



**UK National
Screening Committee**

Screening for Citrullinaemia and Argininosuccinate lyase deficiency

External review against programme appraisal criteria
for the UK National Screening Committee (UK NSC)

Version: 3

Bazian Ltd

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Key points

Citrullinaemia and argininosuccinate lyase deficiency are rare inborn metabolic conditions.

The technique used for screening for citrullinaemia and argininosuccinate lyase deficiency is currently used to screen for other conditions.

Screening for citrullinaemia has been associated with high false positive rates, and the early onset form of the disorder can become symptomatic before the results of the screening test are available.

The sensitivity and specificity of screening for argininosuccinate lyase deficiency are reportedly 100% when argininosuccinic acid is used as a marker. Although many of the infants identified with argininosuccinate lyase deficiency in newborn screening programs were asymptomatic at the time of diagnosis, there has been one report of an infant showing symptoms before the results of the screening test were available.

As the timing of the onset of symptoms varies there could still be value in screening for these disorders.

Both disorders can be treated with long term dietary protein restriction, often in combination with a nitrogen scavenging agent such as sodium phenylbutyrate.

This treatment can help prevent episodes of high ammonia concentration (hyperammonaemia). However, it is unclear whether this treatment is successful in preventing the development of neurocognitive deficiencies and liver disease in argininosuccinate lyase deficiency.

A Canadian study estimated the incremental cost-effectiveness ratio per additional life year saved to be \$1,753,719 for citrullinaemia and \$1,440,777 for argininosuccinate lyase deficiency, excluding start-up costs (as MS/MS is already used in the UK newborn screening program). It is unclear how applicable this study would be to the UK. The economic evaluation in the 2004 HTA report estimated that screening for urea cycle disorders to cost £2,965 per life-year gained. No additional UK based studies of cost effectiveness were identified.

The policy on newborn screening for these conditions should not change.

Summary

Both citrullinaemia and argininosuccinate lyase deficiency are urea cycle disorders. The urea cycle is a sequence of chemical reactions that take place in liver cells to process the excess nitrogen that is produced when protein is used by the body. The product of the urea cycle is a compound called urea, which is excreted in urine.

Citrullinaemia is caused by mutations in both copies of the *ASS* gene which codes for an enzyme, argininosuccinate synthase, which is responsible for the third step of the urea cycle.

Argininosuccinate lyase deficiency is caused by mutations in both copies of the *ASL* gene, which codes for an enzyme, argininosuccinate lyase, which is responsible for the fourth step of the urea cycle.

Mutations which reduce the activity of either of these enzymes disrupt the urea cycle and prevent the body from processing nitrogen effectively. This causes nitrogen, in the form of ammonia, and other toxic substances to accumulate in the blood.

Children with citrullinaemia or argininosuccinate lyase deficiency typically appear normal at birth. There are two forms of both conditions:

- An early onset form, in which symptoms become evident in the first few days of life and can be fatal
- A late-onset form, which is normally milder

In the early onset form of citrullinaemia, as ammonia builds up symptoms including lack of energy (lethargy), poor feeding, vomiting, seizures and loss of consciousness appear, which can be life threatening. Even with treatment patients can have significant neurological defects. Symptoms of the late-onset form include lethargy and intellectual disability, and other symptoms related to high concentrations of ammonia (hyperammonaemia).

Similarly, in the early onset form of argininosuccinate lyase deficiency children present with symptoms including lethargy, poor feeding, vomiting, and poorly controlled body temperature. Without treatment symptoms can worsen and be fatal. Symptoms of the late onset form vary, from symptoms of hyperammonaemia to cognitive impairment, behavioural abnormalities, and/or learning difficulties or to no symptoms. Argininosuccinate lyase deficiency can also cause hepatitis, cirrhosis, and brittle hair.

Argininosuccinate lyase deficiency is different to citrullinaemia in that in this disorder some of the symptoms appear to be unrelated to hyperammonaemia, suggesting that ammonia is not the only toxic compound in argininosuccinate lyase deficiency. Symptoms of argininosuccinate lyase deficiency that appear to be unrelated to the severity or duration of episodes of hyperammonaemia include neurocognitive deficiencies, liver disease and brittle hair.

It is not known how many people in the UK have citrullinaemia or argininosuccinate lyase deficiency. From studies from Europe, the US and Australia, the incidence of citrullinaemia can be estimated as between less than one case per 650,000 births to more than one case per 60,000 births; and the incidence of argininosuccinate lyase deficiency at between less than one case per 940,000 births to more than one case per 50,000 births.

Both citrullinaemia and argininosuccinate lyase deficiency can be detected through newborn screening by measuring the levels of amino acids in the dried blood spot using a technique called tandem mass spectrometry (MS/MS), which is already used to screen for other conditions in the UK.

In citrullinaemia, the amino acid citrulline builds up and the concentration of citrulline in the dried blood spot can be used to screen for this condition. However, other conditions, including argininosuccinate lyase deficiency, pyruvate carboxylase deficiency and citrin deficiency (also known as citrullinaemia type II) also cause elevations in citrulline levels, and further testing is required to distinguish between these conditions. Screening for citrullinaemia using citrulline as a marker has been associated with high false positive rates in some studies, and it has been reported that newborn screening results arrive after the onset of symptoms in some cases of early onset citrullinaemia.

The concentrations of the amino acids argininosuccinic acid and citrulline can be used to screen for argininosuccinate lyase deficiency. Argininosuccinic acid has the advantage of being a specific marker for argininosuccinate lyase deficiency. When argininosuccinic acid was used as a marker for argininosuccinate lyase deficiency, a study reported 100% sensitivity (the percentage of newborns with argininosuccinate lyase deficiency given a positive screening result) and 100% specificity (the percentage of healthy newborns given a negative result). Although many of the infants identified with argininosuccinate lyase deficiency in newborn screening programs were asymptomatic at the time of diagnosis, one study was identified that reported that an infant

developed symptoms (on day four of life) before the results of a newborn blood spot screen were available.

For both citrullinaemia and argininosuccinate lyase deficiency there could be value in the early diagnosis and treatment of patients with forms of the condition that develop symptoms later.

Both conditions are treated with long term dietary protein restriction, often in combination with a nitrogen scavenging agent such as sodium phenylbutyrate.

For argininosuccinate lyase deficiency it is unclear whether this treatment is successful in preventing the development of neurocognitive deficiencies and liver disease, even if metabolic decompensations are avoided.

No randomised controlled trials of screening were identified.

One cost-effectiveness study for screening for citrullinaemia and argininosuccinate lyase deficiency individually was identified. This study was published in 2007 and was performed from a Canadian perspective. Citrullinaemia and argininosuccinate lyase deficiency were amongst the least cost-effective disorders to screen for, with the incremental cost-effectiveness ratio per additional life year saved estimated at \$1,753,719 for citrullinaemia and \$1,440,777 for argininosuccinate lyase deficiency, excluding start-up costs (as MS/MS is already used in the UK newborn screening program). It is unclear how applicable this study would be to the UK. The economic evaluation in the 2004 HTA report estimated that screening for urea cycle disorders to cost £2,965 per life-year gained. No additional UK based studies of cost effectiveness were identified.

There is no reason for the policy on systematic population screening for citrullinaemia to change, as there are still uncertainties over the epidemiology of this condition in the UK, and there are concerns over the reliability of the test and the timing of the test in relation to the presentation of the acute form of the condition.

There is no reason for the policy on systematic population screening for argininosuccinate lyase deficiency to change, as there are still uncertainties over the epidemiology of this condition in the UK, the timing of the test in relation to the presentation of the acute form of the condition, and whether treatment improves outcomes for patients with later-onset forms of the condition.

Introduction

This review will assess screening for citrullinaemia (citrullinaemia type I) and argininosuccinate lyase deficiency, two disorders of amino acid metabolism.

Citrullinaemia and argininosuccinate lyase deficiency are autosomal recessive disorders resulting in deficiency of two different enzymes of the urea cycle. Both these disorders can manifest themselves as acute-early onset forms or milder, late onset forms. Defects in the urea cycle lead to the accumulation of ammonia. Infants with the neonatal forms develop hyperammonaemia within the first few days of life accompanied by vomiting, lethargy, hypothermia and poor feeding. In the absence of treatment symptoms can worsen and lead to death. Symptoms of the late-onset forms vary. Patients with the milder late-onset form of citrullinaemia can present with recurrent lethargy and somnolence, intellectual disability, and/or chronic or recurrent hyperammonaemia. Patients with argininosuccinate lyase deficiency may present with episodic hyperammonaemia triggered by acute infection or stress, or cognitive impairment, behavioural abnormalities and/or learning difficulties. Long term complications of argininosuccinate lyase deficiency include hepatomegaly, elevated transaminases, liver fibrosis or cirrhosis, neurocognitive deficits and trichorrhexis nodosa (course and brittle hair that breaks easily).

Both of these conditions can also be detected through MS/MS analysis of newborn dried blot spots. The mainstay of treatment is long-term dietary protein restriction. Treatment may also include oral administration of nitrogen scavenging agents such as sodium phenylbutyrate. Newborn screening results may not be available soon enough to prevent onset of symptoms in patients with the neonatal forms, but early detection and treatment could be beneficial for patients with late-onset forms.

Current policy

Citrullinaemia and argininosuccinate lyase deficiency are not currently screened for in the UK. Currently, screening is offered to all babies in the UK for phenylketonuria, congenital hypothyroidism, sickle cell disease and medium-chain acyl-coenzyme A dehydrogenase deficiency. The UK NSC also recommends that screening should be offered for maple syrup urine disease, homocystinuria, isovaleric acidaemia and glutaric aciduria type I.

This report

Screening for citrullinaemia and argininosuccinate lyase deficiency on the basis of clinical and cost-effectiveness was last reviewed in 2004 by the Health Technology Assessment (HTA) NHS R&D HTA Programme. The HTA study did not recommend screening for citrullinaemia and argininosuccinate lyase deficiency as there was limited evidence regarding the epidemiology, the reliability of the test and its timing in relation to presentation of the acute form of the condition, and the effectiveness of treatment.

In 2006, on behalf of the US Health Resources and Services Administration, the American College of Medical Genetics (ACMG) published an analysis of the scientific literature and gathered expert opinion on the effectiveness of newborn screening and on newborn screening programme optimisation to produce:

- a uniform condition panel (including implementation methodology);

- model policies and procedures for State newborn screening programmes (with consideration of a national model);
- model minimum standards for State newborn screening programmes;
- a model decision matrix for consideration of State newborn screening programme expansion;
- and consideration of the value of a national process for quality assurance and oversight.

Twenty-nine conditions were assigned to the core panel. Citrullinaemia and argininosuccinate lyase deficiency were included in the core panel.

A 2012 report on the practices of newborn screening for rare disorders implemented in member states of the European Union, candidate, potential candidate and European Free Trade Association countries reported that screening for citrullinaemia type I is undertaken in six countries (Austria, Hungary, some regions of Italy, Portugal, Spain and Iceland) and that screening for argininosuccinate lyase deficiency is undertaken also undertaken in the same six countries.¹Newborn screening for citrullinaemia and argininosuccinate lyase deficiency was re-assessed against the UK National Screening Committee (NSC) criteria for appraising the viability, effectiveness and appropriateness of a screening programme (National Screening Committee 2003).

Particular areas of interest identified by the National Screening Committee included:

- the conditions, including the timing of their presentation
- the test, including its timing, benign/uncertain/additional cases detected, and performance in premature infants
- treatment, including information on outcome, benefits and harms

For this review an updated systematic search has been performed for relevant publications from 2004 to August 2012. Overall, 457 citations were judged to be relevant (see Methodology section for study breakdown). The full texts of selected papers were retrieved after a first pass appraisal at abstract level. Non-systematic reviews, editorials, other opinion pieces, reports of case series of fewer than four patients, and those with nonhuman data were excluded, as were conference abstracts. Priority was given to studies from Europe, North America and Australia. Additional relevant references identified during the preparation of the report were also included. An overview of the most informative and relevant references regarding the individual screening criteria is given below.

Appraisal against UK NSC Criteria

These criteria are available online at <http://www.screening.nhs.uk/criteria>.

1. The condition should be an important health problem

Citrullinaemia Type I

Citrullinaemia (citrullinaemia type I) has several forms: an acute neonatal form and a milder late onset form.² Infants with the neonatal form develop hyperammonaemia within the first few days of life, become lethargic, feed poorly, vomit, and develop tachypnea or stroke. Without treatment, the condition can cause increased intracranial pressure, increased neuromuscular tone, spasticity, ankle clonus, seizures, loss of consciousness and death. Even with prompt intervention, patients normally have significant neurological defects. The milder late-onset form can present with recurrent lethargy and somnolence, intellectual disability, and/or chronic or recurrent hyperammonaemia.²

Argininosuccinate lyase deficiency

Like citrullinaemia, argininosuccinate lyase deficiency, or argininosuccinic aciduria, has an acute neonatal onset form and a late onset form.³ The neonatal onset form is characterized by hyperammonaemia in the first days of life accompanied by vomiting, lethargy, hypothermia and poor feeding. In the absence of treatment symptoms can worsen and lead to death. The late onset form has variable presentation, from episodic hyperammonaemia triggered by acute infection or stress, to cognitive impairment, behavioural abnormalities and/or learning difficulties, even if hyperammonaemia episodes have been avoided.³

Long term complications of argininosuccinate lyase deficiency include: hepatomegaly, elevated transaminases, liver fibrosis or cirrhosis, neurocognitive deficits and trichorrhexis nodosa (course and brittle hair that breaks easily).³

Citrullinaemia and argininosuccinate lyase deficiency are important health problems and are screened for in the US and other European countries

In 2006, on behalf of the US Health Resources and Services Administration, the American College of Medical Genetics (ACMG) published an analysis of the scientific literature and gathered expert opinion on the effectiveness of newborn screening and on newborn screening programme optimisation to produce a uniform condition panel. Citrullinaemia and argininosuccinate lyase deficiency were included in the core panel.

A 2012 report on the practices of newborn screening for rare disorders implemented in member states of the European Union, candidate, potential candidate and European Free Trade Association countries reported that screening for citrullinaemia type I is undertaken in six countries (Austria, Hungary, some regions of Italy, Portugal, Spain and Iceland) and that screening for argininosuccinate lyase deficiency is undertaken also undertaken in the same six countries.¹

Summary: Criterion 1 met. Citrullinaemia and argininosuccinate lyase deficiency are rare but if left untreated the acute early onset forms of the conditions can be fatal. The late onset forms are milder but can lead to hyperammonaemia and associated sequelae, and neurocognitive

deficits. Both citrullinaemia and argininosuccinate lyase deficiency were included in the US core panel of disorders by the ACMG and are screened for by a number of European countries.

2. The epidemiology and natural history of the condition, including development from latent to declared disease, should be adequately understood and there should be a detectable risk factor, disease marker, latent period or early symptomatic stage

Citrullinaemia and argininosuccinate lyase deficiency are both urea cycle defects. Urea cycle defects are often considered together, and therefore data for both of these conditions is presented below.

Natural history

Citrullinaemia type I is an autosomal recessive disease caused by mutations in the gene encoding argininosuccinate synthase (*ASS*).² This enzyme catalyses the third step in the urea cycle. The urea cycle is the principle mechanism for the clearance of waste nitrogen, and deficiency of argininosuccinate synthase leads to the accumulation of ammonia.

As mentioned in Criterion 1, citrullinaemia has several forms: an acute neonatal form and a milder late onset form.² Infants with the neonatal form develop hyperammonaemia within the first few days of life, become lethargic, feed poorly, vomit, and develop tachypnea or stroke. Without treatment, the condition can cause increased intracranial pressure, increased neuromuscular tone, spasticity, ankle clonus, seizures, loss of consciousness and death. Even with prompt intervention, patients normally have significant neurological defects. The milder late-onset form can present with recurrent lethargy and somnolence, intellectual disability, and/or chronic or recurrent hyperammonaemia.²

It is reported that certain mutations in *ASS* are associated with either the neonatal, severe form or the mild, late-onset form.²

Argininosuccinate lyase deficiency is also an autosomal recessive disease, in this case caused by mutations in the gene encoding argininosuccinate lyase (*ASL*).³ Argininosuccinate lyase catalyses the fourth step in the urea cycle. In common with other urea cycle disorders, deficiency in this enzyme leads to hyperammonaemia. However, in addition, patients with argininosuccinate lyase deficiency often also suffer from neurocognitive deficiencies, hepatitis, cirrhosis, and trichorrhaxis nodosa. Some of these outcomes appear to be unrelated to the severity or duration of hyperammonaemic episodes, suggesting that plasma ammonia is not the only toxic compound in argininosuccinate lyase deficiency.³ For example, Ficicioglu et al. (2009) reported outcomes after 13 to 33 years of follow up of 13 patients with argininosuccinate lyase deficiency who had been detected by newborn screening, or due to an affected older sibling, who were asymptomatic when diagnosed.⁴ All patients received treatment with a low protein diet, and certain patients also received arginine or sodium benzoate. Despite the fact that no incidences of hyperammonaemic coma or acute encephalopathy requiring hospitalisation occurred during the follow up period, five patients had learning disabilities, borderline IQ or mild developmental delay or low normal development with the need for special classes. Six of the thirteen patients had electroencephalogram (EEG) abnormalities, and three patients experienced seizures of various types.⁴

As with the other disorders considered, argininosuccinate lyase deficiency has an acute neonatal onset form and a late onset form.³ Like citrullinaemia, the neonatal onset form is characterized by hyperammonaemia in the first days of life accompanied by vomiting, lethargy, hypothermia and poor feeding. In the absence of treatment symptoms can worsen and lead to death. The late onset form has variable presentation, from episodic hyperammonaemia triggered by acute infection or stress, to cognitive impairment, behavioural abnormalities and/or learning difficulties.³

It is known that mutations in *ASL* are responsible for argininosuccinate lyase deficiency, but so far there is no correlation between clinical presentation and genotype.³ Correlation between residual enzyme activity and clinical phenotype has also not been established.³

The 2004 HTA report considered all urea cycle defects together. It stated that:⁵

“The urea cycle is a metabolic pathway, confined to the liver, that leads to the detoxification of ammonia by the synthesis of arginine and urea. There are five urea cycle disorders, each relating to a defect in one of the enzymes of the urea cycle: carbamoyl phosphate synthase deficiency, ornithine carbamoyltransferase (transcarbamylase) deficiency, argininosuccinate synthase deficiency/citrullinaemia, argininosuccinate lyase deficiency/argininosuccinic aciduria, and arginase deficiency/argininaemia.”

“Any disruption in the synthesis of urea leads to accumulation of the ammonium ion, which is highly toxic; therefore, most of the urea cycle disorders share a similar spectrum of clinical presentation. Hyperammonaemia is thought to be the main damaging factor in the first four disorders and they share many common features. These will be discussed as a group distinct from arginase deficiency, which has a somewhat different presentation.

The disorders carbamoyl phosphate synthase deficiency, ornithine carbamoyltransferase (transcarbamylase) deficiency, argininosuccinate synthase deficiency/citrullinaemia and argininosuccinate lyase deficiency/argininosuccinic aciduria have variable severity with a neonatal acute form that is rapid and usually fatal. They all have a range of milder variants that present as chronic conditions later in infancy and childhood. The acute neonatal presentation of these disorders is seen in full-term infants who present with the effects of hyperammonaemia in the first days of life. Acute presentation involves respiratory distress, poor feeding, lethargy, vomiting, hypotonia, spasticity, convulsions and coma. Pulmonary and gastric haemorrhages can also occur and in untreated cases most patients will die in the neonatal period. Those who survive the first few days of life probably have some residual enzyme activity.

The chronic forms of these urea cycle disorders often present with a history of episodic vomiting, lethargy and irritability, and there can be seizures or even periods of coma. These episodes are often associated with high-protein meals or minor infections. Some of these late-onset or chronic presentations of the disorders remain healthy until later childhood, when they suffer acute illness often associated with an infection. Undiagnosed and untreated chronic cases may prove fatal.”

Three studies were identified with further information about the natural history of urea cycle disorders. Information pertaining to citrullinaemia and argininosuccinate lyase deficiency has been extracted.

Nassogne et al. (2005) described the presentation and outcome of patients with urea cycle defects diagnosed between 1972 and 2000 at the Necker-Enfants Malades Hospital, Paris.⁶ During that period, there were 33 cases of citrullinaemia, of which 26 were the neonatal onset

form and seven were the late-onset form, and 20 cases of argininosuccinate lyase deficiency, of which 14 were the neonatal onset form and six were the late onset form.

Nineteen of the 26 patients with the neonatal form of citrullinaemia died. Eight of the 14 patients with the neonatal form of argininosuccinate lyase deficiency died, and two further patients died aged eight and 20 years old respectively. Age at follow-up and neurological outcome for the surviving patients with the neonatal forms of citrullinaemia and argininosuccinate lyase deficiency is shown in Table 1. No details of how the patients were treated were given.

Table 1: Long term neurological outcomes of patients with the neonatal forms of citrullinaemia and argininosuccinate lyase deficiency diagnosed between 1972 and 2000 at the Necker-Enfants Malades Hospital, Paris.⁶ Each line represents one patient. Good outcome indicates absence of neurological signs or psychomotor delay. Moderate outcome indicates persistent learning disorders or mild neurological signs. Bad neurological outcome indicates severe neurological symptoms, bedridden patients, or severe mental retardation.

	Age at follow up (years)	Neurological outcome
Citrullinaemia (ASS deficiency)	1	Good
	2	Good
	3	Good
	5	Good
	10	Good
	14	Good
	27	Bad
Argininosuccinate lyase deficiency (ASL deficiency)	0.5	Good
	8	Bad
	8 (died)	Moderate
	9	Moderate
	13	Moderate
	20 (died)	Moderate

Of the patients with late-onset citrullinaemia, four had chronic presentation. The predominant symptoms were hepatogastric (two patients) or psychomotor retardation (two patients). The remaining three patients presented with acute neurological signs. Of the patients with late-onset argininosuccinate lyase deficiency, three patients had chronic presentation with the predominant symptom being psychomotor retardation and three patients had acute neurological signs. No patient with the late-onset forms of citrullinaemia or argininosuccinate lyase deficiency died. Long-term neurological outcomes for patients with the late-onset forms of these disorders are shown in Table 2. Again, no details of how the patients were treated were given.

Table 2: Long term neurological outcomes of patients with the late-onset forms of citrullinaemia and argininosuccinate lyase deficiency diagnosed between 1972 and 2000 at the Necker-Enfants Malades Hospital, Paris.⁶ Each line represents one patient. Good outcome indicates absence of neurological signs or psychomotor delay. Moderate outcome indicates persistent learning disorders or mild neurological signs. Bad neurological outcome indicates severe neurological symptoms, bedridden patients, or severe mental retardation.

	Age at follow up (years)	Neurological outcome
Citrullinaemia (ASS deficiency)	2	Good
	4	Good
	5	Good
	11	Good
	17	Good
	17	Bad
	26	Bad
Argininosuccinate lyase deficiency (ASL deficiency)	4	Good
	5	Good
	7	Good
	8	Moderate
	16	Moderate
	20	Moderate

To gain more information about the incidence, prevalence, morbidity, mortality and response to therapy of rare diseases, the US set up the Rare Diseases Clinical Research Network, funded by the National Institutes of Health. A consortium within this network is the Urea Cycle Disorder Consortium (UCDC).⁷ The UCDC has set up a National Registry for patients, and is conducting long-term studies on the outcome of patients with urea cycle disorders. It also aims to develop and test new treatments for urea cycle disorders, and to conduct other studies to answer specific questions about urea cycle disorders, such as studies into brain changes in response to ammonia toxicity.

Patients with urea cycle disorders were recruited into a longitudinal study from various sources including clinics, patient advocacy groups (National Urea Cycle Disorders Foundation, NUCDF) and a contact registry created by the Data and Technology Coordinating Centre (DTCC). To be included, patients had to fulfil diagnostic criteria. For citrullinaemia these included ≥ 10 fold elevation of citrulline in plasma and/or decreased argininosuccinate synthase enzyme activity in cultured skin fibroblasts or other appropriate tissues and/or identification of a pathogenic mutation. For argininosuccinate lyase deficiency patients had to have argininosuccinic acid in blood or urine and/or decreased argininosuccinate lyase enzyme activity in cultured skin fibroblasts or other appropriate tissues and/or a pathogenic mutation. Tuchman et al. (2008) reported cross-sectional information obtained when patients enrolled in the trial, between February 2006 and December 2007.⁸

Twenty-nine patients with argininosuccinate lyase deficiency were enrolled, including 18 neonatal cases, who presented clinically during the neonatal period. The median age at enrolment was eight years old, and the median time since diagnosis was seven years. Twenty-six patients with citrullinaemia were enrolled, including 19 neonatal cases. The median age at enrolment was 6.5 years, and the median time since diagnosis was 2.5 years. Self-reported developmental disabilities at enrolment are shown in Table 3. Abnormal findings reported during neurological evaluation are shown in Table 4. Patients also reported their long term treatment (Table 5).

Table 3: Number of enrolled patients with argininosuccinate lyase deficiency or citrullinaemia with self-reported developmental disabilities and psychiatric disorders. Modified from Tuchman et al. (2008)⁸

Disorder	Argininosuccinate lyase deficiency (n=29)	Citrullinaemia (n=26)
Attention deficit hyperactivity disorder	8	5
Autism spectrum disorder	0	0
Communication disorder	2	4
Cerebral palsy	1	1
Intellectual disability	19	14
Learning disability	14	9
Mood disorder	0	0
Other psychiatric disorder	4	0
Seizure disorder	8	4
Visual or hearing impairment	3	3

Table 4: Number of enrolled patients with argininosuccinate lyase deficiency or citrullinaemia with abnormal findings reported on neurological evaluation. Modified from Tuchman et al. (2008)⁸

Disorder	Argininosuccinate lyase deficiency (n=29)	Citrullinaemia (n=26)
Abnormal movement	4	4
Asymmetry	2	2
Contractures	3	3
Cerebellar findings	0	0
Not ambulatory	0	1
Tone changes	6	3
Reflex abnormalities	10	3
Visual/hearing deficits	1	3

Table 5: Number of patients receiving different types of long term therapy. Modified from Tuchman et al. (2008)⁸

Disorder	Argininosuccinate lyase deficiency (n=29)	Citrullinaemia (n=26)
Phenylbutyrate	6	13
Arginine	23	15
Benzoate	2	1
Protein restriction	22	17

Keskinen et al. (2008) reported on the clinical course and outcomes of 20 patients with argininosuccinate lyase deficiency and one patient with citrullinaemia diagnosed by 2007 in Finland.⁹

The symptoms at diagnosis, vital status, experience of hyperammonaemic episodes and mental development for the 20 patients with argininosuccinate lyase deficiency are shown in Table 6. Patients diagnosed by 2001 had mainly been treated with dietary protein restriction (1.2 to 1.6g/kg/day protein) and arginine replacement therapy (0.1 to 0.8g/kg/day). Only two patients were regularly receiving ammonia scavenging agents. By 2007 standard treatment had changed. Patients with early-onset argininosuccinate lyase deficiency were treated with less arginine and an ammonia-scavenging agent was added to the treatment regimen.

One female case of citrullinaemia was identified, who died at 1.5 months of age.

Table 6: Characteristics of patients with argininosuccinate lyase deficiency diagnosed in Finland by March 2007. Modified from Keskinen et al. (2008)⁹

Patient	Gender	Year of diagnosis	Age at diagnosis	Symptoms at diagnosis	Age at follow-up	Hyperammonaemia during treatment	Mental development
1	Male	1977	Prenatal	None	29 years	Some (between 1 and 4 episodes)	Severe disability
2	Male	1996	Prenatal	None	10 years	Some (between 1 and 4 episodes)	Moderate disability
3	Female	1966	NR	Unknown	Died during neonatal period	Died of first episode	
4	Female	1968	NR	Unknown	Died during neonatal period	Died of first episode	
5	Female	1991	9 days	Poor appetite, drowsiness, impaired consciousness, grunting	15 years	Frequent (more than 4 episodes)	Mild disability
6	Male	1982	10 days	Convulsions, irritability, hypotonia, impaired consciousness	Died at 3 months of age	Severely affected by first episode	
7	Male	2003	Post-mortem	Unconsciousness	Died at 3 days of age	Died of first episode	
8	Male	1974	11 days	Unknown	Died at 13 years of age	Frequent (more than 4 episodes)	Moderate disability
9	Female	1974	2 months	Drowsiness, vomiting, impaired consciousness	32 years	Some (between 1 and 4 episodes)	Severe disability
10	Male	1990	6 months	Vomiting, right-sided twitching, crying	16 years	None	Mild disability
11	Female	1984	6 months	Dyspnoea, fever, convulsions	Died at 7 months of age	Died of first episode	

12	Female	1989	11 months	Restlessness, tiredness, drowsiness	18 years	Frequent (more than 4 episodes)	Severe disability
13	Male	1992	11 months	Vomiting, impaired consciousness, convulsions	15 years	Some (between 1 and 4 episodes)	Moderate disability
14	Female	1991	1 year 5 months	Tiredness, vomiting	17 years	None	Mild disability
15	Male	1979	1 year 8 months	Passivity, poor appetite, convulsions, sleepiness	29 years	Some (between 1 and 4 episodes)	Severe disability
16	Male	1987	5 years	Delayed development of speech, clumsiness	24 years	None	Mild disability
17	Male	1993	11 years	Developmental delay, ataxia, epileptic seizures	24 years	None	Mild disability
18	Female	1993	14 years	Developmental delay	27 years	Some (between 1 and 4 episodes)	Mild disability
19	Female	1981	49 years	Paranoid schizophrenia, developmental disability, ataxia	Died at 68 years of age	Unknown	Moderate disability
20	Male	1987	61 years	Paranoid schizophrenia, developmental disability	Died at 64 years of age	Unknown	Moderate disability

Incidence

The HTA report did not include any studies reporting on the incidence of urea cycle disorders in the UK. It concluded that *“the expected incidence of urea cycle disorders, based on estimates from the number of cases clinically diagnosed in the UK (compared with similar disorders of established incidence), is 2.5 cases per 100,000 births.”*⁵

Sanderson et al. (2006) reported the incidence (assumed to be equivalent to birth prevalence) of urea cycle disorders in the West Midlands, UK between 1999 and 2003, although incidences for individual disorders was not given.¹⁰ Based on definitive diagnosis resulting from the investigation of a clinically-presenting patient the birth prevalence of urea cycle defects was 1 in 22,179.

No studies investigating the incidence of citrullinaemia or argininosuccinate lyase deficiency in the UK were identified. Studies which have assessed epidemiology, screening or treatment can offer some estimate of the incidence of the condition. The incidences calculated from these studies in European, North American and Australian populations are shown in Table 7 and Table 8.

Incidences of citrullinaemia are shown in Table 7. Incidences ranged from 1:58,355 when both mild and classic forms of citrullinaemia were considered together (South-West Germany, screened cohort between 1999 and 2005)¹¹ and 1:1,616,000 (Finland, between 1981 and 2007, not identified by screening).⁹ However, the Finish study noted that “Finland is a country with a small and genetically isolated population, and the prevalence of many genetic diseases differ markedly from those in other countries.” The second lowest reported incidence was 1:674,754 (Denmark, the Faroe Islands and Greenland, 1992 to end of 2001, no screening in operation).¹² It was not possible to calculate incidences in studies that did not identify any cases.

Two studies present results which allow the impact of screening programmes on incidence to be assessed.

In Australia, the incidence of citrullinaemia was 1:517,067 in a cohort of unscreened infants born between 1994 and 2002 who were either born before the introduction of screening or were born in a region that had not yet introduced screening.¹³ In the screened cohort (born between 1998 and 2002), no cases of citrullinaemia were identified.

Lund et al. (2012) compared the incidence of citrullinaemia in Denmark, the Faroe Islands and Greenland in the decade before newborn screening and the decade after the introduction of newborn screening.¹² The incidence increased after the introduction of screening. In the decade before the screening, the incidence was 1:674,754. After the introduction of screening the incidence was 1:363,538. Screening for citrullinaemia stopped in 2009. The incidence of citrullinaemia was even higher if false negatives during the period of screening and cases in unscreened children up to March 2011 were considered, at 1:148,823, more than four times the incidence in the decade before screening.

In conclusion, the incidence of citrullinaemia varies widely between reports from different countries, from more than one case per 60,000 births to less than one case per 650,000 births. If a screened cohort was compared to a contemporaneous or historical unscreened cohort in the same country, one study found that the incidence of citrullinaemia was reduced and another study found the incidence increased. It should be noted that individual cases of the disease have a big impact on calculated incidences as citrullinaemia is a rare disease.

Incidences of argininosuccinate lyase deficiency are shown in Table 8. Incidences ranged from approximately 1:49,000 (Massachusetts, US, 1969 to 1978, identified by screening)⁴ and 1:940,000 (North West Carolina, US, screened cohort between 1997 and 2005).¹⁴ It was not possible to calculate incidences from studies which identified no cases.

Two studies present results which allow the impact of screening programmes on incidence to be assessed.

In Australia, the incidence of argininosuccinate lyase deficiency was 1:258,534 in a cohort of unscreened infants born between 1994 and 2002 who were either born before the introduction of screening or were born in a region that had not yet introduced screening.¹³ In the screened cohort (born between 1998 and 2002) the incidence was similar, at 1:230,750.

Lund et al. (2012) compared the incidence of argininosuccinate lyase deficiency in Denmark, the Faroe Islands and Greenland in the decade before newborn screening and the decade after the introduction of newborn screening.¹² In the decade before the screening no cases of argininosuccinate lyase deficiency were diagnosed. After the introduction of screening the incidence was 1:168,016. The incidence was lower if false negatives and cases in unscreened children during this period were considered, at 1:195,659.

In conclusion, the incidence of argininosuccinate lyase deficiency varies widely between reports from different countries, from more than one case per 50,000 births to less than one case per 940,000 births. If a screened cohort was compared to a contemporaneous or historical unscreened cohort in the same country, one study found that the incidence of argininosuccinate lyase deficiency was similar and another study found the incidence increased from no identified cases in the decade before screening to more than one case per 170,000 births in a screened cohort. It should be noted that individual cases of the disease have a big impact on calculated incidences as argininosuccinate lyase deficiency is a rare disease.

Table 7: Estimates of the incidence of citrullinaemia. NR, not reported; NA, not applicable.

Study	Country and time period	Screening programme in operation?	Number of identified cases	Incidence	Notes
Lund et al. (2012) ¹²	Demark, the Faroe Islands and Greenland January 1992 to December 2001	No	NR	1:674,754	
Lund et al. (2012) ¹²	Demark, the Faroe Islands and Greenland 2002 to 2009	Yes	1 true positive 1 false negative 1 case in unscreened cohort	1:363,538 true positives identified by screening between 2002 and 2009	If false negatives and cases in unscreened children were included, the incidence was 1:148,823
Couce et al. (2011) ¹⁵	Galicia, Spain 2000 to 2010	Yes	1	1:210,165	
Lindner et al. (2011) ¹¹	South-West Germany January 1999 to April 2005	Yes	4 ('classic', symptoms developed) 6 ('mild') Both forms: 10	1:145,889 ('classic') 1:97,259 ('mild') 1:58,355 (both forms)	
Kasper et al. (2010) ¹⁶	Austria 2002 to 2010	Yes	8	1:77,811	
Wilcken et al. (2009) ¹³	Australia Born between 1994 and 2002	No	3	1:517,067	
Wilcken et al.	Australia	Yes	0	NA	461,500 infants screened

Study	Country and time period	Screening programme in operation?	Number of identified cases	Incidence	Notes
(2009) ¹³	Born between 1998 and 2002				
Keskinen et al. (2008) ⁹	Finland January 1981 to March 2007	No	1	1:1,616,000	“Finland is a country with a small and genetically isolated population, and the prevalence of many genetic diseases differ markedly from those in other countries.” Incidence estimated by dividing the number of diagnoses by the number of live births
la Marca et al. 2008 ¹⁷	Tuscany, Italy January 2002 to end of 2007	Yes	2	1:80,000	
Auray-Blais et al. (2007) ¹⁸	Quebec, Canada 1973 and 2006	Yes	5	1:493,986	
Feuchtbaum et al. 2006 ¹⁹	California, US January 2002 to June 2003	Yes	0	NA	353,894 infants screened
Frazier et al. (2006) ¹⁴	North Carolina, US July 1997 to July 2005	Yes	5	1:190,000	
Comeau et al. (2004) ²⁰	New England Screening Program, US January 1999 to February 2003	Yes	1	1:318,535	

Table 8: Estimates of the incidence of argininosuccinate lyase deficiency. NR, not reported; NA, not applicable.

Study	Country and time period	Screening programme in operation?	Number of identified cases	Incidence	Notes
Lund et al. (2012) ¹²	Demark, the Faroe Islands and Greenland January 1992 to December 2001	No	0	NA	Of 674,754 live births from 1 January 1992 to 31 December 2001
Lund et al. (2012) ¹²	Demark, the Faroe Islands and Greenland 2002 to 2011	Yes	3 true positives	1:168,016	If false negatives and cases in unscreened children were included, the incidence was 1:195,659
Couce et al. (2011) ¹⁵	Galicia, Spain 2000 and 2010	Yes	1	1:210,165	
Lindner et al., (2011) ¹¹	South-West Germany January 1999 and April 2005	Yes	1	1:583,553	
Kasper et al. (2010) ¹⁶	Austria 2002 to 2010	Yes	2	1:311,245	
Mercmek-Mahmutoglu et al. (2010) ²¹	Austria 1973 to 2000	Yes	24 (23 identified by screening)	1:95,600 (identified by screening) 1:91,667 if symptomatic infant included	One additional infant was diagnosed on a symptomatic basis.
Ficioglu et al. (2009) ⁴	Massachusetts, US 1969 to 1978	Yes	13	Approximately 1:49,000	One infant was diagnosed shortly after birth due to an older sibling previously identified by screening

Study	Country and time period	Screening programme in operation?	Number of identified cases	Incidence	Notes
Wilcken et al. (2009) ¹³	Australia Born between 1994 and 2002	No	6	1:258,534	
Wilcken et al. (2009) ¹³	Australia Born between 1998 and 2002	Yes	2	1:230,750	
Keskinen et al. (2008) ⁹	Finland January 1968 to March 2007		20	1:144,000	“Finland is a country with a small and genetically isolated population, and the prevalence of many genetic diseases differ markedly from those in other countries.” Incidence estimated by dividing the number of diagnose by the number of live births
la Marca et al. (2008) ¹⁷	Tuscany, Italy January 2002 to end of 2007	Yes	0	NA	160,000 infants screened
Auray-Blais et al. (2007) ¹⁸	Quebec, Canada 1973 and 2006	Yes	17	1:145,290	
Feuchtbaum et al. (2006) ¹⁹	California, US Jan 2002 to June 2003	Yes	0	NA	353,894 infants screened
Frazier et al. (2006) ¹⁴	North Carolina, US July 1997 to July 2005	Yes	1	1:940,000	
Comeau et al. (2004) ²⁰	New England Screening Program, US January 1999 to February 2003	Yes	0	NA	318,535 infants were screened

Detectable risk factor or disease marker

Citrullinaemia results from the deficiency of argininosuccinate synthase, which condenses citrulline with aspartate to form argininosuccinic acid. Deficiency in argininosuccinate synthase results in elevated citrulline concentration which can be detected in blood and urine. Citrulline levels in dried blood spots can be analysed by MS/MS in newborn screening programmes (see Criterion 5). However, other conditions, including argininosuccinate lyase deficiency, pyruvate carboxylase deficiency and citrin deficiency (also known as citrullinaemia type II) also cause elevations in citrulline levels, and further testing is required to distinguish between these conditions.

Argininosuccinate lyase deficiency results from the deficiency of argininosuccinate lyase. This enzyme catalyses the cleavage of argininosuccinic acid to produce arginine and fumarate. Deficiency in this enzyme results in elevated levels of argininosuccinic acid and citrulline, which can be detected in blood and urine. The levels of both compounds in dried blood spots can be analysed by MS/MS in newborn screening programmes (see Criterion 5). Argininosuccinic acid has the advantage of being a specific marker for argininosuccinate lyase deficiency.

Latent period or early symptomatic stage

As mentioned previously, both conditions have an early onset form and a late onset form.

There is debate over whether the early onset forms of these conditions have enough of a latent period/early symptomatic stage to allow for screening. Although infants with the early onset form of these diseases appear normal at birth, symptoms can develop rapidly. A number of screening studies have reported that patients were symptomatic before the screening results were available.

Lindner et al. (2011) reported on the efficacy and outcome of a pilot expanded newborn screening programme for metabolic diseases in South-West Germany (1999-2005).¹¹ Four cases of the 'classic' form of citrullinaemia type I were identified and six cases of mild citrullinaemia. Of the four cases of 'classic' citrullinaemia, three presented clinically before the results of newborn screening were available. These three cases experienced a metabolic decompensation before newborn screening was performed (see Table 9). The early onset of the condition raises the question of how early a blood sample would have to be taken for a result to be available in order to prevent outcomes such as metabolic decompensation. In this study one case of argininosuccinate lyase deficiency was identified, due to known family risk. This child was identified before the onset of symptoms.

Table 9: Patients with 'classic' citrullinaemia type I (CIT I) who developed symptoms before blood sampling or the screening result was available during a pilot expanded newborn screening programme in South-west Germany. From Lindner et al. (2011)¹¹

Diagnosis	Age at decompensation (days)	Age at blood sampling for screening (days)	Age at first screening result (days)	Age at confirmed diagnosis (days)	Age at the start of treatment (days)
CIT I classic	1.0	3.0	12.0	2.0	2.0
CIT I classic	1.0	3.0	10.0	5.0	Not available
CIT I classic	2.0	2.7	8.6	2.0	2.0

Frazier et al. (2006) described the MS/MS screening programme in North Carolina between 1997-2005.¹⁴ During that period, five infants with citrullinaemia (argininosuccinate synthase deficiency) were identified. Two patients died in the first days of life from overwhelming metabolic decompensation and/or prematurity. The screening results were only available after death. One case of argininosuccinate lyase deficiency was identified. This patient was alive when the screening result was available.

Table 10: Patients who died before screening results were available due to metabolic decompensation/the effects of prematurity diagnosed with citrullinaemia (argininosuccinate synthase deficiency). From Frazier et al. (2006)¹⁴

Diagnosis	Time of sample collection	Day screening report available	Day of death
Citrullinaemia	2 days	16	7
Citrullinaemia	2 days	16	7

Lund et al. (2012) presented the results of expanded newborn screening in Denmark, the Faroe Islands and Greenland between 2002 and 2011.¹² During the trial period (2002-2009), the median age at screening was five days, and results from the screening lab were available between two and seven days later. Screening for citrullinaemia was again found to be problematic. During the trial period, one true positive, 11 false positives, and one false negative cases of citrullinaemia were identified. The true positive case was diagnosed prenatally due to an affected sister, and was treated from birth. MS/MS analysis of the dried blood spot was abnormal, but the authors report that the results would have probably arrived too late to start treatment before onset of symptoms. The false negative case presented before the results of screening were available. An additional infant with citrullinaemia died before a blood sample could be taken. Screening for citrullinaemia was stopped in 2009, at the end of the trial period, because results were available too late to be useful in preventing disease and due to the high false positive rates. Three cases of argininosuccinate lyase deficiency were identified in this study. None of the patients presented clinically before screening results were available.

Couce et al. (2011) reported on the results of newborn screening in Galicia, Spain between 2000 and 2010.¹⁵ In Galicia, blood and urine samples are collected and tested for all newborns. Samples of blood and urine were collected between the 5th and 8th day of life between 2000 and 2002. In 2003, this changed to the 3rd day of life. One case of citrullinaemia was identified, although screening results were only available after the onset of clinical symptoms. One case argininosuccinate lyase deficiency was identified; this patient was asymptomatic at the time of diagnosis.

In addition, Mercmek-Mahmutoglu et al. (2010) reported on outcomes of infants with argininosuccinate lyase deficiency identified during a 27-year period (between 1973 and 2000) in Austria, during which newborn screening for argininosuccinate lyase deficiency was performed using an enzyme-auxotroph test on dried blood spots collected between day three and five of life.²¹ The age at diagnosis of identified cases was 1.8 ± 1.1 months. Patients identified by screening were asymptomatic until four weeks of life or later when diagnosis and treatment was initiated, and therefore all of the cases identified had late onset argininosuccinate lyase deficiency with presumable onset beyond the newborn period. One additional patient was diagnosed on a symptomatic basis with hyperammonaemic coma on day 4 of life. The screening protocol did not allow for the detection of early onset cases before symptomatic presentation.

Summary:

Citrullinaemia: Criterion 2 partly met. No studies were identified that reported the incidence or prevalence of citrullinaemia in the UK. The natural history of the condition is well established. Citrullinaemia can be detected through MS/MS analysis of newborn dried blood spots. The concentration of citrulline can be used to screen for citrullinaemia, however, other conditions, including argininosuccinate lyase deficiency, pyruvate carboxylase deficiency and citrin deficiency (also known as citrullinaemia type II) also cause elevations in citrulline levels, and further testing is required to distinguish between these conditions. Several studies were identified that reported that infants can develop symptoms before the results of a newborn blood spot screen are available. However, there still could be value in the early diagnosis and treatment of patients with more mild forms of the condition.

Argininosuccinate lyase deficiency: Criterion 2 partly met. No studies were identified that reported the incidence or prevalence of argininosuccinate lyase deficiency in the UK. The natural history of the condition is well established, although no correlation between clinical presentation and genotype or residual enzyme activity has been established. Argininosuccinate lyase deficiency can be detected through MS/MS analysis of newborn dried blood spots. The concentrations of argininosuccinic acid and citrulline can be used to screen for argininosuccinate lyase deficiency. Argininosuccinic acid has the advantage of being a specific marker for argininosuccinate lyase deficiency. The duration of the latent asymptomatic period varies between patients in this disorder as well, and symptoms can develop within the first days of life. Although many of the infants identified with argininosuccinate lyase deficiency in newborn screening programs were asymptomatic at the time of diagnosis, one study was identified that reported that an infant developed symptoms (on day four of life) before the results of a newborn blood spot screen were available. However, there still could be value in the early diagnosis and treatment of patients with more mild forms of the condition.

3. All the cost-effective primary prevention interventions should have been implemented as far as practicable

Criterion 3 not applicable. Both conditions are genetic diseases.

4. If the carriers of a mutation are identified as a result of screening the natural history of people with this status should be understood, including the psychological implications.

This report will focus on the use of tandem mass spectrometry (MS/MS) to quantify levels of markers in punches from dried blood spots.

The screening test identifies individuals with abnormal levels of markers in the blood, rather than screening for the presence of a mutation. No reports of the identification of individuals heterozygous for mutations (carriers) through MS/MS screening were identified.

If a child is diagnosed with either of the disorders, it would mean that the parents are obligate carriers. This would also be the case if infants with these conditions are identified due to

presentation with symptoms, although more infants with these conditions may be diagnosed if newborn screening is implemented (i.e. fewer infants may remain undiagnosed).

Criterion 4 not applicable. The screening test does not identify carriers of a mutation.

5. There should be a simple, safe, precise and validated screening test

This report will focus on the use of tandem mass spectrometry (MS/MS) to quantify levels of markers in punches from dried blood spots. MS/MS was the only technology considered in the 2004 HTA of the clinical and cost-effectiveness of neonatal screening for inborn errors of metabolism, and has been introduced by several newborn-screening programmes around the world for the detection of urea cycle disorders.⁵

Since 2009 all UK laboratories have used tandem mass spectrometry as the screening technology for screening for phenylketonuria.²² Multiple analytes can be simultaneously assayed, allowing the detection of a range of metabolic disorders using the same dried blood-spot sample collected as part of the current screening programme.

The 2004 HTA report concluded that the “evidence regarding the sensitivity and specificity of neonatal screening for urea cycle disorders using tandem MS is limited.”⁵

Since the publication of the HTA report the results of several expanded screening programmes which have used MS/MS on a dried blood spot to screen for citrullinaemia and/or argininosuccinate lyase deficiency have been published.

Reports of screening programmes for multiple disorders

The results of several expanded newborn screening programmes using MS/MS have been published since 2004. Unfortunately, most of the reports do not present enough detail for the sensitivity and the specificity of MS/MS screening for individual disorders to be calculated. In addition, many screening programmes have used the same analyte as a marker for multiple disorders. For example, elevated citrulline has been used for a marker for both citrullinaemia and argininosuccinate lyase deficiency. In this instance, even if the number of cases initially screened positive due to elevated citrulline is known, it is difficult to know how to ascribe these false positives to screening for citrullinaemia or screening for argininosuccinate lyase deficiency.

The results of the screening studies identified are summarised below and in Table 12 and Table 13. Where possible, if insufficient data was presented to calculate the sensitivity and specificity of screening for particular conditions, the sensitivity and specificity of the screening programme as a whole has been calculated.

Lund et al. (2012) described the results of expanded newborn screening in Denmark, the Faroe Islands and Greenland (2002 to March 31st 2011).¹² Flagged samples were re-analysed in duplicate. If abnormal profiles were reproduced, The Centre for Inherited Metabolic Disorders, Copenhagen University Hospital, was immediately contacted. A specialist in metabolic disorders subsequently contacted the child's local paediatric department, which then contacted the families and initiated confirmatory testing of the child. Between 2002 and 2009, the trial period, citrullinaemia was screened for using levels of citrulline as a marker and argininosuccinate lyase deficiency was screened for using levels of argininosuccinic acid as a marker. Screening for

citrullinaemia was stopped at the end of the trial period, but screening for argininosuccinate lyase deficiency was continued. The results of screening are shown in Table 11. Whilst citrullinaemia was being screened for there were one true positive, 11 false positives, and one false negative, and one infant with citrullinaemia who died before screening could take place. Citrullinaemia was removed from the screening panel in 2009 because the screening results were available too late to be useful in preventing disease and due to the high false positive rates. Screening for argininosuccinate lyase deficiency identified three true positives, and no false positives or false negatives.

Table 11: Screening results, and sensitivity and specificity of MS/MS screening for citrullinaemia and argininosuccinate lyase deficiency using results from Lund et al. (2012)¹²

Disorder	Number screened	True positives	False Positives	False Negatives	True negatives	Sensitivity	Specificity
Citrullinaemia	363,538	1	11	1	363,525	50%	99.997%
Argininosuccinate lyase deficiency	504,049	3	0	0	504,046	100%	100%

Couce et al. (2011) reported on newborn screening in Galicia, Spain between 2000 and 2010.¹⁵ During this period, 210,165 infants were screened and 137 cases of inborn errors of metabolism were identified. There were 43 false positives and four false negative results. Therefore the screening programme as a whole had a sensitivity of 97.16%, a specificity of 99.98% and a positive predictive value 76.11%. Citrulline was responsible for the most false positive results- 14 false positives among the 16 cases of elevation (the other cases of elevation were due to one true positive case of citrullinaemia and one true positive case of argininosuccinate lyase deficiency).

Lindner et al. (2011) reported on expanded newborn screening for metabolic diseases in South-West Germany.¹¹ Citrullinaemia and argininosuccinate lyase deficiency were initially included in the screening panel, but these conditions were not part of the legal screening panel in Germany implemented from April 2005 onwards. The authors report that for the programme overall (until June 2009, 1,084,195 children screened) confirmatory testing was recommended for 377 cases and in 373 a metabolic disorder was confirmed. In addition they report that there have been no false-negative cases of any disorder reported. If it is assumed that confirmatory testing was recommended in all screen positives, we can calculate that the programme overall had a sensitivity of 100% and a specificity of 99.9996%.

Kasper et al. (2010) described the MS/MS screening programme in Austria between April 2002 and December 2009.¹⁶ During this period, 622,489 infants were screened. In the Austrian programme, if a dried blood spot screened positive, another disk from the same dried blood spot was punched. If the result indicated that an infant was at risk of acute metabolic decompensation the infant was immediately recalled for confirmatory/diagnostic testing, otherwise a repeat dried blood spot was obtained prior to confirmatory/diagnostic testing. The results for the screening programme of a whole are reported. 1,728 newborns had positive result on initial screening, and 218 were diagnosed with an inborn error of metabolism. A total of four infants with false negative results were reported (two cases of long-chain 3 – hydroxyacyl-CoA dehydrogenase deficiency and two cases of methylmalonic academia). The overall sensitivity was 98.20% and specificity was 99.76%. The positive predictive value of the screening programme was 12.62%.

Wilcken et al. (2009) described the results of screening in Australia between 1998 and 2002, during which period 461,000 infants were screened.¹³ The programme had a false positive rate of 0.18%, and seven cases were missed by screening (false-negatives). The number of false positives per condition was not reported.

la Marca et al. (2008) described the six year experience of screening (January 2002 to October 2004 pilot, all infants since November 2004) for more than 40 inborn errors of metabolism in Tuscany.¹⁷ Infants who screen positive for disorders with possible acute metabolic decompensation are immediately recalled and clinical examinations and confirmatory tests are performed. Infants who screen positive for other disorders provide a second bloodspot. If this also screens positive clinical examinations and confirmatory tests are performed. Not enough details were provided to calculate the sensitivity or specificity of the test.

Feuchtbaum et al. (2006) described a pilot MS/MS screening programme in California between January 2002 and June 2003.¹⁹ During the pilot, 353,894 infants were screened. No cases of citrullinaemia or argininosuccinate lyase deficiency were identified. For the whole programme, 701 results were flagged, and 461 were classified as screen positive and were referred. Of these, 51 were diagnosed with a disorder. Three cases of MS/MS detectable diseases were missed. For the screening programme as a whole, the sensitivity was 94.4% and the specificity was 99.9%.

Frazier et al. (2006) described the MS/MS screening programme in North Carolina between 1997 and 2005.¹⁴ For most analytes, both a 'border line' and 'diagnostic' cut-off were established. If the screening result was above the borderline cut-off, another disc was punched from the same sample card and the analysis was repeated before a report was generated. If the screening result was above the diagnostic cut-off, a metabolic specialist was contacted who immediately contacted the infant's primary care provider with results and recommendations. Results shown in Table 12 and Table 13 are from 2003 to 2004 only, the year that the cut-offs were implemented. During this period 239,415 newborns were screened. For the programme as a whole, 27 infants had repeat samples above the borderline cut-off and were screen positive, and 82 infants had levels above the diagnostic cut-off and were screen positive. There were a total of 58 confirmed diagnoses. The positive predictive value for all disorders screened for was 53%.

Comeau et al. (2004) reported results from the New England Screening Programme between January 1999 and February 2003.²⁰ Data was presented for 19 metabolic disorders together, including citrullinaemia and argininosuccinate lyase deficiency. Considering the panel of 19 metabolic conditions as a whole, 425 infants screened positive; 121 were referred to a specialist, and 28 infants were diagnosed with a condition (318,535 infants screened). Therefore there were 15 screen positives per case and four specialist referrals per case.

The timing of the test

The timing of blood spot sampling is a consideration. The levels of markers may vary physiologically, and the optimal time period for sampling may vary between conditions. Where reported, the age at which blood spots were taken was reported in Table 12 and Table 13. An additional consideration is the need for screening results before the onset of symptoms. As discussed in Criterion 2, several studies reported that patients were symptomatic or died before screening results were available.

In the UK, the blood spot sample is taken on day five of life (in exceptional circumstances between day five and day eight) for all babies regardless of medical condition, milk feeding and

prematurity. Premature infants are retested at 28 days of age.²³ The time of blood spot collection may need to be earlier to screen for citrullinaemia and argininosuccinate lyase deficiency.

The sensitivity and specificity of the test in premature infants

No studies were identified that analysed the sensitivity and specificity of the test in premature infants. Several screening programmes reported that additional samples were taken from premature infants later in life (see Table 12 and Table 13). In addition, we identified one study that looked at the normal ranges of analytes in premature and acutely ill infants (Oladipo et al. [2011]²⁴). This study found that citrulline concentrations were not significantly elevated in premature or acutely ill infants. Variation in argininosuccinic acid was not explored, but levels of this metabolite should be low in all normal infants (see Criterion 6).

Summary:

Criterion 5 not met for citrullinaemia. Several studies reported high false positive rates when screening for citrullinaemia using citrulline levels as a marker, or the arrival of screening results after symptomatic presentation.

Criterion 5 met for argininosuccinate lyase deficiency. When argininosuccinic acid was used as a marker for argininosuccinate lyase deficiency, a study reported 100% sensitivity and 100% specificity. Argininosuccinic acid levels should be low in all infants without argininosuccinate lyase deficiency, including premature infants.

Table 12: Screening for citrullinaemia. NR, not reported.

Study	Country, period	Age at which sample collected	Analyte	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
Lund et al. (2012) ¹²	Denmark, the Faroe Islands and Greenland 2002 to March 31st 2009	5 days (median)	Elevated citrulline Cut-off: >115µM Elevated citrulline/arginine ratio (>6.0)	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters (derivatized)	363,538	1	11	1	All preterm newborns had their expanded biochemical screening test repeated at gestational age 32 weeks or when oral feeding had been established. One infant with citrullinaemia died before screening could take place
Couce et al. (2011) ¹⁵	Galicia, Spain 2000 to 2010	Between the 5 and 8 days of life (between 2000 and 2002) 3 days of life (2003)	Elevated citrulline (blood) Cut-off: NR Orotic acid (Urine) Cut-off: NR	NR	210,165	1	Uncertain. 14 cases had citrulline levels above the cut off and were not diagnosed with a disorder, but citrulline levels were also used to screen for argininosuccinate	0	In Galicia, blood and urine samples are collected and tested for all newborns.

Study	Country, period	Age at which sample collected	Analyte	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
		onwards)					lyase deficiency (in addition, 1 case of argininosuccinate lyase deficiency diagnosed)		
Lindner et al. (2011) ¹¹	South-West Germany January 1999 to April 2005	Between 3 and 5 days of life (before 2002) and between 36 and 72 hours of life thereafter	Details given in Schulze et al. (2003) ²⁵ : Citrulline Cut-off: >65µmol/L Ornithine/Citrulline ratio <1.5 Citrulline/Arginine ratio >15	Details given in Schulze et al. (2003) ²⁵ : Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters (derivatized)	583,553	7 (6 mild cases and 1 classic case)	NR	0	3 cases of classic citrullinaemia presented clinically before a screening sample was taken or before the screening results were available.
Kasper et al. (2010) ¹⁶	Austria April 2002 to December 2009	Between 36 and 72h of life.	Elevated citrulline Cut-off: NR Decreased ornithine/ citrulline ratio Cut-off: NR	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl	622,489	8	NR	0	A second screening sample was obtained and assayed after 14 days of life from all infants born prior to 32 weeks

Study	Country, period	Age at which sample collected	Analyte	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
				esters (derivatized)					gestational age
Wilcken et al. (2009) ¹³	Australia 1998 and 2002	Between 48 to 72 hours of age	NR	NR	461,500	0	NR	0	
la Marca et al. (2008) ¹⁷	Tuscany, Italy 2002 to 2008	Between 48 and 72 hours of life	Elevated Citrulline Cut-off: >25µmol/L	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters. From 2007, samples extracted with hydrazine hydrate in water and methanol and then converted to their butyl esters	160,000	2	NR	NR	For premature infants, a sample is collected between 48 hours and 72 hours of life, then 2 additional samples at 15 and 30 days. For babies on parenteral nutrition, including premature babies, a second sample at 48 hours after the ending of parenteral nutrition is collected. In all transfused newborns, a new

Study	Country, period	Age at which sample collected	Analyte	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
				(derivatized)					sample is collected seven days after the end of transfusion
Feuchtbaum et al. (2006) ¹⁹	California, US January 2002 to June 2003	NR	NR	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters (derivatized)	353,894	0	NR	0	
Frazier et al. (2006) ¹⁴	North Carolina, US 2003 to 2004	39 hours	Elevated citrulline Cut-off: Borderline: >76µmol/L; Diagnostic: >150µmol/L	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters (derivatized)	239,415	1	Uncertain. Three patients had citrulline levels above the 'diagnostic' cut off for citrulline and were not diagnosed with a disorder, but citrulline levels were also used to screen for argininosuccinate	0	Cut-offs used from January 2003

Study	Country, period	Age at which sample collected	Analyte	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
							lyase deficiency		
Comeau et al. (2004) ²⁰	New England, US January 1999 to February 2003	NR	NR	NR	318,535	1	NR	NR	

Table 13: Screening for argininosuccinate lyase deficiency. NR, not reported.

Study	Country, period	Age at which sample collected	Analyte	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
Lund et al. (2012) ¹²	Denmark, the Faroe Islands and Greenland 2002 to March 31st 2011	5 days (median, between 2002 and 2009) 2.5 days (median, February 2009 to March 2011)	Argininosuccinic acid Cut-off: >7.6U (at start of programme, derivatized extracts) >1U (at end of reported period, extracts underivatized)	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters (derivatized) (2002 to 2009). From February 2009 PerkinElmer NeoBase non-derivatized MS/MS kit	504,049	3	0	0	All preterm newborns had their expanded biochemical screening test repeated at gestational age 32 weeks or when oral feeding had been established.
Couce et al. (2011) ¹⁵	Galicia, Spain 2000 to 2010	Between the 5 and 8 days of life (between 2000 and 2002)	Elevated citrulline (blood) Cut-off: NR Argininosuccinic acid (urine) Cut-off: NR	NR	210,165	1	Uncertain. 14 cases had citrulline levels above the cut off and were not diagnosed with a disorder, but citrulline levels	0	In Galicia, blood and urine samples are collected and tested for all newborns

Study	Country, period	Age at which sample collected	Analyte	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
		3 days of life (2003 onwards)					were also used to screen for citrullinaemia (in addition 1 case of citrullinaemia diagnosed)		
Lindner et al. (2011) ¹¹	South-West Germany January 1999 to April 2005	Between 3 and 5 days of life (before 2002) and between 36 and 72 hours of life thereafter	Details given in Schulze et al. (2003) ²⁵ : Argininosuccinic acid Cut-off: >1µmol/L	Details given in Schulze et al. (2003) ²⁵ : Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters (derivatized)	583,553	1	NR	0	
Kasper et al. (2010) ¹⁶	Austria April 2002 to December 2009	Between 36 and 72h of life.	Elevated citrulline Cut-off: NR Decreased ornithine/ citrulline ratio Cut-off: NR	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters (derivatized)	622,489	2	NR	0	A second screening sample was obtained and assayed after 14 days of life from all infants born prior to 32

Study	Country, period	Age at which sample collected	Analyte	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
									weeks gestational age
Wilcken et al. (2009) ¹³	Australia 1998 and 2002	Between 48 to 72 hours of age	NR	NR	461,500	2	NR	0	
la Marca et al. (2008) ¹⁷	Tuscany, Italy 2002 to 2008	Between 48 and 72 hours of life	Argininosuccinic acid Cut-off: >1µmol/L (Citrulline a secondary marker, with a cut off >25µmol/L)	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters. From 2007, samples extracted with hydrazine hydrate in water and methanol and then converted to their butyl esters (derivatized)	160,000	0	NR	NR	For premature infants, a sample is collected between 48 hours and 72 hours of life, then 2 additional samples at 15 and 30 days. For babies on parenteral nutrition, including premature babies, a second sample at 48 hours after the

Study	Country, period	Age at which sample collected	Analyte	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
									ending of parenteral nutrition is collected. In all transfused newborns, a new sample is collected seven days after the end of transfusion
Feuchtbaum et al. (2006) ¹⁹	California, US January 2002 to June 2003	NR	NR	Amino acids and acylcarnitines extracted with methanol and then converted to their butyl esters (derivatized)	353,894	0	NR	0	
Frazier et al. (2006) ¹⁴	North Carolina, US 2003 to 2004	39 hours	Elevated citrulline Cut-off: Borderline: >76µmol/L;	Amino acids and acylcarnitines extracted with methanol and then	239,415	0	Uncertain. Four patients had citrulline levels above the 'diagnostic' cut off for citrulline, but	0	Cut-offs used from January 2003

Study	Country, period	Age at which sample collected	Analyte	Method of extraction	Number screened	Number of true positives	Number of false positives	Number of false negatives	Notes
			Diagnostic: >150µmol/L	converted to their butyl esters (derivatized)			no patient was diagnosed with argininosuccinate lyase deficiency. One patient was diagnosed with citrullinaemia-citrulline levels were also used to screen for citrullinaemia		
Comeau et al. (2004) ²⁰	New England, US January 1999 to February 2003	NR	NR	NR	318,535	0	NR	NR	

6. The distribution of test values in the target population should be known and a suitable cut-off level defined and agreed

A US Region 4 project, a Regional Genetics and Newborn Screening Collaborative, aimed to achieve uniformity of testing panels by MS/MS, improve overall analytical performance and set and sustain the lowest achievable rates of false positive and false negative results. It initially included seven US states, but has expanded into an international collaboration, including 80 programmes in 45 countries, in addition to 47 US states and Puerto Rico. The Region 4 Stork has collected data on:

- Five selected percentiles of individual markers and ratios in the normal population,
- Cut-off values used in routine screening practice
- The complete set of all available amino acid and acylcarnitine results in true positive cases (defined as cases meeting the case definition established by local protocols and/or professional guidelines)
- Performance metrics (detection rate, false positive rate, and positive predictive values)
- Other information relating to newborn screening, including source of reagents, use of derivatization, date of collection and punch size.

The collaboration recently published a paper which aimed to clinically validate cut-off values for newborn screening using tandem mass spectrometry.²⁶

From the data submitted, amino acid cumulative percentiles in normal neonatal dried blood spots were calculated. The ranges for citrulline and argininosuccinic acid are shown in Table 14. The authors note that similar values for normal ranges were seen for most markers between sites, with the exception of argininosuccinic acid and succinylacetone. The levels of argininosuccinic acid were significantly different between sites, with approximately half of participants reporting normal values at much higher levels than that seen in plasma, where argininosuccinic acid is normally undetectable. However, the authors state that argininosuccinic acid is essential for the reliable detection of argininosuccinic acidaemia (argininosuccinate lyase deficiency). As noted previously, elevated citrulline concentrations can be markers for several conditions.

Table 14: Cumulative percentiles of citrulline and argininosuccinic acid in the normal population, as submitted by participants of the Region 4 Stork Collaborative project (as of December 1, 2010). CV coefficient of variation (calculated from values within the interquartile range: median \pm [(75 percentile – 25 percentile) \times 1.5]), n= number of participants that submitted percentile values. Modified from McHugh et al. (2011)²⁶

			Normal population ($\mu\text{mol/L}$)					
			1 percentile		50 percentile		99 percentile	
Marker	Condition(s) marker screens for	N	Value	CV	Value	CV	Value	CV
Argininosuccinic acid	Argininosuccinate lyase deficiency	26	0.04	140%	0.19	98%	0.66	94%
Citrulline	Citrullinaemia type I Citrin deficiency Argininosuccinate lyase deficiency Pyruvate carboxylase deficiency Maternal citrullinaemia	93	6.0	28%	12	24%	28	22%

Amino acid ranges in true positive cases identified by the participants of the Region 4 Stork Project were also reported (Table 15).

Table 15: Marker ranges in neonatal dried blood spots analysed by tandem mass spectrometry in affected newborn cases, from participants of the Region 4 Stork Collaborative Project (as of December 1, 2010). Modified from McHugh et al. (2011)²⁶

			Percentiles of disorder ranges ($\mu\text{mol/L}$)							
Condition	Marker	N	1%	5%	10%	25%	50%	75%	90%	99%
Argininosuccinate lyase deficiency	Argininosuccinic acid	51	0.12	0.25	0.42	1.5	3.8	12	61	110
Argininosuccinate lyase deficiency	Citrulline	108	22	32	40	65	95	141	177	342
Citrullinaemia	Citrulline	215	54	72	102	172	366	745	1107	2213

Using this data, the authors calculated clinically relevant cut-off ranges. For citrulline and argininosuccinic acid, screens above the cut-off would be considered positive (Table 16). The cut-off ranges were defined as the interval between the cumulative 99th percentile of the normal population and the lowest 5th percentile of all disorder ranges of the same marker (if the analyte is informative for multiple conditions). One or both limits of the target range were adjusted depending on the degree of overlap between the normal population and the disorder

range, to balance sensitivity and specificity. The 5th percentile in the cumulative disorder range was 'over-ridden' for argininosuccinic acid and citrulline to increase specificity and reduce the occurrence of false positive results.

Table 16: Cut-offs for citrulline and argininosuccinic acid suggested by the Region 4 Stork Collaborative Project.²⁶ The cut-off ranges were defined as the interval between the cumulative 99th percentile of the normal population (lower end of range, 'low' in the table) and the lowest 5th percentile of all disorder ranges of the same marker (if the analyte is informative for multiple conditions, 'high' in the table). One or both limits of the target range were adjusted depending on the degree of overlap between the normal population and the disorder range, to balance sensitivity and specificity. The 5th percentile in the cumulative disorder range (the high end of the cut-off range) was 'over-ridden' for argininosuccinic acid and citrulline to increase specificity and reduce the occurrence of false positive results.

Marker	Number of cases	Number of conditions	Cut-off range (µmol/L)	
			Low	High
Argininosuccinic acid	51	1 (Argininosuccinate lyase deficiency)	0.66	0.90
Citrulline	385	5 (Citrullinaemia type I, citrin deficiency, argininosuccinate lyase deficiency, pyruvate carboxylase deficiency, maternal citrullinaemia)	28	40

The ratio between amino acids may also be used to screen for conditions. For example, in this publication, the authors also report normal values, values in affected cases, and potential cut-offs for argininosuccinic acid/arginine (to screen for argininosuccinate lyase deficiency), citrulline/arginine (to screen for citrullinaemia and argininosuccinate lyase deficiency among other conditions), citrulline/phenylalanine (to screen for citrullinaemia and argininosuccinate lyase deficiency among other conditions), glutamine/citrulline (N.B. levels below cut-off used to screen for citrullinaemia) and glutamic acid/citrulline (N.B. levels below cut-off used to screen for citrullinaemia).²⁶

Recently, this collaboration has reported the development of multivariate pattern-recognition software designed to convert metabolic profiles into a composite score driven by the degree of overlap between the normal population and the disease range.²⁷ The software has been developed using the MS/MS profiles of 12,077 patients affected with 60 metabolic disorders and 644 heterozygotes carriers for 12 conditions. The authors report that, as of 15 December 2011, a total of 90 active tools were available, 37 of which were applicable to the differential diagnosis of two or more conditions. The authors report that an "all conditions" tool, designed to evaluate a full amino acid and acylcarnitine profile to suggest any possible diagnosis is soon to be released. The tools are intended to generate a score that drives the interpretation and resolution of cases with potentially abnormal MS/MS results.²⁷

Cut offs from individual screening studies

Individual screening studies have also reported cut-offs (see Table 17 and Table 18). As can be seen, the marker analysed and the cut-offs used vary. This may be linked to the age at which specimens are collected and the protocol used to extract markers from the dried blood spot. The differences in cut-offs and markers used may explain some of the variation in the number of false-positives and false-negatives identified by different screening programmes (see Criterion 5).

Table 17: Markers and cut-offs used to screen for citrullinaemia

Study	Primary marker	Cut-off	Secondary marker	Cut-off	Notes
Lund et al. (2012) ¹²	Citrulline	>115µM	Citrulline/ arginine ratio	>6.0	Cut-offs at start of pilot programme in 2002 (derivatized)
la Marca et al. (2008) ¹⁷	Citrulline	>25µmol/L			
Frazier et al. (2006) ¹⁴	Citrulline	Borderline: >76µmol/L; Diagnostic: >150µmol/L			Cut-offs used from January 2003

Table 18: Markers and cut-offs used to screen for argininosuccinate lyase deficiency

Study	Primary marker	Cut-off	Secondary marker	Cut-off	Notes
Lund et al. (2012) ¹²	Argininosuccinic acid	>7.6U	Argininosuccinic acid/ arginine ratio or Citrulline	>0.58 > 115µM	Cut-offs at start of pilot programme in 2002 (derivatized)
Lund et al. (2012) ¹²	Argininosuccinic acid	>1U	Argininosuccinic acid/ arginine ratio or Citrulline	>0.2 >160µM	Cut-offs at end of reported period (March 31 st , 2011) (underivatized)
la Marca et al. (2008) ¹⁷	Argininosuccinic acid	>1µmol/L	Citrulline	>25µmol/L	
Frazier et al. (2006) ¹⁴	Citrulline	Borderline: >76µmol/L; Diagnostic: >150µmol/L			Cut-offs used from January 2003

Cut-offs in premature infants

As mentioned in Criterion 5, Oladipo et al. (2011) tried to define the range of amino acid concentrations encountered in normal, premature and acutely ill infants in the US by measuring the concentrations of 25 amino acids in residual plasma samples by MS/MS.²⁴

Argininosuccinate, which is not normally detectable in neonatal specimens, was not part of the analysis. Although this study was done on plasma samples, the authors say that citrulline is equally distributed between erythrocyte and plasma water, so that the results should hold for newborn screening. Samples were from full term infants (>38 weeks) with uncomplicated nursery stays and discharge in less than 72 hours (n=206); premature babies born between 32 and 37 weeks gestation that remained in nursery for fewer than 10 days and were discharged home without major health concerns (n=50); and acutely ill infants in neonatal intensive care (regardless of gestational age, n=98), diagnoses included sepsis, respiratory distress, cardiac malformation/malfunction, and gastrointestinal disorders. The distributions of 13 amino acids were significantly different in premature infants when compared to healthy, full-term infants. The distribution of 16 amino acids was significantly different in the acutely ill population when compared to healthy, full-term infants. The median concentration and 2.5 to 97.5 percentile distributions of citrulline in healthy, premature and acutely ill infants are shown in Table 19. Citrulline concentrations were not significantly elevated in premature or acutely ill infants.²⁴

Table 19: Median concentrations and 2.5 to 97.5 percentile distributions of citrulline in plasma from healthy, premature and acutely ill infants less than 10 days of age.²⁴

Amino acid ($\mu\text{mol/L}$)	Healthy (n=206)	Premature (n=50)	Acutely ill (n=98)
Median (2.5 to 97.5 percentiles)			
Citrulline	14 (6 to 40)	14 (1 to 31)	12 (5 to 35)

Summary: Criterion 6 uncertain. Although an international collaboration has suggested cut-offs for both citrulline and argininosuccinic acid, individual studies have used different cut-offs to screen for citrullinaemia and argininosuccinate lyase deficiency. In addition, the cut-offs proposed by the international collaboration were based on screening for a panel of disorders, which is an issue as citrulline is elevated in a number of conditions. Levels of argininosuccinic acid, a marker specific for argininosuccinate lyase deficiency, were found to vary between sites.

The distribution of markers in a UK population after specimen collection at day five of life is unknown.

7. The test should be acceptable to the population

MS/MS is performed on amino acids extracted from dried blood spots on Guthrie cards, which are already collected as part of the newborn screening programme.

Summary: Criterion 7 met. MS/MS is performed on extracts from the dried blood spot already collected as part of the newborn screening programme.

8. There should be an agreed policy on the further diagnostic investigation of individuals with a positive test result and on the choices available to those individuals

The US National Academy of Clinical Biochemistry (NACB) has produced laboratory medicine practice guidelines for the follow-up testing for metabolic diseases identified by the expanded newborn screening programme, grading the strength of recommendations using criteria adopted from the US Preventative Services Task Force.²⁸ The NACB recommendations for confirmatory testing are shown in Table 20.

Table 20: NACB recommendations.²⁸ Evidence: A-I, the highest level of evidence, the NACB strongly recommends adoption, there is good evidence that it improves important health outcomes and the NACB concludes that benefits substantially outweigh harms. The evidence includes consistent results from well-designed, well-conducted studies in representative populations.

	Screening marker	Follow-up analyses	Follow-up markers	Additional testing	Evidence
Argininosuccinate lyase deficiency	Citrulline	Plasma/urine amino acids	Argininosuccinate, citrulline	No additional testing indicated	A-I
Citrullinaemia Type I (ASA synthase deficiency)	Citrulline	Plasma/urine amino acids	Citrulline, argininosuccinate	Ammonia, bilirubin Alkaline phosphatase, γ -glutamyl transferase	A-I

The New York Mid-Atlantic Consortium for Genetic and Newborn Screening Services (NYMAC, region 2), one of the seven US regional collaboratives, has developed guidelines for the confirmation of diagnoses of conditions in the newborn screening panel- the NYMAC Newborn Screening Diagnostic Guidelines.^{29,30} Extracts from the relevant guidelines (last updated November 30, 2010) are displayed in Table 21 and Table 22.

Table 21: Diagnostic guideline for argininosuccinate lyase deficiency. Extracted from the NYMAC Diagnostic Guidelines for Confirmation of Screen-Positive Newborn Screening Results.³⁰

Argininosuccinate lyase deficiency	
<i>Abnormal newborn screening metabolite(s):</i>	Elevated citrulline In some states elevated argininosuccinic acid
<i>Initial diagnostics at referral centre</i>	Plasma amino acids (PAA) Urine orotic acid
<i>Recommended additional testing to consider at time of initial consultation</i>	Liver function tests Ammonia
<i>Abnormal Metabolites Expected</i>	Elevated citrulline, glutamine, and argininosuccinic acid (PAA) Decreased arginine (PAA) Elevated urine orotic acid Normal liver function tests expected Elevated ammonia expected
<i>If initial testing is negative has the disorder been ruled out?</i>	Yes
<i>Diagnostic Confirmation</i>	Elevated argininosuccinic acid
<i>Differential Diagnosis</i>	Citrullinaemia type I Citrin deficiency (Citrullinaemia type II)

Table 22: Diagnostic guideline for citrullinaemia type I. Extracted from the NYMAC Diagnostic Guidelines for Confirmation of Screen-Positive Newborn Screening Results.³⁰

Citrullinaemia Type I	
Abnormal newborn screening metabolite(s):	Elevated citrulline
Initial diagnostics at referral centre	Plasma amino acids (PAA) Urine orotic acid
Recommended additional testing to consider at time of initial consultation	Liver function tests Ammonia
Abnormal Metabolites Expected	Elevated citrulline (and alanine and glutamine if sick) (PAA) Decreased arginine (PAA) Elevated urine orotic acid Normal liver function tests expected Elevated ammonia expected
If initial testing is negative has the disorder been ruled out?	Yes
Diagnostic Confirmation	Elevated citrulline (and alanine and glutamine if sick) and orotic acid levels Enzyme analysis in fibroblasts Mutation analysis is required to differentiate between mild citrullinaemia type I and citrin deficiency (citrullinaemia type II)
Differential Diagnosis	Argininosuccinate lyase deficiency Citrin deficiency (Citrullinaemia type II)

The American College of Medical Genetics have also produced ACTION sheets and algorithms.³¹

In addition, some screening papers have given the criteria they have used to make a diagnosis. Kasper et al. (2010) report that for amino acid disorders, confirmatory testing included a repeated newborn screen and plasma amino acid analysis in Austria.¹¹ Additional testing is performed for some disorders.¹¹ Frazier et al. (2006) similarly report that a repeat newborn screen and plasma amino acid analysis is performed for suspected amino acid disorders, and ammonia is assayed for argininosuccinate lyase deficiency and citrullinaemia.¹⁴ Diagnostic criteria used in South West Germany (Lindner et al. [2011]¹¹) and the confirmatory tests employed in Denmark, the Faroe Islands and Greenland (Lund et al. [2012]¹²) were also described (see Table 23 and Table 24).

Table 23: Minimal criteria to accept diagnosis as confirmed. South West Germany. Lindner et al. (2011)¹¹

Disorder	Minimal criteria to accept diagnosis as confirmed
Argininosuccinate lyase deficiency	Characteristic amino acid profile in plasma and urine
Citrullinaemia type I (classic)	Characteristic amino acid profile in plasma and ammonia in blood
Citrullinaemia type I (mild)	Characteristic amino acid profile in plasma and ammonia in blood, molecular genetic analysis

Table 24: Confirmatory tests employed in Denmark, the Faroe Islands and Greenland. Lund et al. (2012)¹²

Disorder	Confirmatory tests
Citrullinaemia	Urine Organic Acids Urine Amino Acids Plasma Amino Acids Mutation analysis
Argininosuccinate lyase deficiency	Urine Organic Acids Urine Amino Acids Plasma Amino Acids Mutation analysis

Summary: Criterion 8 met. Example guidance on the diagnostic investigations that that should be performed if citrullinaemia or argininosuccinate lyase deficiency is suspected is available from the US, and this guidance tallies with the confirmatory tests reported in studies of newborn screening.

9. If the test is for mutations the criteria used to select the subset of mutations to be covered by screening, if all possible mutations are not being tested, should be clearly set out

Criterion 9 not applicable.

10. There should be an effective treatment or intervention for patients identified through early detection, with evidence of early treatment leading to better outcomes than late treatment

The aim of treatment is to prevent manifestations of the disease. For argininosuccinate lyase deficiency and citrullinaemia type I treatment aims to prevent hyperammonaemia and/or metabolic decompensations.

The HTA report identified one study that evaluated the effectiveness of dietary interventions for citrullinaemia. The report concluded that “therapeutic interventions for urea cycle disorders, namely ornithine transcarbamylase deficiency and citrullinaemia, included long-term dietary protein restriction with oral administration of sodium phenylbutyrate. This treatment regimen improved the clinical management of patients with ornithine transcarbamylase deficiency and improved survival rates for patients with citrullinaemia. However, mental retardation, growth retardation, risk of hyperammonaemic episodes and the need for lifetime adherence to strict medication and dietary management accompanied survival for patients with citrullinaemia. No additional evidence was available for the effectiveness of treatments for other urea cycle disorders, such as argininosuccinic aciduria, arginase deficiency and hyperornithinaemia.”

Treatment options for argininosuccinate lyase deficiency and citrullinaemia include:^{2,3}

- Diet- restriction of dietary protein intake and consumption of a high calorie diet to induce anabolism and prevent the breakdown of protein
- Arginine base supplementation – promotes the synthesis of citrulline in citrullinaemia and argininosuccinic acid in argininosuccinate lyase deficiency, which serve as waste nitrogen products
- Oral nitrogen scavenging therapy
- Orthotopic liver transplantation

A phase 3 double blind crossover RCT of a new drug to promote waste nitrogen excretion, glycerol phenylbutyrate, compared to sodium phenylbutyrate, was identified in the update search.³² It found that glycerol phenylbutyrate was non-inferior to sodium phenylbutyrate with respect to ammonia control. Glycerol phenylbutyrate and sodium phenylbutyrate both contain phenylbutyric acid, but glycerol phenylbutyrate consists of three molecules of phenylbutyric acid joined to glycerol that is hydrolysed in the small intestine by pancreatic lipases to release phenylbutyric acid, contains no sodium, has minimal taste and no odour.³²

In the RCT, patients received placebo and sodium phenylbutyrate or glycerol phenylbutyrate and placebo for 14 days and then crossed over to receive the alternative treatment. Forty six patients were enrolled, three with citrullinaemia type I. Patients were taking an average of 14.54g/day of sodium phenylbutyrate for an average of approximately 11 years at enrolment. Levels of ammonia and plasma and urine levels of metabolites were monitored at the end of each treatment period. Glycerol phenylbutyrate was non-inferior to sodium phenylbutyrate with respect to ammonia control (mean blood ammonia 976.6µmol.h/L with sodium phenylbutyrate and 865.9µmol.h/L with glycerol phenylbutyrate). The results of this trial were also compared and combined with the results of two short Phase 2 trials (one in paediatric patients and one in adults). Each study found lower blood ammonia levels with glycerol phenylbutyrate, and the difference was significant when the results of all three studies were pooled. Blood glutamine levels were also significantly lower with glycerol phenylbutyrate in the pooled analysis.³²

In this study, the results of a 12 month open label safety trial of glycerol phenylbutyrate for urea cycle disorders were also presented. Seventy seven (51 adult and 26 paediatric) patients were enrolled on the trial, six with citrullinaemia and three with argininosuccinate lyase deficiency. During the 12 month trial, the patients' ammonia levels were similar to mean fasting values seen in the short term studies. During the period of treatment with glycerol phenylbutyrate, patients had fewer hyperammonaemic crises: 15 patients reported 24 hyperammonaemic crises in the 12 months preceding enrolment whilst they were receiving sodium phenylbutyrate, whereas 12 patients experienced 15 crises while being treated with glycerol phenylbutyrate. Neuropsychological tests were also performed. All neuropsychological test results remained stable in adults, as did Wechsler Abbreviated Scale of Intelligence and Child Behaviour Checklist scores in paediatric patients. Among the 22 paediatric patients who completed the neuropsychological testing after 12 months, all Behaviour Rating Inventory of Executive Function domains were significantly improved, suggesting an improvement in executive function.³²

Common adverse events reported in at least 10% of patients during long term treatment included vomiting, upper respiratory tract infection, nausea, nasopharyngitis, diarrhoea, headache, hyperammonaemia, decreased appetite, cough, fatigue, dizziness, and oropharyngeal pain.³²

Despite the fact that traditionally patients with argininosuccinate lyase deficiency have been supplemented with high doses of arginine (400 to 700mg/kg/day) to replenish the arginine pool and to facilitate nitrogen excretion via conversion to argininosuccinate, the results of a recent cross-over randomised controlled trial suggest that low-dose arginine therapy in combination with sodium phenylbutyrate should be used to treat argininosuccinate lyase deficiency.³³ In this trial, 12 patients with argininosuccinate lyase deficiency were randomised to receive low-dose (100mg/kg/day or 2g/m² body surface area if weight >20kg) arginine therapy in combination with sodium phenylbutyrate or high dose (500mg/kg/day or 10g/m² body surface area if weight >20kg) arginine therapy in combination with placebo for one week. After a median period of 54 days, patients crossed over to the other arm of the trial. At the end of the week of each treatment arm liver function tests, plasma amino acids and serum chemistries were assayed. High dose arginine therapy resulted in increased levels of the aminotransferases AST and ALT, which may indicate hepatic cell injury. There were no differences in hepatic synthetic function detected.³³

Early versus late treatment

Ficioglu et al. (2009) and Mercmek-Mahmutoglu et al. (2010) reported long term outcomes for patients diagnosed with argininosuccinate lyase deficiency.^{4,21}

Ficioglu et al. (2009) reported outcomes after 13 to 33 years of follow up of 13 patients with argininosuccinate lyase deficiency who had been detected by newborn screening or due to an affected older sibling between 1969 and 1978 in Massachusetts.⁴ Patients were between four and six weeks old at diagnosis and all patients were asymptomatic when diagnosed. They received treatment with a low protein diet, initiated between 3 and 26 weeks of age. Nine patients also received arginine supplementation. One patient received a trial of sodium benzoate at 11 years of age.

After 13 to 33 years follow up, patients had:

- Normal overall growth

- Eight had normal intellectual and psychomotor development. Five patients had learning disabilities, borderline IQ or mild developmental delay or low normal development with the need for special classes.
- Six had electroencephalography (EEG) abnormalities.
- Three patients experienced seizures of various types
- No liver dysfunction
- No incidences of hyperammonaemic coma during the follow-up period; or acute encephalopathy requiring hospitalization

The authors compared these outcomes to outcomes for a cohort of patients diagnosed with late-onset argininosuccinate lyase deficiency due to symptomatic presentation between 1.5 years and 15 years of age. All patients in this cohort developed mental retardation, generalized seizures, and intermittent ataxia. However, it is difficult to tell if the differences observed are due to early treatment of the screened cohort or due to differences in the severity of the condition.⁴

Mercmek-Mahmutoglu et al. (2010) reported on outcomes of infants with argininosuccinate lyase deficiency identified during a 27-year period (between 1973 and 2000) in Austria, during which newborn screening of argininosuccinate lyase deficiency was performed using an enzyme-auxotroph test on dried blood spots collected at day three to five of life.²¹ Twenty-three patients were diagnosed through newborn screening. The age at diagnosis was 1.8 ± 1.1 months. Patients identified by screening were asymptomatic until four weeks of life or later when diagnosis and treatment was initiated, thus all of the cases identified had late onset argininosuccinate lyase deficiency with presumable onset beyond the newborn period. One additional patient was diagnosed on a symptomatic basis with hyperammonaemic coma on day four of life. The screening protocol did not allow for the detection of early onset cases before symptomatic presentation. Despite treatment, this patient had developmental delay and autistic behaviour and died at the age of 14 years from liver failure.

Patients detected by screening were treated with a low protein diet and arginine supplements. Stable plasma arginine and ammonia levels led both treatments to be stopped in most of patients after the third to fifth year of life. Long-term outcome data were available for 17 patients (median age 13 years).

At follow up:

- IQ values were within or above average in eleven, low average in five and in the mild intellectual disability range in one patient. Five patients had learning disabilities and three patients had behavioural problems
- Patients did not experience metabolic decompensation despite unrestricted protein intake.

Nine patients had an extended follow-up. Of these:

- Four had an abnormal electroencephalography (EEG) without evidence of clinical seizures
- Three patients had abnormal liver function tests and/or evidence of hepatic steatosis.

The outcomes were again better than the normal natural history of late onset argininosuccinate lyase deficiency. However, again it is unclear whether the differences observed are due to early

treatment of the screened cohort or due to differences in the severity of the condition, especially as three patients had undetectable levels of argininosuccinic acid in their urine at diagnosis.²¹

No additional studies were identified that analysed the benefit of early treatment for citrullinaemia.

Summary:

Criterion 10 met for citrullinaemia. Although no studies were identified that assessed the efficacy of treatment for citrullinaemia type I, it was found in the previous review that therapy with the long-term dietary protein restriction with oral administration of sodium phenylbutyrate improved survival rates for patients with citrullinaemia.

Criterion 10 partially met for argininosuccinate lyase deficiency. Treatment seems to be effective in preventing metabolic decompensations. It is difficult to assess whether early treatment is beneficial as studies which have compared outcomes for early versus treatment after symptom onset have identified patients for early treatment by screening, and these patients might have had more mild forms of the condition. In addition, it is unclear whether treatment is successful in preventing the development of neurocognitive deficiencies and liver disease even if metabolic decompensations are avoided. It has been suggested that another toxic compound is present in argininosuccinate lyase deficiency in addition to ammonia. Additional therapies/regimes specific for argininosuccinate lyase deficiency rather than urea cycle disorders in general may need to be developed.

11. There should be agreed evidence based policies covering which individuals should be offered treatment and the appropriate treatment to be offered

Criterion 11 not assessed.

12. Clinical management of the condition and patient outcomes should be optimised in all health care providers prior to participation in a screening programme

Criterion 12 not assessed.

13. There should be evidence from high quality Randomised Controlled Trials that the screening programme is effective in reducing mortality or morbidity. Where screening is aimed solely at providing information to allow the person being screened to make an “informed choice” (eg. Down’s syndrome, cystic fibrosis carrier screening), there must be evidence from high quality trials that the test accurately measures risk. The information that is provided about the test and its outcome must be of value and readily understood by the individual being screened

The 2004 HTA report stated that “RCTs of screening for rare disorders are difficult because of the enormous numbers that would be needed for adequate power.”⁵

No RCTs assessing whether screening for citrullinaemia or argininosuccinate lyase deficiency is effective in reducing mortality or morbidity were identified in the update search. However a study comparing outcomes between screened and unscreened cohorts was identified, as well as several studies that have described the outcomes of cases identified by screening.

Wilcken et al. (2009) and Norman et al. (2009a) compared the outcomes for patients with amino acid, organic acid and fatty acid metabolism (excluding PKU) disorders identified in a screened cohort and those identified in contemporaneous and historical cohorts of unscreened patients in Australia.^{13,34} Before 1998, no MS/MS screening was performed. It was introduced to three of the five Australian laboratories in 1998, 1999 and 2002. Cases identified during the period (1998 to 2002) and in the areas that screening was available were compared to a contemporaneous cohort in an unscreened region, and to a historical unscreened cohort (1994 to 1998). In Wilcken et al. data for specific conditions was not presented.¹³ However, the study found that, overall, MS/MS newborn screening is associated with a better outcome for patients at 6 years of age, with fewer deaths and fewer clinically significant disabilities.¹³ In Norman et al. disorders were divided. Although data was not presented separately for citrullinaemia and argininosuccinate lyase deficiency, data was presented for deaths in patients with urea cycle disorders (including citrullinaemia and argininosuccinate lyase deficiency) (see Table 25).³⁴

Table 25: Identification of urea cycle disorders and mortality from these diseases. From Norman et al. (2009a)³⁴ †Urea cycle disorders included 15 cases of ornithine transcarbamylase deficiency. ‡Number of patients screened taken from Wilcken et al. (2009)¹³

	Newborn Screening Diagnosis 1998-2002			Clinical Diagnosis Contemporaneous Control Group, 1998-2002			Clinical Diagnosis Historical control Group, 1994-1998					
	Number of patients screened‡	Total cases	Death by 7 days	Death later	Total cases	Death by 7 days	Death later	Total cases	Death by 7 days	Death later		
Disorders												
Urea cycle disorders†	461,500	7	2	1	533,400	7	1	1	1,017,800	16	5	3

If deaths before the age of 7 days are assumed to occur too early for screening to have an impact, and only deaths after the age of 7 days are considered, it can be seen that there was one death from urea cycle disorders during the period of screening (1: 461,500 infants screened) and four deaths in the pooled cohort that presented clinically (1:387,800 unscreened infants).

Ficioglu et al. (2009) and Mercmek-Mahmutoglu et al., (2010) reported on the long term outcomes of patients with argininosuccinate lyase deficiency who had been detected by newborn screening (see Criterion 10).^{4,21} In both studies, outcomes were better for patients identified by screening compared to the natural history/an historical cohort of patients with argininosuccinate lyase deficiency. However, it is difficult to tell if the differences observed are due to early treatment of the screened cohort or due to differences in the severity of the condition, i.e. that screening detected patients with mild variants that would not have presented symptomatically.

Couce et al. (2011) reported on the results of newborn screening in Galicia (Spain) between 2000 and 2010.¹⁵ One case of citrullinaemia and one case of argininosuccinate lyase deficiency were identified by newborn screening. The case of citrullinaemia was symptomatic at diagnosis and therefore would have presented clinically even without screening. Long term follow up for the patients identified by screening is shown in Table 26. No comparison with an unscreened cohort was performed.

Table 26: Characteristics of cases of citrullinaemia and argininosuccinate lyase deficiency identified by newborn screening in Galicia, Spain. From Couce et al. (2011)¹⁵. PDI/IQ Psychomotor Development Index/Intellectual Quotient

Diagnosis	Number of subjects	Clinical symptoms at diagnosis	Mean follow-up (months)	PDI/IQ (mean)	Present status
Citrullinaemia type I	1	Yes	24	85	Clinical symptoms
Argininosuccinate lyase deficiency	1	No	26	86	Free of symptoms

Summary: Criterion 13 not met. No randomised controlled trials of screening were identified. An observational study comparing outcomes for patients with urea cycle disorders (including citrullinaemia and argininosuccinate lyase deficiency) identified by screening with patients in unscreened cohorts suggests that screening is associated with better outcomes. This finding was independent of the detection rate, suggesting that the identification of mild variants was not responsible for the improvements seen. However, details for citrullinaemia and argininosuccinate lyase deficiency were not presented separately. Other identified studies have looked at long term outcomes of patients with argininosuccinate lyase deficiency identified by screening. Although outcomes for patients identified by screening are better when compared to the normal natural history of argininosuccinate lyase deficiency or historical cohorts, in these incidences it is unclear whether the improvements are due to early treatment of the screened cohort or due to the identification of patients with less severe variants.

14. There should be evidence that the complete screening programme (test, diagnostic procedures, treatment/ intervention) is clinically, socially and ethically acceptable to health professionals and the public

Criterion 14 not assessed.

15. The benefit from the screening programme should outweigh the physical and psychological harm (caused by the test, diagnostic procedures and treatment)

No formal assessments of the balance of benefits and harms of screening were identified.

Harms of screening

As with all screening tests there is likely to be harm from false-positive results and results with unknown clinical significance.

Carriers are not identified by the screening test. The presence of a family member with the disease could lead to the genetic testing of other members of the family and the identification of individuals carrying the mutation. This is likely to be the case whether screening is implemented or whether infants are identified due to presentation with symptoms, although more infants may receive a diagnosis if newborn screening is implemented (i.e. fewer cases may remain undiagnosed).

Harms of treatment

The European Public Assessment Report (EPAR) for the public lists the following as common side effects of sodium phenylbutyrate:³⁵

- Amenorrhoea (absence of periods) and irregular menstruation (irregular periods)
- Abnormal kidney function
- Abnormal blood cell counts

Other factors

Current treatment for both conditions involves dietary management. This may affect patients' quality of life. This was not assessed, but studies which assessed the effectiveness of treatment reported variable compliance with treatment.

Summary:

Criterion 15 uncertain for citrullinaemia and argininosuccinate lyase deficiency. No formal assessments of the balance of benefits and harms of screening were identified. Testing for citrullinaemia and argininosuccinate lyase deficiency can be done on a dried blood spot. There is likely to be some harm caused by the identification of false positives and false negatives. The adverse effects associated with treatment do not outweigh the benefits.

16. The opportunity cost of the screening programme (including testing, diagnosis and treatment, administration, training and quality assurance) should be economically balanced in relation to expenditure on medical care as a whole (ie. value for money). Assessment against this criteria should have regard to evidence from cost benefit and/or cost effectiveness analyses and have regard to the effective use of available resource

The economic evaluation in the 2004 HTA report estimated that screening for urea cycle disorders costs £2,965 per life-year gained.⁵

No UK based cost-effectiveness analyses were identified.

From the update search, only one study was identified that considered the cost-effectiveness of screening for citrullinaemia and argininosuccinate lyase deficiency individually using MS/MS. Cipriano et al. (2007) performed a cost effectiveness analysis from a societal perspective of replacing screening for phenylketonuria using the Guthrie bacterial inhibition assay with expanded screening for up to 21 inherited metabolic disorders using MS/MS in Ontario, Canada.³⁶ Two programme strategies were assessed: each disease was assessed independently, including the entire capital cost of investing in the MS/MS technology, the cost of screening and the cost of programme maintenance; and diseases were assessed in bundles, as MS/MS allows several diseases to be screened for at the same time. Using a decision analytic model, with life years saved as the outcome, the analysis considered:

- the incidence and the severity of the conditions
- the sensitivity, specificity and positive predictive rate of the test
- the health benefits
- the start-up costs of MS/MS screening
- the cost of confirmatory testing
- the cost of treatment, hospitalisation, social services and education

The incremental cost and the incremental cost-effectiveness ratios (ICERs) for screening for citrullinaemia and argininosuccinate lyase deficiency individually are shown in Table 27.

Table 27: Incremental cost effectiveness of each disease evaluated independently and a breakdown of incremental costs, savings and life years gained per patient screened. All costs given in 2004 Canadian dollars. From Cipriano et al. 2007³⁶

Disease	Incremental cost (\$) (including start-up*)	Incremental cost (\$) (excluding start-up)	Incremental life years gained (\$) ($\times 10^{-5}$)	ICER† (\$) (including start-up)	ICER (\$) (excluding start up)	Order of cost-effectiveness‡
Argininosuccinate lyase deficiency	62.89	44.52	3.09	2,035,275	1,440,777	18
Citrullinaemia	60.81	42.44	2.42	2,512,810	1,753,719	19

*Programme start-up and base operation costs (whether screening for one or more diseases) is \$18.37 per infant

† The incremental cost-effectiveness ratio (ICER) describes the incremental cost required to acquire the benefit of one additional life-year. It is calculated by dividing the total incremental cost by the incremental life years gained

‡Position relative to the 21 inherited metabolic disorders considered

Argininosuccinate lyase deficiency and citrullinaemia were amongst the least cost-effective disorders to screen for. The authors of the review state that urea cycles disorders are the least cost-effective disorders to screen for, and that screening for argininosuccinate lyase deficiency and citrullinaemia is not cost-effective even when costs and benefits are averaged across multiple diseases in a screening programme, for example if all 21 metabolic conditions are screened for. They suggest that this is mainly due to the low number of life years gained combined with a high incremental cost associated with treating patients with sodium-phenylbutyrate. They state that urea cycle disorders frequently result in death before a clinical diagnosis is made. Although early diagnosis leads to enhanced survival in some cases, it also results in an expensive treatment regimen.

Several other studies were identified that considered the cost-effectiveness of screening.

Norman et al. (2009b)³⁷ performed a systematic review of studies examining the cost-effectiveness of screening for rare metabolic conditions using tandem mass spectrometry published between January 1997 and March 2008.³⁷ The systematic review found that despite the substantial differences in the methods employed, the consensus is positive in favour of MS/MS screening.

Norman et al. (2009a)³⁴ analysed the cost-effectiveness of screening for a panel of disorders including argininosuccinate lyase deficiency and citrullinaemia in Australia, using the phased introduction of screening using MS/MS in Australia.³⁴

The cost effectiveness per life years gained and deaths averted are shown in Table 28.

Table 28: Cost-effectiveness outcomes. Costs given in Australian dollars.

Outcome measure	Outcome, number per 100,000 screens	Cost per 100,000 screens. A\$	Cost effectiveness A\$ per outcome
Life years gained	32.378	349,010 (cost of screening [218,000] plus additional cost of treatment [131,010])	10,779
Deaths averted	0.738		472,913

Feuchtbaum and Cunningham (2006) performed cost-effectiveness and cost-utility analyses from a payer perspective (costs to the public, i.e. excluding additional costs borne by families) of MS/MS screening for all MS/MS detectable disorders in California, US.³⁸ Costs and benefits were derived from the results of the California MS/MS screening pilot.¹⁹

The model found that incremental cost of screening was approximately \$5.7 million per 540,000 births. Per 540,000 births screened, 83 affected newborns would be identified, and in the base case analysis eight deaths would be prevented. The incremental cost of screening per life saved was \$708,063. An estimated 949 QALYs would be saved, and the saving per QALY would be \$1,628. These calculations and other calculations relating to medical costs saved are shown in Table 29.

Table 29: Summary of economic impact of MS/MS screening in California, in the base case scenario and estimating total lifetime medical care costs at \$1,000,000 for a severely mentally retarded person. Results were calculated per 83 diagnosed cases.

Lives saved (<i>a</i>)	8
Incremental programme costs (<i>b</i>)	\$5,664,500
Incremental costs per life saved (<i>b/a</i>)	\$708,063
Incremental costs per case detected (<i>b/83</i>)	\$68,247
Lifetime medical costs with screening (<i>c</i>)	\$5,321,052
Total costs with screening (<i>d=b+c</i>)	\$10,985,552
Total costs per case detected (<i>d/83</i>)	\$132,356
Total costs without screening (<i>e</i>)	\$12,530,204
Total costs saved with screening (<i>f=e-d</i>)	\$1,544,652
QALY saved (<i>g</i>)	969
Saving per QALY (<i>h=f/g</i>)	\$1,628
Values of lives saved (<i>i=a x \$5,700,000</i>)	\$45,600,000
Net incremental benefit (<i>j=f+i</i>)	\$47,144,652

Summary: Criterion 16 not met. The economic evaluation in the 2004 HTA report estimated that screening for urea cycle disorders to cost £2,965 per life-year gained. No UK based studies of cost effectiveness were identified. Analyses that have considered screening for a panel of MS/MS detectable disorders have found screening to be cost effective. However, in the one study that considered screening for disorders individually, screening for argininosuccinate lyase deficiency and citrullinaemia were amongst the least cost-effective disorders to screen

for. This analysis was published in 2007 and performed from a Canadian perspective and it is unclear how applicable this study would be to the UK.

17. All other options for managing the condition should have been considered (eg. improving treatment, providing other services), to ensure that no more cost effective intervention could be introduced or current interventions increased within the resources available

Summary: Criterion 17 not assessed.

Other potential options for improving outcomes for citrullinaemia and argininosuccinate lyase deficiency include interventions to improve awareness of these conditions so that these diseases are suspected and diagnosed in a timely manner.

18. There should be a plan for managing and monitoring the screening programme and an agreed set of quality assurance standards

The European Research Network for the evaluation and improvement of screening, Diagnosis and treatment of Inherited disorders of Metabolism (ERNDIM) aims to reach a consensus between European Biochemical Genetics Centres on reliable and standardised procedures for diagnosis, treatment and monitoring of inherited metabolic diseases.³⁹ This is achieved through provisions of quality control schemes on a European wide scale. Quantitative schemes, for example for amino acids, are planned and managed by members of the ERNDIM Scientific Advisory Board and organised in partnership with SKML (the Dutch Foundation for Quality Assessment in Medical Laboratories), a Quality Assurance (QA) provider based in the Netherlands. SKML dispatches QA samples to scheme participants and provides a website for on-line submission of results and access to scheme reports by participants. In 2011, the scheme consisted of 8 lyophilised samples.⁴⁰

The US Centres for Disease Control and Prevention (CDC) provides QA for dried blood spot screening tests. All laboratories in the US that test dried blood spots participate voluntarily in the Newborn Screening Quality Assurance Program (NSQAP).⁴¹ The CDC accepts international participants into the QA programme. The CDC provides Quality Control materials, proficiency testing services and technical support. The proficiency testing programme provides laboratories with quarterly panels of blind-coded dried blood spot specimens and gives the laboratory an internal assessment of performance.

Summary: Criterion 18 not directly assessed. US and European quality assurance systems are in place, which could provide examples for UK practice.

19. Adequate staffing and facilities for testing, diagnosis, treatment and programme management should be available prior to the commencement of the screening programme

Criterion 19 not assessed.

20. Evidence-based information, explaining the consequences of testing, investigation and treatment, should be made available to potential participants to assist them in making an informed choice

Criterion 20 not assessed.

21. Public pressure for widening the eligibility criteria for reducing the screening interval, and for increasing the sensitivity of the testing process, should be anticipated. Decisions about these parameters should be scientifically justifiable to the public

Children Living with Inherited Metabolic Diseases (Climb), the National Information Centre for Metabolic Diseases, reports on their website that they have been working alongside medical professionals and families to add Inherited Metabolic Diseases to the Newborn Screening Programme.⁴²

Other stakeholders with an interest in screening for these conditions include:

- Genetic Alliance UK
- Institute of Child Health
- Royal College of General Practitioners
- Royal College of Midwives
- Royal College of Paediatrics and Child Health

Summary: Criterion 21 uncertain. Climb report that they have been working alongside medical professionals and families to add Inherited Metabolic Diseases to the Newborn Screening Programme, but specific disorders are not mentioned.

22. If screening is for a mutation the programme should be acceptable to people identified as carriers and to other family members

Criterion 22: not applicable.

Screening flow chart

Based on the results of screening programmes that gave values for the number of true positives, the number of false positives and the number of false negatives identified, and that screened for citrullinaemia using citrulline as a marker and argininosuccinic acid as a marker for argininosuccinate lyase deficiency, flow charts showing what happens to 100,000 babies screened are shown in Figure 1 and Figure 2.

Figure 1: Screening flow chart for citrullinaemia. Based on the results of screening programmes that used citrulline as a marker for citrullinaemia and that reported the number of true positive, false positives and false negatives identified (Couce et al. [2011]¹⁵ and Lund et al. [2012]¹²)

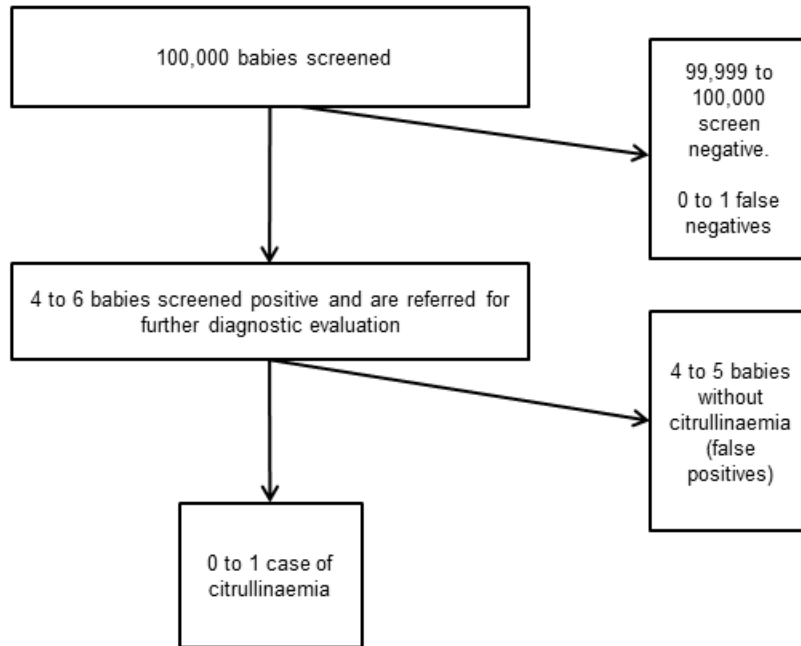
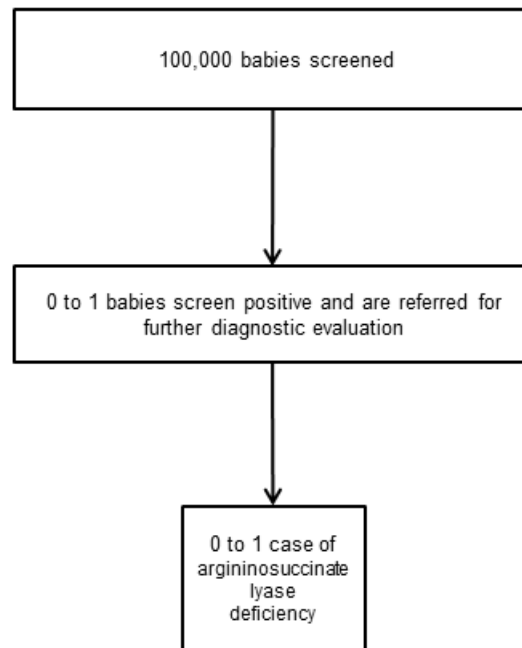


Figure 2: Screening flow chart for argininosuccinate lyase deficiency. Based on the results a screening programmes that used argininosuccinic acid as a marker for argininosuccinate lyase deficiency and that reported the number of true positive, false positives and false negatives identified (Lund et al. [2012]¹²)



Conclusions

The condition

Citrullinaemia

Citrullinaemia type I is an autosomal recessive disease caused by mutations in the gene encoding argininosuccinate synthase (ASS), an enzyme in the urea cycle. Deficiency in argininosuccinate synthase leads to the accumulation of ammonia. Infants with the neonatal form develop hyperammonaemia within the first few days of life, become lethargic, feed poorly, vomit, and develop tachypnea or stroke. Without treatment, the condition can cause increased intracranial pressure, increased neuromuscular tone, spasticity, ankle clonus, seizures, loss of consciousness and death. Even with prompt intervention, patients normally have significant neurological defects. The milder late-onset form can present with recurrent lethargy and somnolence, intellectual disability, and/or chronic or recurrent hyperammonaemia.

Citrullinaemia can be detected through MS/MS analysis of newborn dried blood spots. The concentration of citrulline can be used to screen for citrullinaemia, however, other conditions, including argininosuccinate lyase deficiency, pyruvate carboxylase deficiency and citrin deficiency (also known as citrullinaemia type II) also cause elevations in citrulline levels, and further testing is required to distinguish between these conditions. It is questionable whether screening will be able to prevent the onset of symptoms in patients with the early onset form, as several studies were identified that reported that infants can develop symptoms before the results of a newborn blood spot screen are available. However, there still could be value in the early diagnosis and treatment of patients with more mild forms of the condition.

The incidence of citrullinaemia in the UK is uncertain. Using studies from Europe, the US and Australia, the incidence can be calculated as between less than one case per 650,000 births to more than one case per 60,000 births. Different studies have found that the introduction of screening has reduced or increased the incidence of citrullinaemia. Individual cases of the disease in small populations contribute to the wide variations in calculated incidences, as citrullinaemia is a rare disease.

Argininosuccinate lyase deficiency

Argininosuccinate lyase deficiency is also an autosomal recessive disease, in this case caused by mutations in the gene encoding argininosuccinate lyase (*ASL*), another enzyme of the urea cycle. In common with other urea cycle disorders, deficiency in this enzyme leads to hyperammonaemia associated sequelae, and has an early onset and late-onset form. However, in addition, patients with argininosuccinate lyase deficiency often also suffer from neurocognitive deficiencies, hepatitis, cirrhosis, and trichorrhexis nodosa. Some of these outcomes appear to be unrelated to the severity or duration of hyperammonaemic episodes, suggesting that plasma ammonia is not the only toxic compound in argininosuccinate lyase deficiency.

Argininosuccinate lyase deficiency can be detected through MS/MS analysis of newborn dried blood spots. The concentrations of argininosuccinic acid and citrulline can be used to screen for argininosuccinate lyase deficiency. Argininosuccinic acid has the advantage of being a specific marker for argininosuccinate lyase deficiency. The duration of the latent asymptomatic period varies between patients in this disorder as well, and symptoms can develop during the first days of life. Although many of the infants identified with argininosuccinate lyase deficiency in newborn screening programs were asymptomatic at the time of diagnosis, one study was identified that reported that an infant developed symptoms (on day four of life) before the results of a newborn blood spot screen were available. However, there still could be value in the early diagnosis and treatment of patients with more mild forms of the condition.

The incidence of argininosuccinate lyase deficiency in the UK is uncertain. From European, US and Australian studies the incidence can be estimated at between less than one case per 940,000 births to more than one case per 50,000 births. As with citrullinaemia, the detection of a single case in small populations contributes to the wide variations in the estimates of incidence.

The screening test

Screening for all three conditions can be performed using MS/MS on dried blot spots. Dried blood spots are already collected as part of the newborn screening programme, and MS/MS technology is used to screen for phenylketonuria in the UK.

Screening for citrullinaemia using citrulline as a marker has been associated with high false positive rates in some studies, and it has been reported that newborn screening results arrive after the onset of symptoms in some cases of early onset citrullinaemia.

When argininosuccinic acid was used as a marker for argininosuccinate lyase deficiency, a study reported 100% sensitivity and 100% specificity.

An international collaboration has attempted to define the normal range of markers of these disorders and to establish cut-offs. However, citrulline is elevated in multiple conditions and argininosuccinic acid levels were found to vary between sites.

Treatment

Citrullinaemia

Although no studies were identified that assessed the efficacy of treatment for citrullinaemia type I, it was found in the previous review that therapy with long-term dietary protein restriction plus oral administration of sodium phenylbutyrate improved survival rates for patients with citrullinaemia. New nitrogen scavenging therapies are being developed and tested.

Argininosuccinate lyase deficiency

Argininosuccinate lyase deficiency is also treated with long-term dietary protein restriction with oral administration of sodium phenylbutyrate. New nitrogen scavenging therapies are being developed and tested. Treatment seems to be effective in preventing metabolic decompensations. It is difficult to assess whether early treatment is beneficial as studies which have compared outcomes for early versus treatment after symptom onset have identified patients for early treatment by screening, which means that these patients might have more mild forms of the condition. In addition, it is unclear whether treatment is successful in preventing the development of neurocognitive deficiencies and liver disease even if metabolic decompensations are avoided. It has been suggested that another toxic compound is present in argininosuccinate lyase deficiency in addition to ammonia. Additional therapies/regimes specific for argininosuccinate lyase deficiency rather than urea cycle disorders in general may need to be developed.

The screening programme

No randomised controlled trials of screening were identified. An observational study comparing outcomes for patients with urea cycle disorders (including citrullinaemia and argininosuccinate lyase deficiency) identified by screening with patients in unscreened cohorts suggests that screening is associated with better outcomes. This finding was independent of the detection rate, suggesting that the identification of mild variants was not responsible for the improvements seen. However, details for citrullinaemia and argininosuccinate lyase deficiency were not presented separately. Other identified studies have looked at long term outcomes of patients with argininosuccinate lyase deficiency identified by screening. Although outcomes for patients identified by screening are better when compared to the normal natural history of argininosuccinate lyase deficiency or historical cohorts, in these incidences it is unclear whether the improvements are due to early treatment of the screened cohort or due to the identification of patients with less severe variants.

The economic evaluation in the 2004 HTA report estimated that screening for urea cycle disorders to cost £2,965 per life-year gained. No additional UK based studies of cost effectiveness were identified. Analyses that have considered screening for a panel of MS/MS detectable disorders have found screening to be cost effective. However, in the one study that considered screening for disorders individually, argininosuccinate lyase deficiency and

citrullinaemia were amongst the least cost-effective disorders to screen for. This analysis was published in 2007 and performed from a Canadian perspective and it is unclear how applicable this study would be to the UK.

US and European quality assurance systems are in place. No UK based reports were identified as screening for these disorders is not currently provided in the UK. However, if screening was to be implemented in the UK, plans for managing and monitoring the screening programme and quality assurance standards could be formulated based on the systems used in Europe or the US, or the UK could join one of these systems.

Implications for research

The following areas could provide useful avenues for further research:

- Large European based epidemiological studies, as uncertainty remains over the epidemiology of citrullinaemia and argininosuccinate lyase deficiency.
- Studies determining the feasibility of blood-spot screening for these conditions in the UK, including:
 - Studies to determine whether the UK bloodspot screening process can detect early-onset cases before they are symptomatic. Reports from screening programmes have found that acute-neonatal forms of the disorders can present symptomatically before the results of screening tests are available. For these disorders testing earlier than at five days of age may be beneficial.
 - Studies to determine the distribution of markers in a UK population on day five of life
 - Studies to determine the predictive value of screening in the UK.
- Development of a screening marker specific for citrullinaemia
- Development of treatments that prevent the development of neurocognitive deficiencies and liver disease associated with argininosuccinate lyase deficiency
- Studies which determine the value of early treatment
- Further studies into the long term outcomes of treating citrullinaemia and argininosuccinate lyase deficiency, particularly to determine the value of screening and treating the mild forms of these conditions.

Methodology

The draft update report was prepared by Bazian Ltd., and then adapted in line with comments from the National Screening Committee.

Search strategy

BACKGROUND: A systematic review on this topic was published in 2004: Pandor A et al, Clinical effectiveness and cost effectiveness of neonatal screening for inborn errors of metabolism using tandem mass spectrometry: a systematic review, March 2004

SOURCES SEARCHED: Medline, Embase, Cochrane Library.

DATES OF SEARCH: Medline 2004- July Week 4 2012; Embase 2004-2012 Week 31, Cochrane Library 2012 Issues 7 and 3.

SEARCH STRATEGY:

Medline (OVID interface)

- 1 Neonatal Screening/
- 2 ((neonat* or newborn*) adj2 screen*).tw.
- 3 Mass Screening/
- 4 exp Infant, Newborn/
- 5 1 or 2 or (3 and 4)
- 6 Tandem Mass Spectrometry/
- 7 exp spectrum analysis, mass/
- 8 (tandem adj2 mass).tw.
- 9 or/6-8
- 10 5 and 9
- 11 Tyrosinemias/
- 12 tyrosin?emi*.tw.
- 13 (((fumarylacetoacetate adj hydrolase) or fumarylacetoacetase or fah) adj2 deficien*).tw.
- 14 or/11-13
- 15 Citrullinemia/
- 16 citrullin?emi*.tw.
- 17 citrullinuri*.tw.
- 18 (argininosuccinate adj2 (synthase or synthetase) adj2 deficien\$).tw.
- 19 ass deficien*.tw.
- 20 or/15-19
- 21 Argininosuccinic Aciduria/
- 22 ((Argininosuccinic adj Aciduria) or Argininosuccinicaciduria).tw.
- 23 ((Argininosuccinate or Argininosuccinase or asl or asal) adj deficien*).tw.
- 24 or/21-23
- 25 amino acid metabolism, inborn errors/
- 26 14 or 20 or 24 or 25
- 27 prevalence/
- 28 incidence/
- 29 (prevalen* or inciden*).tw.
- 30 exp epidemiological studies/
- 31 "predictive value of tests"/

- 32 "sensitivity and specificity"/
- 33 ((positive or negative) adj predictive value*).tw.
- 34 (false adj (positive* or negative*)).tw.
- 35 (sensitiv* or specific*).tw.
- 36 early diagnosis/
- 37 Delayed Diagnosis/
- 38 disease progression/
- 39 prognosis/
- 40 "quality of life"/
- 41 exp treatment outcome/
- 42 morbidity/
- 43 mortality/
- 44 Tyrosinemias/di, dh, dt, ep, mo, su, th [Diagnosis, Diet Therapy, Drug Therapy, Epidemiology, Mortality, Surgery, Therapy]
- 45 Citrullinemia/di, dh, dt, ep, mo, su, th
- 46 Argininosuccinic Aciduria/di, dh, dt, ep, mo, su, th
- 47 exp Diet Therapy/
- 48 Liver Transplantation/
- 49 nitisinone.mp.
- 50 Phenylbutyrates/
- 51 exp Renal Dialysis/
- 52 Phenylacetates/
- 53 5 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 47 or 48 or 49 or 50 or 51 or 52
- 54 26 and 53
- 55 44 or 45 or 46 or 54
- 56 10 or 55
- 57 amino acid metabolism, inborn errors/di, dh, dt, ep, mo, su, th
- 58 56 or 57
- 59 limit 58 to yr="2004 -Current"

A similar search strategy was used in Embase, and a simplified strategy in the Cochrane Library.

RESULTS

All results were downloaded into an Excel spreadsheet, and 491 duplicates removed. A total of 1916 citations remained.

Database	No. citations retrieved	Exclusive
Medline	1009	1005
Embase	1333	857
Cochrane Library	65	54
	Total=2407	Total = 1916

The title and abstracts of these citations, and where necessary and available the full text, were examined for relevance to newborn screening for amino acid disorders or expanded newborn screening using tandem mass spectrometry. Articles commenting on other papers are listed with the original paper. 457 citations remained, and have been classified as follows:

Category	No. of citations
Systematic reviews	
– general/amino acid disorders	6
– Tyrosinaemia I	1
Guidelines	
– general/amino acid disorders	6
– Tyrosinaemia I	1
Non-systematic reviews	
– general/amino acid disorders	58
– Argininosuccinate lyase deficiency	2
– Citrullinaemia	3
– Tyrosinaemia I	6
Natural history	
– general/amino acid disorders	4
– Argininosuccinate lyase deficiency	6
– Citrullinaemia	45
– Tyrosinaemia I	27
Prevalence of condition	
– general/amino acid disorders	27
Outcomes	
– general/amino acid disorders	6
– Argininosuccinate lyase deficiency	2
– Citrullinaemia	1
– Tyrosinaemia I	5
Delayed diagnosis	
– general/amino acid disorders	1
Screening test	
– general/amino acid disorders	55
– Citrullinaemia	2
– Tyrosinaemia I	23
Treatment	

– general/amino acid disorders	7
– Argininosuccinate lyase deficiency	8
– Citrullinaemia	22
– Tyrosinaemia I	56
Screening programme	
– general/amino acid disorders	69
– Argininosuccinate lyase deficiency	2
– Citrullinaemia	2
– Tyrosinaemia I	4
Total	457

Quality

Non-systematic reviews, editorials, other opinion pieces, reports of case series of fewer than four patients, and those with nonhuman data were excluded. Conference abstracts were also excluded. Additional relevant references identified during the preparation of the report were also included. Priority was given to studies from Europe, North America and Australia.

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