and hoping to extend. Fridge storage space an issue for one lab.
Kit problems + supplier response	Some kits received without calibrator wells – resolved promptly by IIVD. An issue with the frame not fitting properly on the filter plate – also resolved promptly. Significant lot to lot variation – no evidence of clinical impact. 1 lab had 2 incidents of freezer failures requiring kits to be discarded. Requirement to introduce split	Generally issues resolved promptly. 1 lab required replacement of an entire lot number – response/investigation was lengthy but replacements sent out in a reasonable timeframe.	Pre go-live the initial kit formulation generated a large number of false positives which would have been overwhelming for the clinical service. This caused a delay in the original go-live date. Once changes were made, new method required validation. Following go-live one lab experienced high plate failure rate due to sub-

	reagent deliveries (since no OOH temp monitoring available) to avoid recurrence. All 3 labs highlighted that immunoIVD are excellent at responding to e-mail enquiries or problems very promptly.		optimal extraction/amplification. Revvity were pro-active in troubleshooting.
	ImmunoIVD	**EnLite**	**EONISQ**
Software problems and algorithm changes	No problems. All 3 labs use Omnilab IT system which is a user definable software package. It was noted however that the SCID algorithm is complex and the changes to V2 (introduction of geometric mean and factoring in LOB) made user acceptance testing somewhat complicated & labour intensive.	No software problems. 2 labs using PE/Revvity IT system noted that IT changes required take a long time to be implemented.	Software version changes required V&V work to be repeated and data re-assessed. Some restrictions to software configuration for 1 lab who use general departmental LIMS system. Revvity IT system can be configured as required but implementation of IT changes in Revvity system requires a long lead time.
General Experience with supplier	Supplier scored highly in all categories	Same supplier for EnLite and EONISQ. Generally satisfactory but occasional issues with timeliness of response.	