UK National Screening Committee

Newborn Screening for Biotinidase Deficiency

An evidence map on screening for biotinidase deficiency for the UK National Screening Committee

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The UK National Screening Committee secretariat is hosted by the Department of Health and Social Care

About the UK National Screening Committee (UK NSC)

The UK NSC advises ministers and the NHS in the 4 UK countries about all aspects of population screening and supports implementation of screening programmes.

Conditions are reviewed against evidence review criteria according to the UK NSC's evidence review process.

Read a complete list of UK NSC recommendations.

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Summary

This document discusses the findings of the evidence map on screening for biotinidase deficiency.

Evidence maps are a way of scanning published literature to look at the volume and type of evidence in relation to a specific topic. They inform whether the evidence is sufficient to commission a more sustained analysis on the topic under consideration.

Based on the findings of this evidence map, no further work on screening for biotinidase deficiency should be commissioned at the present time.

The UK National Screening Committee (UK NSC) will return to screening for biotinidase deficiency in 3-years' time.

Introduction and approach

Background & Objectives

The UK National Screening Committee (UK NSC) external reviews (also known as evidence summaries or evidence reviews) are developed in keeping with the UK NSC evidence review process to ensure that each topic is addressed in the most appropriate and proportionate manner. Further information on the evidence review process can be accessed online.

Screening for biotinidase deficiency is a topic currently due for an update external review.

Biotinidase deficiency is an autosomal recessive metabolic disorder which affects the BTD gene; this gene is responsible for producing an enzyme called biotinidase.[1] The disorder occurs due to an absence of biotinidase activity, which results in the body's inability to breakdown and recycle biotin, a B vitamin that is often found in food groups such as carbohydrates, fats and proteins. Newborns may inherit this disorder if a mutation is seen in both the paternal and maternal gene.[1, 2] There are 2 types of biotinidase deficiency: profound (affected individuals have less than 10% mean normal serum enzyme activity) and partial (affected individuals maintain approximately 10–30% mean normal serum enzyme activity).[3] The incidence of profound and partial biotinidase deficiency worldwide is estimated to be approximately 1 in 60,000.[4] However, there is currently no data on the prevalence or incidence of biotinidase deficiency in the UK. Loss of biotinidase activity, if left untreated, usually leads to a number of neurologic, sensorineural and cutaneous symptoms.[5] As a consequence of these complications, patients require life-long therapy with pharmacological agents to manage the symptoms of biotinidase deficiency.[1]

Symptoms

Newborns with biotinidase deficiency often appear healthy at birth; symptoms typically manifest between 2 and 5 months of age but can often present after several years, depending on the classification of biotinidase deficiency diagnosis.[6] In the absence of normal biotinidase activity, babies tend to develop primary neurologic symptoms such as seizures, hypotonia, vision problems and hearing loss, along with cutaneous abnormalities, including skin rashes, alopecia and recurrent viral or fungal infections.[5, 7] Common cutaneous symptoms such as skin rashes and alopecia can affect more than 70% of clinically ascertained children.[7] Children with untreated partial biotinidase deficiency usually only experience mild symptoms, which can develop particularly during

periods of metabolic stress.[5] However, almost all children with profound biotinidase deficiency are at risk of developing symptoms, if left untreated.[5]

Screening and diagnosis methods

The confirmed diagnosis of biotinidase deficiency depends on demonstrating deficient activity of the enzyme through serum or plasma samples.[8] Additional genotyping is beneficial to confirm the deficient enzymatic activity, and to differentiate between individuals with profound and partial biotinidase deficiency.[5, 6] Methods for biotinidase deficiency screening were first developed in 1984; these have entailed the determination of biotinidase activity through dried blood spot samples using qualitative testing, whereby the dried blood spot samples are used for colorimetric enzymatic assays.[6] The colorimetric assay has become the most widespread method for dried blood spot screening in comparison with methods such as fluorescence-based enzymatic assays and multiplex plate testing due to its relative simplicity and limited expense. A limitation to this method is that a large proportion of cases suffer from partial enzyme deficiency, which may be more challenging to detect if the method has low sensitivity. An alternative method, semi-quantitative fluorescence-based enzymatic assays, measures biotinidase activity using an artificial substrate of biotinyl-6-aminoquinoline;[6] this is a more expensive substrate than those used in colorimetric methods, but fluorescence-based methods can be advantageous as they have demonstrated higher precision during newborn screening for biotinidase.[9, 10] Additional methods for determining biotinidase activity include measuring the release of biotin from biocytin and other radioisotopic biotinylated analogues, but these are considerably more expensive and labour intensive and are therefore undesirable in screening settings.[6] Therefore, the currently preferred screening methods for biotinidase deficiency are the colorimetric and fluorescence-based enzymatic assays.

Treatment

For individuals diagnosed with biotinidase deficiency, the current treatment options consist of oral supplementation with unbound (free) biotin. Biotin supplementation is a life-long therapy. Children diagnosed before symptom manifestation generally remain asymptomatic and appear to have a normal development, if adequate adherence to biotin supplementation is maintained.[5, 11] Previous UK NSC reviews found that children with symptomatic biotinidase deficiency have improved following treatment with 5 to 10 mg oral biotin supplementation per day, with no known side effects;[3, 12, 13] however, certain neurologic symptoms such as hearing loss, visual abnormalities and developmental delays are irreversible and do not subside after the initiation of treatment.[5, 7] Early diagnosis and treatment initiation of biotinidase deficiency may therefore be important to prevent symptom manifestation in pre-symptomatic children with profound biotinidase deficiency.

Global screening for biotinidase deficiency

Worldwide, countries have been establishing their own screening regimes for biotinidase deficiency in newborns; the USA have recommended screening for biotinidase deficiency as part of the recommended uniform screening panel (RUSP) core conditions since 1984.[14, 15] Likewise, the northeast of Italy has been conducting biotinidase deficiency screening in newborns since 1986;[15] Italy has incorporated a semi-quantitative method of analysis using a solid phase time-resolved immunofluorescence assay;[15] Maguolo 2021 successfully demonstrated that qualitative colorimetric methods followed by semi-quantitative methods can accurately identify biotinidase deficiency in cases of borderline enzymatic activity.[15] The incorporation of biotinidase deficiency in neonatal screening programmes has been implemented in a number of European countries, including Austria, Belgium, Czech Republic, Denmark, Germany, Hungary, Italy, Latvia, Netherlands, Norway, Poland, Sweden and Switzerland.[16, 17] As of 2021, Spain, Ukraine and Uzbekistan, are conducting pilot studies/regional screening for biotinidase deficiency in newborns.[17] Table 1 summarises existing European screening methods used for biotinidase deficiency.

Table 1: Existing European screening methods and programme statistics for biotinidase deficiency

Country	Initiation of screening	Screening method	Cut-off level	Prevalence	Reference
Austria	n.d.	Colorimetric	Visual	1:39,511[a]	Loeber 2007[18]; Kasper 2010[19]
Belgium	n.d.	Colorimetric	10%	1:33,324[a]	Loeber 2007[18]
Czech Republic	2016	Fluorometric	30%	1:8,638	David 2019[20]
Denmark	2009	Enzymatic assays	n.d.	n.d.	Lund 2020[21]
Germany	n.d.	Fluorometric, colorimetric	30%	1:45,436[a]	Loeber 2007[18]
Hungary	1989	n.d.	n.d.	1:20,000	Milánkovics 2007[22]
Italy	1986	Colorimetric	n.d.	n.d.	Loeber 2007[18]
Latvia	n.d.	n.d.	n.d.	n.d.	
Netherlands	n.d.	Colorimetric	30%	n.d.	Wiltink 2016[23]
Poland	n.d.	n.d.	n.d.	1:60,000[11]	Ministry of Health Poland[24]
Norway	2012	Fluorometric	<30% (<60 U/dL)	n.d.	Tangerass 2020[25]
Spain	2021	Colorimetric	n.d.	1:20,420[a]	Loeber 2007[18]
Sweden	2002	Enzymatic assays	20%	1:33,817[a]	Loeber 2007[18]
Switzerland	1983	Colorimetric	n.d.	1:47,486[a]	Weber 2004[26]

Footnotes: [a] prevalence data was determined from screening programmes by Loeber 2007 prior to 2004.[18]
Abbreviations: n.d., no data.

The most commonly used screening cut-off level for sensitive quantitative analysis in biotinidase deficiency newborn screening programmes is 30% biotinidase activity; this activity level should pick up both partial and profound cases of biotinidase deficiency. However, the common detection limit of 30% biotinidase activity has been found to produce a large number of false positives due to low specificity.[23] European countries have started to establish methods with higher sensitivity and the USA have individually established cut-off limits and re-screening methods.[6] One study found a method with a cut-off level of 15%, which successfully eliminated the potential for false positive results.[23] Nevertheless, whilst efforts have been made to improve screening accuracy, problems of false results are still not resolved. For example, the 2018 UK NSC review found that despite the differing enzymatic cut-off levels in global screening methods, false positives were found in roughly half of the newborns tested.[2] These false positive results cause unnecessary stress to families involved, and lead to further expense in confirmatory testing; therefore, the need for including high specificity screening methods for biotinidase deficiency in national screening programmes is currently under consideration.

Previous review on screening for biotinidase deficiency

The 2018 UK NSC review on newborn screening for biotinidase deficiency found that prior to 2018, there were no existing studies that evaluated the performance of newborn screening tests; ultimately, no data on sensitivity, specificity or negative predictive value could be identified.[2] Additionally, none of these studies were conducted in a UK population. This paucity of evidence meant that a cut-off level for the diagnosis of biotinidase activity was unclear and could not be determined. Although the prevalence and incidence of biotinidase deficiency have been reported for global populations, UK prevalence could not be determined based on these figures, due to variation in ethnicity and genetic differences.[2] In addition, there was insufficient evidence to inform: a) whether screen detection improves outcomes compared with clinical detection, and b) which screen-detected children with partial or profound deficiency will develop symptoms and need biotin supplementation, or the optimal dose to give. Subsequently, the 2018 review concluded that screening for biotinidase deficiency in newborns should not be recommended in the UK.[2]

Since 2018, 2 systematic literature reviews (SLR) on biotinidase deficiency have been registered on the International Prospective Register of Systematic Reviews (PROSPERO). Van Winkel et al. registered a protocol for an SLR in July 2020, focusing on the comparison between clinical outcomes of patients diagnosed with biotinidase deficiency by screening methods or due to clinical manifestations later in life.[27] Their SLR aims to assess the existing literature surrounding the influence of newborn screening on clinical courses of patients with biotinidase deficiency, time intervals between symptom manifestation, diagnosis and treatment, and finally the influence of

treatment on long-term outcomes and symptoms. Zeng et al. registered a protocol in November 2020 for a global SLR and meta-analysis, which investigates the prevalence of inherited metabolic diseases such as biotinidase deficiency.[28] It is expected that once published, the evidence summarised by these 2 SLRs should highlight and collate the prevalence and incidence of biotinidase deficiency worldwide, alongside the global approaches to screening and measurement methods for the metabolic disease.

The UK NSC currently does not recommend screening for biotinidase deficiency. The Committee based this recommendation on the evidence provided by the 2018 review carried out by Bazian.

Aims of the evidence map

Evidence maps are rapid evidence products which aim to gauge the volume and type of evidence relating to a specific topic.

This evidence map has been developed to assess whether a more sustained review on screening for biotinidase deficiency should be commissioned and to evaluate the volume and type of evidence on key issues related to screening for biotinidase deficiency. The aim of this document is to present the information necessary for the UK NSC to decide this.

The aim was to address the following questions:

> Q1: What is the prevalence and/or incidence of biotinidase deficiency in the UK?
> Q2: What is the accuracy of available screening tests using dried blood spots to detect biotinidase deficiency?

Currently, there is no available data on the incidence or prevalence of biotinidase deficiency in newborns in the UK. There is also insufficient data on the diagnostic accuracy of the screening test to be used in dried blood spot screening. This evidence map will therefore focus on studies reporting outcomes relating to the prevalence and incidence of biotinidase deficiency in newborns, along with the diagnostic accuracy of screening methods for biotinidase deficiency.

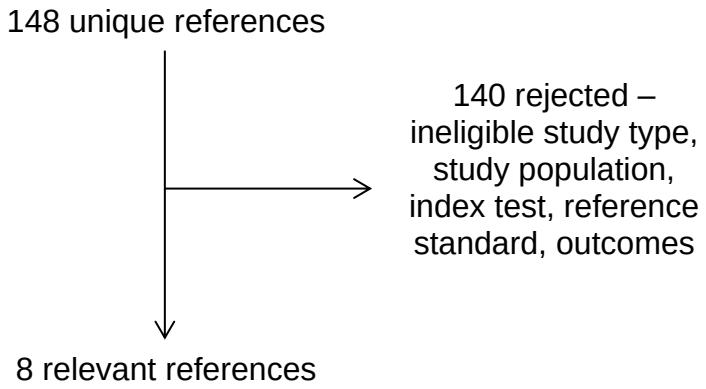
Search methods and results

Searches were conducted on 18 June 2021 in 3 databases: MEDLINE, Embase, Cochrane Database of Systematic Reviews (CDSR) and Cochrane Central Register of Controlled Trials (CENTRAL). The search period was restricted to January 2017 – 18 June 2021. MEDLINE (including MEDLINE In-Process, MEDLINE Daily and Epub Ahead of Print) and Embase were searched simultaneously via the Ovid SP platform. The Cochrane Library databases (CDSR and CENTRAL) were searched via the Wiley Online platform.

The detailed search strategies, as well as the exclusion and inclusion criteria are available in Appendix 1. One reviewer screened all titles and abstracts. All references were reviewed at abstract level, though in some cases full texts were reviewed to clarify uncertain pieces of information. Decisions regarding the eligibility of all included studies and 10% of excluded studies were verified by a second, independent reviewer. A formal quality appraisal of the evidence was not required, given the remit of the evidence map.

The search returned 150 results across MEDLINE, Embase and the Cochrane library databases. After automatic and manual de-duplication, 148 unique references were assessed for relevance to the review question. Eight records were included in the evidence map. Of these, 6 were relevant to questions 1 and 2, and 2 were relevant to question 2 only. A flow diagram summarising the number of studies included and excluded is presented in **Error! Reference source not found.**. The abstract reporting tables are available in Appendix 2.

Figure 1: Summary of included and excluded publications



Summary of findings

Question 1: What is the prevalence and/or incidence of biotinidase deficiency in the UK?

Seven studies were identified as potentially eligible, and for 2 of these, their full texts were reviewed to determine relevance. Of the 2 studies checked, one was excluded. In total, 6 studies were included as being relevant to question 1.

None of the 6 included studies were conducted in the UK, but all were conducted in Organisation for Economic Co-operation and Development (OECD) countries (3 in Italy; one in Czech Republic; one in Denmark and one in Norway). All of these were retrospective cohort studies that analysed newborn dried blood spot samples obtained through newborn screening programmes. One study reported prevalence of biotinidase deficiency both among the study population and in other countries (not specified),[20] and 5 studies reported overall incidence of biotinidase deficiency amongst the study population. Incidence of both partial and profound biotinidase deficiency was reported by one Italian study,[15] and the Norwegian study reported the incidence of partial biotinidase deficiency.[25]

Two of the studies conducted in Italy found similar overall incidence of biotinidase deficiency: 1:6,300[15] and 1:5,966.[29] It was noted in both studies that these figures are much higher than the estimated global incidence of biotinidase deficiency (1:60,000).[14] The third Italian study reported a lower incidence of 1:61,000,[30] however, this was conducted over a longer time period (30 years compared with 12 years[29] and 6 years[15]). The incidence reported in this study overlapped with the reported worldwide incidence.

In the Czech Republic, the screening prevalence of biotinidase deficiency was found to be 1:8,638.[20] Through literature analysis, this study also found the prevalence of biotinidase deficiency in other countries (not further specified) to be 1:30,000–1:60,000. It is unclear whether this figure includes OECD countries.

The Norwegian study reported an overall incidence of 1:35,489 for biotinidase deficiency in newborns screened between 2012 and 2020. Three cases of partial biotinidase deficiency were also identified.[25]

In the Danish study, incidence of biotinidase deficiency was not explicitly reported but could be determined from relevant data. A total of 45 true-positive cases of biotinidase deficiency were identified out of 967,780 newborns screened.[31]

In summary, although there is some evidence on the prevalence and incidence of biotinidase deficiency in high-income countries, where the population, screening methods and technology are expected to be similar to that of the UK, there is currently no evidence on the prevalence and/or incidence of biotinidase deficiency in the UK. Therefore, due to the lack of UK-specific evidence, commissioning an evidence summary is not currently recommended.

Question 2: What is the accuracy of available screening tests using dried blood spots to detect biotinidase deficiency?

Eight studies were identified as potentially relevant and the full texts were consulted for 4 of these to determine relevance. Eventually, all 8 studies were included as relevant to question 2.

Six of the included studies were retrospective cohort analyses with consecutively enrolled populations. One study in Turkey was a retrospective analysis, but the selection of the population into the study was unclear, hence the level of evidence was judged to be of lower priority (Tier 2, see Appendix 1). The remaining study was an update to technical standards and guidelines on laboratory diagnosis of biotinidase deficiency, and therefore deemed to be lower priority evidence (Tier 2).[6] None of the studies were conducted in the UK, but all were conducted in OECD countries (USA; Turkey; Italy; Czech Republic; Denmark and Norway). A variety of index tests were used, including semi-quantitative and quantitative colorimetric assays, fluorometry and spectrophotometric analysis. The reference standard, if reported, was usually confirmatory testing of biotinidase activity and/or genetic analysis of the *BTD* gene.

Positive predictive value (PPV) was reported in 3 studies. Values reported were 0.38;[20] 3.9%[30] and 76%.[21] It should be noted that in the Lund 2020 study, PPV was only reported for one year of the screening programme (2018) and not for the entire screening period.[21]

Sensitivity and specificity were found to be 93.1% and 95.1% respectively, in one study where spectrophotometric methods were used.[32] However, it is unclear how the population in the study was selected, the evidence is at a high risk of bias and not of high quality.

The number of false positive results was reported in 4 studies,[20, 21, 25, 30] along with the false positive rate in 2 of these studies, which were similarly low at 0.0187% and 0.04%.[20, 30] Additionally, one study reported an incidental finding of vitamin B12 deficiency in a patient with biotinidase deficiency.[25]

The included technical standard and guidelines update by Strovel 2017, confirmed that profound deficiency is indicated by less than 10% of biotinidase activity, whereas partial deficiency, by 10 to 30%.[6] It is noteworthy that in the other included screening studies, where reported, the screening programmes also used a cut-off limit of less than 30% of mean normal biotinidase activity.[20, 25, 29, 30]

All included studies presented positive conclusions regarding newborn screening of biotinidase deficiency using dried blood spots. Several studies reported that early

detection of biotinidase deficiency by newborn screening resulted in positive clinical outcomes for patients after follow-up.[15, 25, 30] Many authors also expressed positive opinions about the accuracy and cost-effectiveness of these screening tests in their conclusions.

In summary, the accuracy of available screening tests using dried blood spots to detect biotinidase deficiency has been explored in high-income settings but no UK-specific evidence was found. The limited number of studies currently available, the heterogeneity in the index tests examined, and the lack of consistency in the outcomes reported limits comparability of the evidence. At present there is therefore insufficient evidence to justify commissioning a more extensive evidence summary.

Conclusions

The findings of this evidence map are unlikely to impact current recommendations on screening for biotinidase deficiency as limited new evidence was identified that would change those conclusions.

Recommendations

On the basis of this evidence map, the volume and type of evidence related to screening for biotinidase deficiency is currently insufficient to justify an update review at this stage and so should be re-considered in 3-years' time.

Appendix 1 – Search strategy for the evidence map

Sources searched: Ovid MEDLINE® and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations and Daily 1946 to 17 June 2021, Embase® 1974 to 17 June 2021, and the Cochrane Library (Cochrane Database of Systematic Reviews and Protocols, Issue 6 of 12, June 2021; Cochrane Trials, Issue 6 of 12, June 2021).

Dates of searches: 1 January 2017 to 18 June 2021 for all databases. Searches were run on 18 June 2021.

MEDLINE and Embase (searched simultaneously via the Ovid SP platform)
1. Biotinidase deficiency/ 2. ((BTD or multiple carboxylase or biotinidase) and deficien$).ti,ab,kw,kf. 3. 1 or 2 4. ("Conference Abstract" or "Conference Review" or comment or editorial or note or case reports or news or news release).pt. 5. (case stud$ or case report$).ti,ab. 6. historical article/ or case study/ 7. exp animals/ not exp humans/ 8. or/4-7 9. 3 not 8 10. limit 9 to yr=2017-current 11. remove duplicates from 10
Cochrane Library (searched via the Wiley Online platform)
1. [mh ^"Biotinidase deficiency"] 2. ((BTD or "multiple carboxylase" or biotinidase) and deficien*):ti,ab,kw 3. {or #1-#2} 4. #3 with Cochrane Library publication date Between Jan 2017 and Jun 2021, in Cochrane Reviews, Cochrane Protocols 5. #3 with Publication Year from 2017 to 2021, in Trials

Results by database:

MEDLINE and Embase	148
Cochrane Library	2
Total	150

Inclusions and exclusions:

Studies were included based on the eligibility criteria listed in Table 2 and Table 3 for question 1 and question 2, respectively.

Table 2: Eligibility criteria for question 1

PICOS domain	Inclusion criteria	Exclusion criteria
Patient population	• Newborns, defined as <12 months of age • General population	N/A
Intervention	N/A	N/A
Comparator	N/A	N/A
Outcomes	Prevalence and/or incidence of biotinidase deficiency	Any other outcome
Study design	• Cross-sectional studies • Cohort studies • SLRs/(N)MAs • Peer-reviewed registry data from neonatal screening programmes	Any other study design, including: • Interventional studies • Case reports • Narrative reviews • Editorials • Commentaries • Conference abstracts • Other publication types that have not been peer-reviewed
Setting	**Tier 1:** Studies conducted in the UK **Tier 2:** Studies conducted in high-income countries where the population, screening methods and technology are expected to be similar to that of the UK (OECD and EEA countries excluding South Korea and Mexico)	Studies in ineligible countries, or international studies where outcomes for eligible countries are not presented separately to outcomes from ineligible countries
Other considerations	• Articles published in the English language • Articles published since January 2017	• Studies with abstract not in the English language • Articles published before January 2017

Abbreviations: EEA, European Economic Area; N/A, not applicable; OECD, Organisation for Economic Co-ordination and Development; (N)MA, (network) meta-analysis; SLR, systematic literature review.

Table 3: Eligibility criteria for question 2

PICOS domain	Inclusion criteria	Exclusion criteria
Patient population	Newborns, defined as <12 months of age	Children who are not newborns Adults
Intervention	**Index test:** Any standalone test or any multiplex test used to screen for biotinidase deficiency using dried blood spots **Reference standard:** Repeat testing to measure enzymatic activity and/or genetic analysis of the BTD gene or any other specific "gold standard" as determined by the study authors	**Index test:** Any other index test that is not performed on newborn dried blood spots **Reference standard:** N/A
Comparator	Any or none	N/A
Outcomes	Outcomes relating to diagnostic accuracy, including but not limited to: • Sensitivity • Specificity • PPV • NPV • LR • AUC • Incidental findings, for example other conditions detected by the test	Outcomes not relevant to diagnostic accuracy

PICOS domain	Inclusion criteria	Exclusion criteria
Study design	**<u>Tier 1</u>:** • RCTs • Non-randomised studies with consecutively enrolled populations (e.g. prospective and retrospective cohort studies) • SLR/(N)MAs of these study designs **<u>Tier 2</u>:** • Case-control studies • Cross-sectional studies • Case series • SLR/(N)MAs of these study designs • Any relevant technical standards/guidelines regarding the screening detection and diagnosis of biotinidase deficiency	Any other study design, including: • Case reports • Narrative reviews • Editorials • Commentaries • Conference abstracts • Other publication types that have not been peer-reviewed
Setting	Studies conducted in the UK or other high-income countries where the population, screening methods and technology are expected to be similar to that of the UK (OECD and EEA countries excluding South Korea and Mexico)	Studies in ineligible countries, or international studies where outcomes for eligible countries are not presented separately to outcomes from ineligible countries
Other considerations	• Articles published in the English language • Articles published since January 2017	• Studies with abstract not in the English language • Articles published before January 2017

Abbreviations: AUC, area under the curve; EEA, European Economic Area; LR, likelihood ratio; N/A, not applicable; (N)MA, (network) meta-analysis; NPV, negative predictive value; OECD, Organisation for Economic Co-ordination and Development; PPV, positive predictive value; RCT, randomised controlled trial; SLR, systematic literature review.

Appendix 2 – Abstract reporting tables

Abstracts relevant to Question 1 and 2:

Q1: What is the prevalence and/or incidence of biotinidase deficiency in the UK?
Q2: What is the accuracy of available screening tests using dried blood spots to detect biotinidase deficiency?

TITLE	
Citation	*David* et al*. (2019), Epidemiology of Rare Diseases Detected by Newborn Screening in the Czech Republic, Central European Journal of Public Health, 27(2):154–159.*[20]
BACKGROUND	
Study type	*Retrospective cohort analysis*
Objectives	*To explore the prevalence of 18 rare diseases in newborns in the Czech Republic using analytical techniques on dried blood spot samples, including fluorescence immuno-assay, tandem mass spectrometry and fluorimetry.*
Components of the study	***Population:*** *Dried blood spot samples in newborns (Czech Republic [n=888,891])* ***Index test:*** *Fluorimetry* ***Reference standard:*** *Positive screening results were referred for follow-up at appropriate clinical centres for confirmation* ***Outcomes****: Prevalence of biotinidase (BTD) deficiency in one population and the evaluation of newborn screening (NBS) methods, including specificity, false positive rates and positive predictive values* *[Full text consulted]*
OUTCOMES	
Outcomes reported	*Outcomes relevant to question 1:* • *Screening prevalence was found to be 1:8,638 in the Czech Republic* • *BTD deficiency prevalence in other countries [not further specified] was found to be 1:30,000–1:60,000 through literary data analysis* *Outcomes relevant to question 2:* *BTD activity analysis:*

	• *Screening methods using dried blood spots:* ○ *Decision limit was BTD serum activity <30.0% than median of a healthy population* ○ *Total numbers of false positives and false positive rate were (n=34) and 0.0187%, respectively* ○ *Positive predictive value was 0.38%* • *Confirmatory testing:* ○ *Confirmatory criteria were BTD deficiency or 2 pathogenic mutations in BTD gene using venous blood samples* *[Full text consulted]*
Conclusions	*The prevalence of screened rare diseases in the Czech population was found to be higher for BTD and lower in 6 other rare diseases in comparison to international published data. Additionally, NBS is an efficient tool to improve quality of care in Czech populations with rare diseases.*

Abbreviations: BTD, biotinidase; NBS, newborn screening.

TITLE	
Citation	*Funghini* et al*. (2020) High frequency of biotinidase deficiency in Italian population identified by newborn screening, Molecular Genetics and Metabolism Reports, 25.*[29]
BACKGROUND	
Study type	*Retrospective cohort analysis*
Objectives	*To report 12 years of experience in the newborn screening of biotinidase (BTD) deficiency on 466,182 neonates. When a positive screening result occurred, a clinical evaluation was made of the patient and genetic counselling was offered to the family. Molecular analysis the BTD gene was carried out in all recalled neonates.*
Components of the study	***Population:*** *Dried blood spot samples from newborns born in Umbria and Tuscany, Italy [n=466,182]* ***Index test:*** *Quantitative colorimetric assay of biotinidase activity in dried blood spot. Diagnosis was confirmed by quantitative colorimetric assay of serum biotinidase activity. Plasma acylcarnitines in LC-MS/MS and urinary organic acid profiles in GC-MS were performed to check for abnormalities usually found in patients with biotinidase deficiency* ***Reference standard:*** *Molecular analysis of BTD gene* ***Outcomes:*** *Overall incidence of BTD deficiency, average recall rate*

	The study also reports: • *Mutation analysis results of newborns with BTD enzymatic activity <30%* • *Genetic analysis results of parents of newborns with BTD deficiency* • *Serum BTD activity of newborns with BTD enzymatic activity <30%* • *Mean value of BTD enzyme activity for different genotype groups* *[Full text consulted]*
OUTCOMES	
Outcomes reported	*Outcomes relevant to question 1:* • *Overall incidence of biotinidase deficiency: 1: 6,300 births* *Outcomes relevant to question 2:* • *Average recall rate over 10 years: 0.2%* • *Of recalled newborns, approximately 10% had a confirmed positive result of retesting* *[Full text consulted]*
Conclusions	*NBS introduction had a dramatic impact on BTD deficiency diagnosis, and the incidence has increased significantly compared to other areas. Partial defects are more common than profound in this population and have a distinctive genotype. Early introduction of biotin therapy can prevent clinical symptoms in all patients diagnosed with BTD deficiency by newborn screening.*

Abbreviations: BTD, biotinidase; GC-MS, gas chromatography–mass spectrometry; LC-MS/MS, liquid chromatography tandem mass spectrometry, NBS, newborn screening.

TITLE	
Citation	*Lund* et al*. (2020) Danish expanded newborn screening is a successful preventive public health programme, Danish Medical Journal, 67(1).*[21]
BACKGROUND	
Study type	*Retrospective cohort analysis*
Objectives	*To evaluate the expanded newborn screening (eNBS) programme in Denmark of 17 metabolic diseases in 967,780 newborns. To compare clinical signs of disease in newborns at screening and follow-up.*

Components of the study	***Population:*** *Dried blood spot samples in newborns (Denmark [n=967,780]) born from 1 February 2002–12 February 2019* ***Index test:*** *Biotinidase (BTD) screening using enzymatic assays [not specified] with dried blood spot samples; positive screening results were sent for confirmatory molecular-genetic analyses* ***Reference standard:*** *Positive results during screening were then sent for molecular analysis of BTD gene; an unspecified sample was obtained for confirmatory testing* ***Outcomes:*** *Evaluation of newborn screening methods, including false positive rates and positive predictive values for BTD deficiency. The incidence of BTD was also determined* *The study also reports:* • *BTD deficiency was found more frequently by screening than by clinical presentation* • *Longitudinal clinical evaluation of newborns* • *Most children with BTD deficiency were healthy* • *Overall positive predictive value (PPV) of the eNBS: 62% in 2018* • *Overall false positive rate of the eNBS: 0.024% in 2018* *[Full text consulted]*
OUTCOMES	
Outcomes reported	*Outcomes relevant to question 1:* • *Incidence of BTD deficiency: n=45 in 967,780 screened newborns* *Outcomes relevant to question 2:* *BTD deficiency screening using enzymatic assays:* • *False positives: n=14* • *False negatives: n=1* • *PPV: 76 (%) in 2018* *[Full text consulted]*
Conclusions	*The study concluded that eNBS is a successful preventative public health programme. Additionally, it was concluded that early treatment in a latent phase of disease is effective and*

	screening should be extended to other diseases not currently in the programme.

Abbreviations: BTD, biotinidase; eNBS, expanded newborn screening; NBS, newborn screening; PPV, positive predictive value.

TITLE	
Citation	*Maguolo et al. (2021) Newborn Screening for Biotinidase Deficiency. The Experience of a Regional Center in Italy, Frontiers in Pediatrics, 9.*[15]
BACKGROUND	
Study type	*Retrospective cohort analysis* *[Full text consulted]*
Objectives	*To describe the experience in the diagnosis, treatment and follow-up of patients with biotinidase deficiency identified by newborn screening at the Regional Centre for Newborn Screening of Verona and followed up by the Inherited Metabolic Disease Unit of Verona and Neonatal Intensive Care Unit of Bolzano, Italy, from 2014 to 2020.*
Components of the study	***Population:*** *Dried blood spot samples (DBS) of newborns screened by the Regional Screening Centre of Verona between 2014–2020 ([N=293,784]; Diagnosed with biotinidase (BTD) deficiency: [n=49])* ***Index test:*** *GSP® Neonatal Biotinidase Activity kit* ***Reference standard:*** *Serum BTD activity determination by colorimetric assay and molecular analysis of the BTD gene in all probands and parents.* ***Outcomes:*** *Incidence of biotinidase deficiency among this population, number of samples recalled to repeat DBS in case of BTD deficiency, number of recalled samples that were confirmed BTD deficiency* *The study also reports:* • *Results of genetic analysis of the BTD gene* • *Presentation of symptoms at diagnosis and follow up* *[Full text consulted]*
OUTCOMES	
Outcomes reported	*Outcomes relevant to question 1:* • *Total incidence of BTD deficiency was found to be 1:5,966 newborns* • *Incidence of profound BTD deficiency was found to be 1:58,757*

	• *Incidence of partial BTD deficiency was found to be 1:6,677* *Outcomes relevant to question 2:* • *Number of samples recalled to repeat DBS in case of BTD deficiency: n=287* • *Number of recalled samples diagnosed with BTD deficiency: n=49*
Conclusions	*NBS introduction had a significant impact on BTD deficiency diagnosis, and the incidence increased significantly compared both to other areas and to incidences previously reported. Partial defects were found to be more common than profound and had a distinctive genotype. All patients identified by NBS did not present any clinical signs and symptoms related to BTD deficiency.*

Abbreviations: BTD, biotinidase; NBS, newborn screening.

TITLE	
Citation	*Porta* et al*. (2017) Neonatal screening for biotinidase deficiency: A 30-year single center experience, Molecular Genetics and Metabolism Reports, 13: (80–82).*[30]
BACKGROUND	
Study type	*Retrospective cohort analysis*
Objectives	*To review the outcome of newborn screening for biotinidase deficiency performed at the Regional Reference Center for Newborn Screening of Piemonte and Valle d'Aosta and the Regional Reference Center for diagnosis and treatment of inborn errors of metabolism from January 1987 to December 2016 and the correspondent long-term clinical outcome.*
Components of the study	***Population:*** *Dried blood spot samples from newborns ([N=1,097,894]; diagnosed with biotinidase (BTD) deficiency [n=18]).* ***Index test:*** *First tier test was a semiquantitative colorimetric assay* ***Reference standard:*** *Newborns screened positive were recalled for re-determination of BTD activity on dried blood spot and, in case of confirmed abnormal results, referred to clinical evaluation and quantitative measurement of serum BTD activity. Profound and partial biotinidase deficiency were defined as <10% and 10–30% of median serum enzyme activity, respectively. Molecular analysis was performed by full gene sequencing in affected patients and*

	by targeted mutation analysis in parents after informed consent. Serum BTD activity was also assessed in heterozygous parents of patients with genotyped BTD deficiency ***Outcomes:*** *Overall incidence of BTD deficiency, positive predictive value, false positive rate* *The study also reports:* • *Results of molecular analysis by full gene sequencing in patients diagnosed with BTD deficiency* • *Serum BTD activity in patients diagnosed with BTD deficiency* • *In vivo serum BTD activity in 16 heterozygous parents of patients with profound or partial BTD deficiency* • *Clinical characteristics and outcomes of patients diagnosed with BTD deficiency (clinical follow-up: 13.6 ± 10.8 years)* • *Estimated cost per test (€)* *[Full text consulted]*
OUTCOMES	
Outcomes reported	*Outcomes relevant to question 1:* • *Overall incidence of BTD deficiency: 1;61,000* *Outcomes relevant to question 2:* • *Positive predictive value: 3.9%* • *False positive rate: 0.04% (of 1,097,894 newborns screened, there were 443 false positive results)* *[Full text consulted]*
Conclusions	*The combined incidence of profound and partial BTD deficiency in the region overlapped that reported worldwide. The false positive rate was very low, and was even better than that advocated for expanded newborn screening programmes by tandem mass spectrometry. The positive predictive value was also low for this mass screening programme. Biotin therapy (10–20 mg/day) allowed the full prevention of clinical symptoms in all patients with no adverse effects. These excellent outcomes confirm that newborn screening for BTD deficiency is a very effective secondary prevention programme.*

Abbreviations: BTD, biotinidase; NBS, newborn screening.

TITLE	
Citation	*Tangeraas* et al*. (2020) Performance of expanded newborn screening in Norway supported by post-analytical bioinformatics tools and rapid second-tier DNA analyses, International Journal of Neonatal Screening, 6(3), 51.*[25]
BACKGROUND	
Study type	*Retrospective cohort analysis*
Objectives	*The objective of this paper is to describe the screening results, experience with second-tier mass spectrometry methods and DNA testing, and the clinical outcomes and challenges experienced during the first 8 years after expanding our newborn screening programme (NBS).* *[Full text consulted]*
Components of the study	***Population**: Newborn dried blood spot (DBS) samples (Norwegian population [n=461,369]* ***Index test:** Biotinidase (BTD) activity was initially determined with a Victor Multilabel Plate Reader (PerkinElmer, Turku, Finland) and measured by a semi-quantitative method using abiotin-6-amidoquinoline substrate. From 2013, screening for biotinidase deficiency was performed using the Genetic Screening Processor (GSP®) and the GSP Neonatal Biotinidase kit, both from PerkinElmer* ***Reference standard:** In the case of an abnormal screening result in the first assessment, 2 new DBS punches were re-analysed. Second-tier DNA sequencing was used to resolve abnormal first-tier results. BTD activity was measured in serum as a result of a positive screening call* ***Outcomes:** Incidence of BTD deficiency between 2012-2020, number of true-positive and false-positive cases of BTD detected, incidental detection of B12 deficiency and overall positive predictive value of the screening programme* *The study also reports:* • *Results of genetic analysis of 13 BTD deficiency patients* • *Incidence and clinical presentation of 19 other metabolic conditions* • *Overall PPV of the screening programme* *[Full text consulted]*
OUTCOMES	
Outcomes reported	*Outcomes relevant to question 1:*

	• *Incidence of BTD deficiency between 2012–2020: 1:35,489* • *Incidence of partial BTD: n=3* *Outcomes relevant to question 2:* • *False positive cases: 43 (31-57)* • *True positive cases: 32 (7-58)* • *Screening cut-off value: <60 u/dL* • *Incidental findings: Vitamin B12 deficiency was incidentally detected during follow-up testing in one case of BTD deficiency* *[Full text consulted]*
Conclusions	*The overall performance of the eNBS for inborn error of metabolism (IEMs) improved significantly over the last 8 years, accomplishing one true positive case for every false positive reported. DNA result should override a positive biochemical test. Partial BTD deficiency was more prevalent in the screening programme than severe deficiency, which is consistent with findings from other screening programmes. The majority of cases with IEMs detected by NBS had favourable outcomes and benefitted from pre-symptomatic diagnosis.*

Abbreviations: BTD, biotinidase; DBS, dried blood spot; eNBS, expanded newborn screening; IEMs, inborn error of metabolism; NBS, newborn screening; PPV, positive predictive value.

Abstracts relevant to Question 2 only:

Q2: What is the accuracy of available screening tests using dried blood spots to detect biotinidase deficiency?

TITLE	
Citation	*Ercan* et al*. (2020), Evaluation of the efficiency of serum biotinidase activity as a newborn screening test in Turkey, Journal of Pediatric Endocrinology and Metabolism, 34(1):89–94.*[32]
BACKGROUND	
Study type	*Retrospective cohort analysis*
Objectives	*To evaluate the results of biotinidase (BTD) enzyme activity in accordance with the presence of genetic mutations and investigate the correlation between genotype and biochemical phenotypes together.*

Components of the study	***Population**: Dried blood spot samples in newborns (Turkish population [n=133])* ***Index test:** Trimaris fluorometric biotinidase kit and Thermo Fisher Scientific spectrophotometric analysis measuring at a wavelength of 570 nm. BTD activity levels equal to or greater than 65 Motion Reference Unit (MRU) were accepted as normal* ***Reference standard:** Samples with a BTD activity level of less than 65 MRU underwent repeated measurement. Patients with an activity level lower than 65 MRU in the repeated sample were directed to attend the metabolism outpatient clinic. Genetic analysis was performed with primers containing exons of BTD gene; the sequence data was analysed on Mutation Surveyor Program* ***Outcomes**: Diagnostic sensitivity of fluorometric and spectrophotometric methods by determining BTD activity (%)* *The study also reports:* • *Genotype distribution according to biochemical phenotypes* • *Frequently seen genetic mutations; c.1330 G>C (p.D44H) was the most commonly detected biotinidase variant allele* *[Full text consulted]*
OUTCOMES	
Outcomes reported	*Diagnostic accuracy in a Turkish population:* • *113 newborns produced a positive result for BTD deficiency after mutation analysis* • *Sensitivity and specificity of serum BTD activity were 93.1% and 95.1% respectively, using spectrophotometric methods* • *10 newborns displayed potential BTD deficiency with fluorometric screening, but only one newborn showed partially decreased BTD activity with spectrophotometric methods* *[Full text consulted]*
Conclusions	*Spectrophotometric methods showed better sensitivity than fluorometric analysis. Additionally, the genetic spectrum of BTD deficiency identified may contribute to future studies relating to genotype and biochemical phenotypes.*

Abbreviations: BTD, biotinidase; MRU, Motion Reference Unit.

TITLE	
Citation	*Strovel* et al*. (2017) Laboratory diagnosis of biotinidase deficiency, 2017 update: A technical standard and guideline of the American College of Medical Genetics and Genomics, 19(10):1079–1079.*[6]
BACKGROUND	
Study type	*Technical standards/guidelines regarding screening detection and diagnosis of biotinidase (BTD) deficiency*
Objectives	*These guidelines were developed to define and standardise laboratory procedures for enzymatic BTD testing, to delineate situations for which follow-up testing is required, and to identify variables that may influence test performance and interpretation of results.*
Components of the study	***Population:*** *N/A* ***Index test:*** *Colorimetric enzymatic assay using the artificial substrate biotin-4-amidobenzoic acid, or fluorimetric assays with biotinyl-6-aminoquinoline as an artificial substrate* ***Reference standard:*** *Repeat testing to measure biotinidase activity and/or genetic analysis of the BTD gene* ***Outcomes:*** *Reports current practices for newborn screening of BTD deficiency in the USA:* • *Current screening methods* • *Reporting results* • *Conditions identified by enzymatic BTD testing* *Recommendations for the laboratory diagnosis and newborn screening of BTD deficiency:* • *Preanalytical requirements: sample types, volumes, shipping, handling and storage* • *Method validation: calibration and quantitation, reference ranges, testing personnel* • *Testing for BTD deficiency: sample preparation, analytical methods, quality control, proficiency testing* • *Test interpretation and reporting* *The study also reports:* • *Estimated incidence of BTD deficiency in the USA based on newborn screening outcome data from 2006* *[Full text consulted]*
OUTCOMES	
Outcomes reported	• *Suggested biotinidase activity threshold/screening cut-off: biotinidase activity <10% of mean normal*

	activity is indicative of profound deficiency and activity between 10% and 30% of mean normal activity is indicative of partial deficiency. In the USA, different states have established their own screening cut-offs in addition to rescreening and follow-up protocols • *Colorimetric enzymatic assay using the substrate biotin-4-amidobenzoic acid is the most common screening test using dried blood spots* • *A reference is made to a published comparison of fluorimetric assays and colorimetric assays, that suggests fluorimetric assays may be slightly more specific. More studies are needed to compare them.* • *Genetic analysis of the BTD gene is useful to differentiate between individuals with profound and partial biotinidase deficiency, as well as individuals who are carriers for profound deficiency and those homozygous for a partial deficiency allele* *[Full text consulted]*
Conclusions	*Guidelines for the laboratory diagnosis of BTD deficiency were updated.*

Abbreviations: BTD, biotinidase.

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