Australian Government

Medical Services Advisory Committee

Public Summary Document

Application No. 1531 – Alpha Thalassaemia genetic testing

Applicant: The Royal College of Pathologists of Australasia (RCPA)

Date of MSAC consideration: MSAC 75th Meeting, 28-29 March 2019

Context for decision: MSAC makes its advice in accordance with its Terms of Reference, visit the MSAC website

1.   Purpose of application

An application for genetic deletion testing for the diagnosis of alpha (α) thalassaemia in females of reproductive age with abnormal red cell indices and, in certain circumstances, their reproductive partners of was referred to the MSAC Executive from the Genetics Working Group of the Pathology Clinical Committee of the MBS Review. The Royal College of Pathologists in Australasia has agreed to act as applicant.

The proposed medical service is testing for common gene deletions that cause α thalassaemia.

2.   MSAC's advice to the Minister

After considering the strength of the available evidence in relation to comparative safety, clinical effectiveness and cost-effectiveness, MSAC deferred its advice regarding public funding of alpha thalassaemia genetic testing to seek further information, particularly from the Haematology Society of Australia and New Zealand (HSANZ), on:

- the proposed clinical algorithms for testing in two separate contexts if there are abnormal red cell indices – for couples who are planning to get pregnant and for couples who are already pregnant, with an emphasis on identifying any time-sensitive elements;
- the appropriate test(s) to use (gap-PCR and/or MLPA deletion testing or other diagnostic testing including sequencing) in these two proposed clinical algorithms, the order of testing in each algorithm if sequential testing is appropriate, and the costs per test or sequence of tests, and per patient;
- the cost effectiveness of using different testing approaches; and
- total overall healthcare costs.

MSAC also requested further information on the potential for setting up a national registry along the lines already established in Victoria.

MSAC advised that this further information would need to be considered by the ESC.

MSAC accepted the safety and clinical effectiveness of alpha thalassaemia genetic testing using deletion testing via either gap-PCR or MLPA, however noting that gap-PCR testing is less effective than MLPA testing. MSAC acknowledged the current inequity of access to genetic testing across Australia.

3. Summary of consideration and rationale for MSAC's advice

MSAC noted that the purpose of the application is to seek Medicare Benefits Schedule (MBS) listing of alpha (α) thalassaemia genetic testing – a new item for genetic deletion testing of the α-globin genes – where haematological studies cannot provide a definitive diagnosis.

Alpha thalassaemia is a disease with a complex genotype. Mutations in the two HBA genes (four alleles) result in varying disease severity, from silent carriers (one mutated allele) to haemoglobin Bart's hydrops fetalis syndrome (Hb Bart's; four mutated alleles), which is almost uniformly fatal in newborns. Women carrying an Hb Bart's fetus also have increased morbidity associated with the pregnancy. MSAC noted that the proposal is designed to provide reproductive options for at-risk parents, not to diagnose disease.

MSAC noted that the incidence and mutation spectra of α thalassaemia vary with ethnic background, with some ethnic groups (e.g. those from Asia and Africa) having a carrier rate as high as 60%. MSAC noted the increasing clinical need for α thalassaemia testing, as the incidence of α thalassaemia has risen in Australia with increased migration from South-East Asia, Middle Asia and Africa.

α-Thalassemia is caused by deletion in approximately 95% of cases[1]. In the remaining 5% of cases point mutations, rather than deletions, are found, and these are diagnosed by sequencing (Sanger or next generation).

MSAC acknowledged the current inequity of access to this genetic testing across Australia. Currently, state governments and private out-of-pocket payments fund α thalassaemia genetic testing to different degrees.

MSAC noted the advice that couples carrying an Hb Bart's fetus frequently choose termination of pregnancy (TOP) once diagnosed, and the major benefits of testing are avoiding the birth of a child with an almost uniformly lethal condition and maternal morbidity. MSAC acknowledged that these benefits are difficult to quantify.

MSAC agreed that deletion testing offers superior effectiveness and non-inferior safety compared with no testing, even though the evidence is limited and of poor quality.

However, MSAC noted that the initial proposal did not propose a test methodology for deletion testing. MSAC considered a key area of uncertainty is the type of genetic testing. Deletion testing by gap-PCR is of inferior effectiveness to deletion testing by Multiplex ligation-dependent probe amplification (MLPA). Gap-PCR deletion testing only captures about 84% of deletions. MLPA testing is required to capture the remaining 16% of deletions. Sanger sequencing is the best current method for characterizing globin variants and point mutations causing thalassemia.

The MSAC noted that the application proposed there are two clinical scenarios where genetic testing is appropriate:

1. pre-pregnancy planning: couples at risk of having an Hb Bart's fetus should undergo testing before pregnancy; and
2. at-risk pregnant couples: the application proposed woman could undergo testing first; a positive result would initiate subsequent testing of the male partner.

MSAC considered that clinical input from haematologists is required to address the first scenario. If either parent has suggestive haematological indices, gap-PCR deletion testing may be appropriate. If gap-PCR deletion testing is negative, then MLPA deletion testing

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[1] Am J Clin Pathol July 2017;148:6-15

would be warranted. Alternatively, MLPA deletion testing alone could be used in these individuals, but the comparative cost-effectiveness of this method to sequential gap-PCR plus MLPA in a sub-set of individuals is to be established. Victoria, which currently funds α thalassaemia testing, should be able to provide data regarding numbers of couples requiring MLPA testing.

For the second scenario, MSAC considered these cases more urgent and may require both partners to undergo immediate comprehensive deletion testing concurrently, as the time taken to follow the pathway proposed by the applicant (i.e. sequential deletion testing of the woman, then the man) can be lengthy and not optimal if Bart's hydrops fetalis syndrome is a potential diagnosis.

MSAC recommended that the two different scenarios be assessed, to ensure the order of testing is clinically relevant, and then costed appropriately.

MSAC noted the three main drivers of the economic evaluation are:

- changes in the prevalence of α thalassaemia and haemoglobin H disease genotypes in the population eligible for testing;
- number of partners screened who are eligible for testing; and
- test cost, and how many tests should be standard for each case (e.g. deletion testing, +/– sequencing).

The MSAC considered that the number of individuals or couples that would require testing is uncertain and potentially underestimated. The epidemiological approach using ABS Census of Population and Housing, 2016 (ABS 2018), and AIHW Perinatal data, 2016 (AIHW 2018) may not adequately capture the proportion of women of reproductive age that are pregnant or who plan a pregnancy each year in the ethnic populations known to be at increased risk of α thalassaemia

MSAC recommended seeking further information, including from the HSANZ and the applicant, about proposed clinical algorithms and appropriate tests (including the order of testing) for each, as well as costs per test and the total health care cost. MSAC also requested further information on the potential for setting up a national registry along the lines of the existing registry in Victoria.

MSAC noted the need for education and for culturally relevant services in the context of the potential for consumer confusion around α and beta thalassaemias and the high carrier rates in some ethnic populations.

4. Background

An application for MBS funding of genetic testing of the alpha-globin genes has not previously been made to Medical Services Advisory Committee (MSAC).

This Referral was submitted to the MSAC Executive who recommended assessment via an expedited pathway. This was suggested on the grounds that:
- the States/Territories currently provide funding for α thalassaemia genetic testing; and
- there was an evidence pack submitted with the application.

However, the Department noted that there are very few guidelines describing genetic testing for α thalassaemia, (and thalassaemia in general), and that the target population for testing should be clarified during the contracted assessment (CA).

Current funding arrangements
The CA outlines that genetic testing is currently provided by the States and Territories, often being conducted through hospital genetic services. Different arrangements are used for

funding across the States and Territories. In Victoria, testing for haemoglobinopathies (HbP) is funded through a state funded grant to Monash University. However, Victoria is the only state in which the cost of testing is completely covered and in others, the patient may be obliged to pay at least a proportion of the cost.

5.   Prerequisites to implementation of any funding advice

No information on the regulatory and/or accreditation requirements associated with the provision of genetic deletion testing for α-thalassaemia was provided in the CA, however the test would be covered by current NATA accreditation requirements.

6.   Proposal for public funding

The proposed MBS item descriptor (with ESC recommended revisions highlighted) is presented in Table 1.

The proposed target population for the test is women of reproductive age and their partners if both are at risk of carrying α thalassaemia mutations.

Table 1   Proposed MBS item descriptor

Item 73XXX	Category 6 – PATHOLOGY SERVICES
Deletion testing of HBA1 and HBA2 for: - the diagnosis of alpha thalassaemia in patients of reproductive age with abnormal red cell indices, with thalassaemia screening for beta-thalassaemia not conclusive, without concurrent iron deficiency or with iron deficiency if pregnant and no historic normal cell indices; or - the determination of carrier status in reproductive partners of patients of child bearing potential with diagnosed alpha thalassaemia.	
Fee: TBD	Benefit: 75% = TBD; 85% = TBD
Explanatory note 'Abnormal red cell indices' refers to a mean corpuscular volume <80 fL and/or mean corpuscular haemoglobin <28 pg and HbA2 <3.4% and haematological studies not conclusively diagnostic of thalassaemia.	

7.   Summary of Public Consultation Feedback/Consumer Issues

There was no consultation period as this application followed an expedited pathway.

Feedback from two public state laboratories was received in the course of seeking data on α thalassaemia prevalence and testing. Both sources felt that genetic deletion testing alone (GAP-PCR or MLPA) was insufficient to identify all couples at risk of pregnancies with a clinically significant form of α thalassaemia. A testing regimen that progresses from α thalassaemia deletion testing to HbA gene sequencing and beta (β) thalassaemia testing if necessary is recommended by the laboratories. A centralised laboratory and coordinated testing for couples was also the recommended model for funding, to reduce the number of repeat requests received and performed as a result of individual private laboratory participation. Feedback from the two laboratories advised that α thalassaemia genetics is more complex than some other genetic diseases that have tests funded by Medicare, and requires concerted effort and coordination by experts in the field, along with counselling for couples on their reproductive options.

8.   Proposed intervention's place in clinical management

Genetic deletion testing is proposed as an additional test to those already performed and funded through Medicare. The current testing regimen by which α thalassaemia is diagnosed in the absence of deletion testing involves a full blood count (FBC), ferritin and thalassaemia studies (Items 65078 and 65081). In the current proposal, genetic deletion testing would follow these tests in those who are identified with microcytic hypochromic anaemia, normal ferritin, and are either found positive for haemoglobin H (HbH) inclusions or excluded for β

thalassaemia (raised HbA_2). The proposed algorithm for women of of reproductive age and their partners is at Figure 1, and for pregnant women and their partners is at Figure 2. (Note the additional steps of the proposed pathway are shaded blue).

Clinical and pathology experts advise that a full clinical picture should be obtained and used, in addition to biochemical criteria, to identify those at high risk of carrying significant deletions.
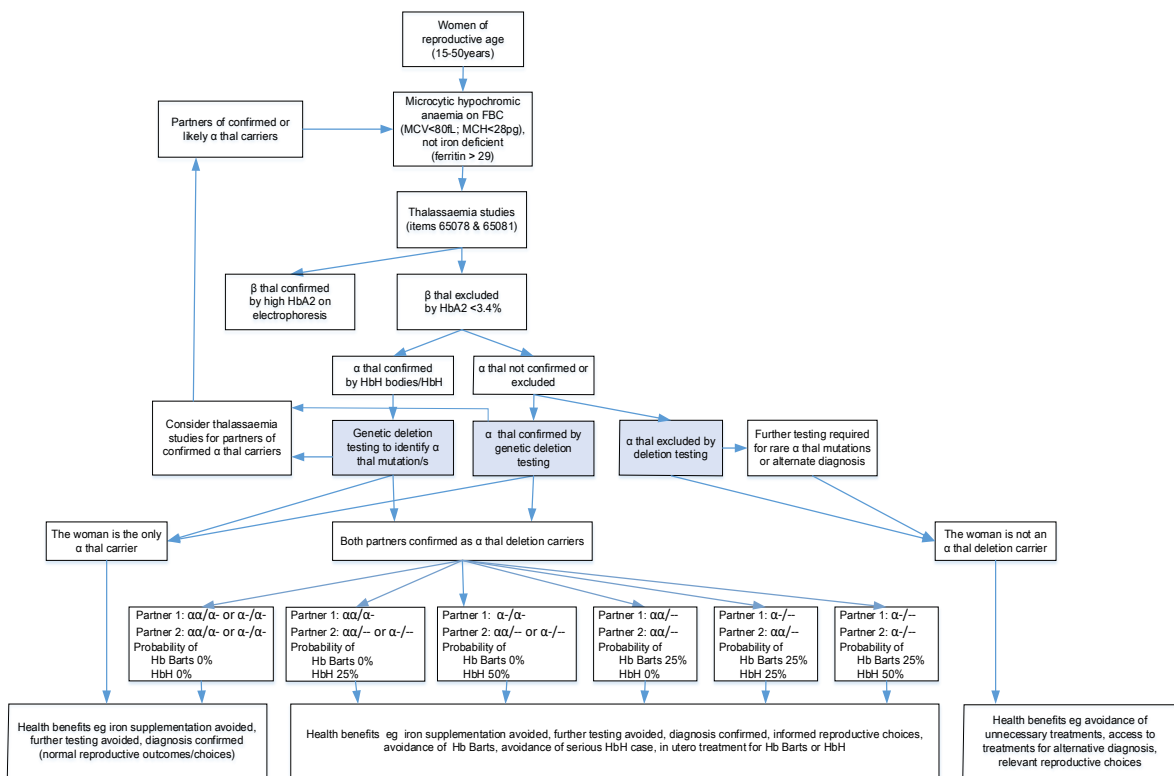
9.   Comparator

Deletion testing for α thalassaemia is not currently available through the MBS.

The comparator for this evaluation is considered to be "no genetic testing".

The "no genetic testing" scenario includes prior tests of full blood count (FBC), for red cell indices, and thalassaemia studies when indicated. MBS item 65078 for tests for diagnosis of thalassaemia was listed on the MBS in 1 November 1998.

The primary outcome for α thalassaemia diagnosis usin/g thalassaemia studies is to exclude those with β thalassaemia by the presence of raised HbA_2 and to identify those carrying two or more gene deletions. Non-genetic testing using HbH inclusions is considered to be of poor sensitivity and specificity for diagnosing α thalassaemia. The cis deletions (α^0 phenotype) are those most critical in pregnancy, as they are the most likely to lead to an Hb Bart's affected fetus, if both parents are carriers.

Figure 1 flowchart

Women of reproductive age (15-50years)

Partners of confirmed or likely α thal carriers

Microcytic hypochromic anaemia on FBC (MCV<80fL; MCH<28pg), not iron deficient (ferritin > 29)

Thalassaemia studies (items 65078 & 65081)

β thal confirmed by high HbA2 on electrophoresis

β thal excluded by HbA2 <3.4%

α thal confirmed by HbH bodies/HbH

α thal not confirmed or excluded

Consider thalassaemia studies for partners of confirmed α thal carriers

Genetic deletion testing to identify α thal mutation/s

α thal confirmed by genetic deletion testing

α thal excluded by deletion testing

Further testing required for rare α thal mutations or alternate diagnosis

The woman is the only α thal carrier

Both partners confirmed as α thal deletion carriers

The woman is not an α thal deletion carrier

Partner 1: αα/α- or -/α-
Partner 2: αα/α- or -/α-
Probability of
Hb Barts 0%
HbH 0%

Partner 1: αα/α-
Partner 2: αα/-- or α-/--
Probability of
Hb Barts 0%
HbH 25%

Partner 1: α-/α-
Partner 2: αα/-- or α-/--
Probability of
Hb Barts 0%
HbH 50%

Partner 1: αα/--
Partner 2: αα/--
Probability of
Hb Barts 25%
HbH 0%

Partner 1: α-/--
Partner 2: αα/--
Probability of
Hb Barts 25%
HbH 25%

Partner 1: α-/--
Partner 2: α-/--
Probability of
Hb Barts 25%
HbH 50%

Health benefits eg iron supplementation avoided, further testing avoided, diagnosis confirmed (normal reproductive outcomes/choices)

Health benefits eg iron supplementation avoided, further testing avoided, diagnosis confirmed, informed reproductive choices, avoidance of Hb Barts, avoidance of serious HbH case, in utero treatment for Hb Barts or HbH

Health benefits eg avoidance of unnecessary treatments, access to treatments for alternative diagnosis, relevant reproductive choices

Figure 1 Proposed diagnostic algorithm for women of reproductive age (15-50 years), and their partners

α thal = alpha thalassaemia; β thal = beta thalassaemia; FBC = full blood count; HbA$_2$ = haemoglobin A2; HbH = haemoglobin H; MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume

Figure 2 flowchart

Pregnant women

Partners of confirmed or likely α thal carriers

Microcytic hypochromic anaemia on FBC (MCV<80fL; MCH<28pg), not iron deficient (ferritin > 29)

Thalassaemia studies (items 65078 & 65081)

β thal confirmed by high HbA2 on electrophoresis

β thal excluded by HbA2 <3.4%

α thal confirmed by HbH bodies/HbH

α thal not confirmed or excluded

Consider thalassaemia studies for partners of confirmed α thal carriers

Genetic deletion testing to identify α thal mutation/s

α thal confirmed by genetic deletion testing

α thal excluded by deletion testing

Further testing required for rare α thal mutations or alternate diagnosis

The woman is the only α thal carrier

Both partners confirmed as α thal deletion carriers

The woman is not an α thal deletion carrier

Partner 1: αα/α- or -/α-
Partner 2: αα/α- or -/α-
Probability of
Hb Barts 0%
HbH 0%

Partner 1: αα/α-
Partner 2: αα/-- or α-/--
Probability of
Hb Barts 0%
HbH 25%

Partner 1: α-/α-
Partner 2: αα/-- or α-/--
Probability of
Hb Barts 0%
HbH 50%

Partner 1: αα/--
Partner 2: αα/--
Probability of
Hb Barts 25%
HbH 0%

Partner 1: α-/--
Partner 2: αα/--
Probability of
Hb Barts 25%
HbH 25%

Partner 1: α-/--
Partner 2: α-/--
Probability of
Hb Barts 25%
HbH 50%

Health benefits eg iron supplementation avoided, further testing avoided, diagnosis confirmed (normal reproductive outcomes/choices)

Health benefits eg iron supplementation avoided, further testing avoided, diagnosis confirmed, informed reproductive choices, avoidance of Hb Barts, avoidance of serious HbH case, in utero treatment for Hb Barts or HbH

Health benefits eg avoidance of unnecessary treatments, access to treatments for alternative diagnosis or rare α thal mutation, relevant reproductive choices

Figure 2 Proposed diagnostic algorithm for pregnant women and their partners

α thal = alpha thalassaemia; β thal = beta thalassaemia; FBC = full blood count; HbA$_2$ = haemoglobin A2; HbH = haemoglobin H; MCH = mean corpuscular haemoglobin; MCV = mean corpuscular volume

ESC noted that "iron deficiency if pregnant and no historic normal cell indicies" were not captured in the clinical pathways above

10. Comparative safety

The Population, Priori tests, Intervention, Comparator and Outcome (PPICO) elements were established in the Application Referral that was submitted to the MSAC Executive, providing pre-specified search criteria for the contracted assessment (CA).

Two non-comparative (level IV) studies (Table 2) met the safety, effectiveness and cost criteria established in the PPICO and provided direct evidence for the safety of deletion testing using gap-PCR. Both reported on large Southeast Asian screening programs for couples who were either pregnant or planning a pregnancy.

Table 2. Studies providing direct evidence for α-thalassaemia deletion testing in people of child bearing age (taken from the CA)

Trial/ Study	N	Design / duration	Risk of bias	Patient population	Key outcome(s)	Result used in economic evaluation
Jiang et al. 2017	11,039 couples	Case series Level IV	Low	Couples of child-bearing age	Prevalence of HbH & Hb Bart's Uptake of PND Uptake of TOP	Yes
Yamsri et al 2010	1,422 couples	Case series Level IV	Moderate	Couples positive for α or β-thal	Uptake of PND Couples at risk of an α^0-thal thalassaemia	No

For linked evidence, the evidence in some cases was broader than that of interest. Twenty-one studies (n=170,233; level III-1 to IV, Table 3) met the sensitivity, specificity, positive/negative predictive value, diagnostic yield, need for re-resting, reliability and reproducibility criteria laid out in the PPICO and the diagnostic performance was assessed. Seven studies assessed comparative clinical validity (n=2,112; level III-2) shown in Table 4. No studies met the PPICO pre-specified criteria of change in management outcomes and therapeutic effectiveness for clinical utility. To address this latter issue, the CA stated that studies, which included people at risk of all HbPs, were used to provide information on changes in management associated with HbP testing, as well as the clinical consequences of those changes in management.

One Australian study (Prior, Bittles & Erber 2004, Table 3) was included in the assessment.

Table 3. Studies reporting on analytical validity

Study Country	Study design Level of evidence[a] Quality appraisal[b]	Study population characteristics	Eligibility criteria Study objective	Intervention	Comparator	Outcomes assessed Statistical analysis	Comments Funding source
Basha, Mularo & Cook 2017 USA	A comparative study without concurrent controls Level IV High risk of bias	N = 423 clinical samples Samples from patients submitted for diagnosis at a clinic	*Criteria* Diagnosis: consecutive cases over 24 months *Objective* To describe the development , validation and implementation of a 2-tube α-thal test	Deletion testing by gap-PCR using fluorescent labelling and CE for 6 common mutations ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $-\alpha^{20.5}$, $--^{SEA}$, $--^{MED}$, $--^{FIL}$)	-	Diagnostic yield	*Funding*: NR
Bergeron et al 2005 Canada	Comparison with reference standard that does not meet criteria for Level II or III-1 evidence. Level IV moderate risk of bias	N = 196 Patients attending a hospital with unexplained microcytosis Age > 18y	*Criteria* Blood samples of patients tested with normal ferritin and HbA$_2$, no abnormal Hb detected by HPLC *Objective* To identify the proportion of patients with unexplained microcytosis who have α-thal	Multiplex PCR for 7 deletions[a] ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $-\alpha^{20.5}$, $--^{SEA}$, $--^{MED}$, $--^{FIL}$, $--^{Thai}$)	-	Diagnostic yield	Group assessed by PCR: Gp 3 (n = 204): negative HbH & MCV ≤82fL, or diagnosed with other HbP– PCR not performed for 7 case due to poor quality DNA, and PCR failed in one case Funding: NR
Chaibunruang et al 2013 Thailand	Case series Level IV Moderate risk of bias	N = 12,525 Samples from a large referral and research centre for HbP	*Criteria* Referral from a hospital to the research centre *Objective* Analysis of molecular, genetic and prevalence data in a large cohort	Deletion testing using gap-PCR or real-time PCR ($--^{SEA}$, $--^{THAI}$), multiplex PCR for deletion and non-deletion mutations ($-\alpha^{3.7}$, $-\alpha^{4.2}$, α^{CS}, α^{Pakse})	-	Diagnostic yield	*Funding*: National Research University program grant, Khon Kaen University & Office of the Higher Education Commission, Ministry of Education, Thailand
Chaibunruang et al 2010 Thailand	Case series Level IV Moderate risk of bias	N = 206 Left over blood samples of suspected carriers used to test screening protocol	*Criteria* Hypochromic microcytic anaemia, excluded for β-thal Objective Tot test an improved screening protocol	Deletion testing by gap-PCR ($--^{SEA}$, $--^{THAI}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$), and non-deletions (α^{CS}, α^{Pakse})	α-thal status by screening test (OF, DCIP, HbH inclusions)	Diagnostic yield Concordance	*Funding*: grants from Khon Kaen University, Office of Higher Education Commission, Ministry of Education, Thailand & the Royal Golden Jubilee

Study Country	Study design Level of evidence[a] Quality appraisal[b]	Study population characteristics	Eligibility criteria Study objective	Intervention	Comparator	Outcomes assessed Statistical analysis	Comments Funding source
							PhD program of the Thailand Research Fund
Galanello et al 1998 Italy	Case series Level IV Low risk of bias	N = 526 Adults of Sardinian descent	*Criteria* Individuals screening negative for β-thal, MCV <79fL, MCH <27pg, normal HbA$_2$ and iron status *Objective* Identification of α-thal carriers using PCR and a simplified screening approach	Deletion testing by PCR for 4 common mutations (-$\alpha^{3.7}$, -$\alpha^{4.2}$, -$\alpha^{20.5}$, --^{MED}) and RE	Red cell indices	Diagnostic yield	*Funding*: Grants from CNR-Target project (#N.91.00012.pf99), Theleton (#E 502), Fondazione Italiana Leonardo Giambrone, Regione Sardegna (#30.04.1990)
Gilad et al 2017 Israel	A comparative study with concurrent controls Level III-2 High risk of bias	N = 975 Samples of patients referred to a single centre in Israel from 1994-2014 Age range (y): 0.5-85	*Criteria* Individuals referred for diagnosis due to microcytosis with or without anaemia, excluded for β-thal and iron deficiency *Objective* Accurate diagnosis of thalassaemia by Gap-PCR, gene sequencing and MLPA	Deletion testing by gap-PCR for common mutations (-$\alpha^{3.7}$, -$\alpha^{4.2}$, -$\alpha^{20.5}$, --^{MED}, --^{SEA}, --^{Thai}, --^{Fil})	Gene sequencing for point mutations MLPA for rare mutations	Sensitivity Specificity Diagnostic yield	Primers for --^{SEA}, --^{Thai} and --^{Fil} were added to the multiplex Gap-PCR if a patients was of Southeast Asian origin. Up until 2010, point mutation analysis was carried out by restriction enzyme analysis, and afterwards by sequencing of the HBA gene. *Funding*: NR
Giordano et al. 2006 Netherlands	Study of diagnostic yield Level IV Moderate risk of bias	N = 139 Pregnant women contracted regarding a pilot test for HbP testing N = 5 Partners of detected α-thal carriers	*Criteria* Women in early pregnancy attending a hospital outpatient department *Objective* To assess the use of a screening program for first-line health-care providers	Gap-PCR for common deletions (-$\alpha^{3.7}$, -$\alpha^{4.2}$, -$\alpha^{20.5}$, --^{MED}, --^{SEA}, --^{Thai}, --^{Fil})	No testing (prior tests only)	Diagnostic yield	Diagnosis of β-thal was also performed *Funding*: NR
Gohari et al 2003	Study of diagnostic yield Level IV	N = 69 Individuals who underwent pre-marital	*Criteria* Individuals with abnormal MCV or MCH and normal HbA$_2$	PCR for 7 common mutations (-$\alpha^{3.7}$, -	No testing (prior tests only)	Diagnostic yield	*Funding*: NR

Study Country	Study design Level of evidence[a] Quality appraisal[b]	Study population characteristics	Eligibility criteria Study objective	Intervention	Comparator	Outcomes assessed Statistical analysis	Comments Funding source
Iran	Moderate risk of bias	carrier screening (initiated in 1992)	*Objective* To explore the spectrum of α-thal mutations in Iran	$\alpha^{4.2}$, $-\alpha^{20.5}$, $--^{MED}$, $--^{SEA}$, $--^{Thai}$, $--^{Fil}$), followed by RE analysis for non-deletion mutations			
Hafezi-Nejad et al. 2014 Iran	Case series Level IV Low risk of bias	N = 754 couples Iranian couples wanting children and at risk of HbP	*Criteria* Couples counselled for HbP screening in Iran *Objective* To determine the need for inclusion of HbP screening other than that for β-thal in the nationwide program	Deletion testing for common 4 mutations ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $-\alpha^{20.5}$, $--^{MED}$), followed by reverse strip assay to 4 deletions ($-\alpha^{20.5}$, $--^{SEA}$, $--^{Thai}$, $--^{Fil}$)	Gene sequencing and MLPA	Diagnostic yield	Insufficient detail to extract results for comparator *Funding*: NR
Henderson et al 2009 UK	Case series Level IV Moderate risk of bias	N = 2,500 Individuals referred to 2 centres with possible α-thal over a four year period	*Criteria* Diagnosed with possible α-thal with MCH<25pg and haematological studies	Gap-PCR for common deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $-\alpha^{20.5}$, $--^{MEDI}$, $--^{MEDII}$, $--^{SEA}$, $--^{Thai}$, $--^{Fil}$)	direct sequencing for non-deletions, MLPA for those testing negative	Diagnostic yield	*Funding*: European Commission project grant RI-2004-026539, & Oxford Partnership Comprehensive Biomedical Research Centre NIHR fund
Hossein et al 2012 Iran	Study of diagnostic yield Level IV Low risk of bias	N = 2000 Age range: 18-36 y Couples referred to a premarital screening program	*Criteria* Microcytic hypochromic anaemia, excluded for β-thal, HbS and iron deficiency *Objective* To evaluate mutations in two provinces of southern Iran	Gap-PCR followed by multiplex PCR and reverse hybridisation test strips for common ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $-\alpha^{20.5}$, $--^{MED}$) and non-deletions (α^{-5nt}, $\alpha^{poly\,A1}$, $\alpha^{poly\,A2}$, $-\alpha^{codon\,19}$, $\alpha\alpha\alpha^{anti\,3.7}$)	-	Diagnostic yield	The article was not explicit about which mutations were detected with each interventional method. *Funding*: NR

Study Country	Study design Level of evidence[a] Quality appraisal[b]	Study population characteristics	Eligibility criteria Study objective	Intervention	Comparator	Outcomes assessed Statistical analysis	Comments Funding source
Huang et al 2017 China	Case control study Level III-2 Moderate risk of bias	N = 1,213 Pre-characterised DNA samples from 3 Chinese hospitals	*Criteria* Clinical samples previously genotyped by multiple Gap-PCR/RBD analysis *Objective* To assess the performance of a real-time PCR technique	Gap-PCR and RBD for common mutations: 4 deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$, $--^{Thai}$), and 3 common non-deletions	Real-time PCR melting curve analysis	Concordance	The clinical samples include 936 samples from whole blood, and 277 samples from amniotic fluid *Funding*: National Natural Science Foundation of China (#81101323 & #81360091), Guangxi Key Laboratory Thalassaemia Research Project (#15-140-11), Liuzhou Science and Technology Development Funds (#2014G020404) & 2011 Collaborative Innovation Centre of Guangxi Biological Medicine
Jiang et al 2017 China	Study of diagnostic yield Level IV Low risk of bias	N = 41,531 Couples of child bearing age	*Criteria* Pre-gestational couples at risk of children with significant thalassaemia disease *Objective* Carrier identification in couples taking up free screening in southern province in China	Gap-PCR for common deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $-^{SEA}$, $--^{Thai}$) and RDB for common non-deletions	No testing (prior tests only)	Incremental diagnostic information	*Funding*: National Natural Science Foundation of China (#81571448) & Guangdong Provincial Department of Science and Technology agency (#2016A020215218)
Kohne & Kleihauer 2010 Germany	Study of diagnostic yield Level IV Low risk of bias	N = 100,621 Retrospective analysis of samples tested from 1971-2007 at a German university	*Criteria* Previous haematological findings *Objective* To determine the occurrence, spectrum and geographical distribution of Hb defects in Germany	Multiplex single-tube PCR for 6 common deletions, MLPA, MAPH, Single strand sequencing for non-deletions	No testing (prior tests only)	Diagnostic yield	Unable to extract data on diagnosis by comparator *Funding*: NR

Study Country	Study design Level of evidence[a] Quality appraisal[b]	Study population characteristics	Eligibility criteria Study objective	Intervention	Comparator	Outcomes assessed Statistical analysis	Comments Funding source
Kipp et al 2011 USA	Case control study Level III-2 Moderate risk of bias	N = 5,386 Specimens previously clinically tested in a clinic and laboratory	*Criteria* Samples from patients tested for α-thal from Jun 2007-Apr 2010 *Objective* To assess a combined assay of Multiplex PCR and MLPA for α-thal diagnosis	Multiplex PCR for 2 common deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$) and MLPA for common deletions and duplications	Southern blot	Concordance Test failure rate	*Funding*: NR
Liu et al 2000 UK	Case control study Level III-2 High risk of bias	N = 52 DNA samples previously genotyped	*Criteria* NR *Objective* To assess a Multiplex PCR method	Multiplex PCR for 7 common deletions ($-\alpha^{20.5}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$, $--^{Thai}$, $--^{Fil}$, $--^{MED}$) and one triplication ($\alpha\alpha\alpha^{anti3.7}$)	Southern blot	Concordance	*Funding*: support from Wellcome Trust, Medical Research Council and a grant to JMO from the International Atomic Energy Agency (Technical Contract no. 15011/RO).
Prior, Bittles & Erber 2004 Australia	Study of diagnostic yield Level IV Low risk of bias	N = 920 Samples submitted to Western Australia pathology laboratories in a 12 month period Mean age: 32 y Age range: 5 days -88 y	*Criteria* Samples submitted for Hb investigation from 1. The Migrant Health Service with abnormal indices & 2. Molecular analysis requests from private and public laboratories following HbH screening *Objective* To estimate prevalence of α-thal in WA	Multiplex PCR for 7 common deletions ($-\alpha^{20.5}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$, $--^{Thai}$, $--^{Fil}$, $--^{MED}$), PCR/RE for non-deletions (Nco1, Hph1 (-5nt), Mse1 and CS)	-	Diagnostic yield	*Funding*: support from the Genomics Directorate of the Western Australia Department of Health
Sorour et al 2007 United Kingdom	Study of diagnostic yield Level IV Low risk of bias	N = 425 Women screened for thalassaemias at an ante-natal clinic	*Criteria* Women screening negative for β-thal, *Objective* To assess Multiplex PCR testing for common α-thal	Multiplex PCR for six common deletions ($-\alpha^{20.5}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$, $--^{Fil}$, $--^{MED}$)	Red cell and indices and ethnicity	Incremental diagnostic information	Diagnostic accuracy only *Funding*: NR

Study Country	Study design Level of evidence[a] Quality appraisal[b]	Study population characteristics	Eligibility criteria Study objective	Intervention	Comparator	Outcomes assessed Statistical analysis	Comments Funding source
			deletions as a routine screening tool, alongside a standard ethnic origin/low MCH approach				
Timmann et al 2005 Ghana	Study of diagnostic yield Level IV Moderate risk of bias	N = 122 Individuals of a West African population with moderate prevalence of the $-\alpha^{3.7}$ deletion	*Criteria* Informed consent *Objective* To validate a melting curve analysis method for α-thal diagnosis	Gap-PCR for six common deletions ($-\alpha^{20.5}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$, $--^{Fil}$, $--^{MED}$)	Red cell and Hb indices, HbH inclusions, MCA ratio	Incremental diagnostic information	MCA is not relevant comparator, however diagnosis by Gap-PCR was reported *Funding*: National Genome Research Network of the German Ministry of Education and Research, Volkswagen Foundation, & a doctoral grant (FM) at the University of Hamburg
Turner et al 2015 United Arab Emirates	Study of diagnostic yield Level IV Moderate risk of bias	N = 167 Archived samples from a single laboratory Known genotypes: n=n105 Unknown genotypes: n= 62	*Criteria* Samples with known genotype to establish a protocol, samples of unknown genotype for validation *Objective* Development of the GRACE-PCR assay	Gap-PCR for 7 common deletions ($-\alpha^{20.5}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$, $--^{Fil}$, $--^{MED}$, $--^{THAI}$) and common non-deletions including ($\alpha\alpha^{CS}$, $\alpha\alpha^{Icara}$, $\alpha\alpha^{polyA-1}$, $\alpha\alpha^{polyA-2}$)	GRACE-PCR screen and MCA analysis	Incremental diagnostic information	GRACE-PCR is not a relevant comparator, however diagnosis by Gap-PCR was reported *Funding*: NR
Waye et al 2001 Canada	Study of diagnostic yield Level IV Moderate risk of bias	N = 116 Patients with HbH disease living in Canada Age range: 1-75 y N = 28 Partners of adults with HbH disease	*Criteria* HbH disease previously diagnosed *Objective* To determine genotypes associated with HbH disease in Canadian patients	PCR for 6 common deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $-^{SEA}$, $--^{Fil}$, $--^{MED}$, $--^{THAI}$) and non-deletions by sequencing	Prior diagnosis with HbH disease (tests not specified)	Incremental diagnostic information	Population is those with HbH disease *Funding*: NR

Table 4. Studies reporting on clinical validity

Study Country	Study design Level of evidence[a] Quality appraisal[b]	Study population characteristics	Eligibility criteria Study objective	Intervention	Comparator	Outcomes assessed Statistical analysis	Comments Funding source
Agarwal et al. 2013 USA	Comparison with reference standard that does not meet criteria for Level II or III-1 evidence. Level III-2 Moderate risk of bias	N = 67 Suspected silent carriers or α-thal trait Age range: 18-31y	*Criteria* Whole blood samples selected based on HbH concentration, HbH <2%: n = 59, HbH 2-5%: n = 3, HbH 0%: n= 5 *Objective* Detection of silent carriers by IFE	Deletion testing by Gap-PCR multiplex assay for 7 common deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $-\alpha^{20.5}$, $--^{MED}$, $--^{SEA}$, $--^{Thai}$, $--^{Fil}$)	HbH concentration (HPLC & IFE)	Diagnostic accuracy	Technicians were blinded to phenotype prior to genotype analysis Population broad *Funding*: NR
Bergstrome Jones & Poon 2002 Canada	Comparison with reference standard that does not meet criteria for Level II or III-1 evidence. Level III-2 Moderate risk of bias	N = 452 Samples from patients referred for HbP analysis Age ≥4y Group 1 - patients referred for thal or Hb investigation in 1 week: n = 89 Group 2 – positive results on HbH prep or MCV ≤82fL: n = 65 Group3 – negative on HbH prep and MCV ≤82fL: n = 297 Post implementation group: n = 298	*Criteria* Peripheral blood samples, positive for HbH, or unconfirmed for α-thal Samples from patients <4 years excluded *Objective* To compare multiplex PCR with current screening approach for detection of α-thal	Deletion testing by Single tube multiplex PCR for 6 common deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $-\alpha^{20.5}$, $--^{MED}$, $--^{SEA}$, $--^{Fil}$)	Diagnosis by standard haematological methods (Red cell & Hb indices, HbH bodies)	Correlation of genotypes with haematology results Sensitivity Specificity	Age group broad *Funding*: NR
Chaibunruang et al 2010 Thailand	Case series Level IV Moderate risk of bias	N = 206 Left over blood samples of suspected carriers used to test screening protocol	*Criteria* Hypochromic microcytic anaemia, excluded for β-thal Objective Tot test an improved screening protocol	Deletion testing by gap-PCR ($--^{SEA}$, $--^{THAI}$, $-\alpha^{3.7}$, $-\alpha^{4.2}$), and non-deletions (α^{CS}, α^{Pakse})	α-thal status by screening test (OF, DCIP, HbH inclusions)	Diagnostic yield Concordance	*Funding*: grants from Khon Kaen University, Office of Higher Education Commission, Ministry of Education, Thailand & the Royal Golden Jubilee PhD

Study Country	Study design Level of evidence[a] Quality appraisal[b]	Study population characteristics	Eligibility criteria Study objective	Intervention	Comparator	Outcomes assessed Statistical analysis	Comments Funding source
							program of the Thailand Research Fund
Colosimo et al 2011 Italy	Case control study Level III-2 Moderate risk of bias	N = 25 Patients referred to a centre in Rome Age range 4-73 y	*Criteria* Samples from patients found doubtful or negative for common mutations *Objective* To assess the MLPA test for identifying α-thal mutations not identified through usual methods	MLPA (-$\alpha^{3.7}$, -$\alpha^{4.2}$, --^{CAL}, --^{FIL}, -^{SEA}, - -^{MED})	Conventional molecular PCR screening for common deletions and variants (-$\alpha^{3.7}$, -$\alpha^{4.2}$, -$\alpha^{20.5}$, - -^{MED}, α^{HphI}, α^{NcoI}, $\alpha\alpha\alpha^{anti3.7I}$)	Concordance	Comparison of 2 DNA analysis methods 21 out of 25 participants were of reproductive age (19-42 years) *Funding*: NR
Ebrahimkhani et al 2011 Iran	Comparison with reference standard that does not meet criteria for Level II or III-1 evidence. Level III-2 Low risk of bias	N = 40 Patients referred to a pathology and genetics centre in Iran Age (± SD): 25.7±16y	*Criteria* Patients with known HbH disease and giving consent *Objective* To determine the relationship between phenotype and genotype of HbH disease in Iran	Deletion testing by gap-PCR (-$\alpha^{3.7}$, -$\alpha^{4.2}$, --^{MED}) and multiplex PCR by StripAssay (--^{SEA}, --^{THAI}, --^{FIL}), and a further 11 mutations by StripAssay	HbH disease severity by transfusion and splenectomy status	Incremental diagnostic information Correlation between genotype and HbH disease severity Statistical analysis by chi-square testing (95%CI) using SPSS11.5.	*Funding*: NR
Gilad et al 2017 Israel	A comparative study with concurrent controls Level III-2 Low risk of bias	N = 975 Samples of patients referred to a single centre in Israel from 1994-2014 Age range (y): 0.5-85	*Criteria* Individuals referred for diagnosis due to microcytosis with or without anaemia, excluded for β-thal and iron deficiency *Objective* Accurate diagnosis of thalassaemia by Gap-PCR, gene sequencing and MLPA	Deletion testing by gap-PCR for common mutations (-$\alpha^{3.7}$, -$\alpha^{4.2}$, -$\alpha^{20.5}$, --^{MED}, --^{SEA}, --^{Thai}, --^{Fil})	Clinical evaluation (including prior tests) Disease severity (silent carrier, thal trait or HbH disease)	Diagnostic accuracy Correlation between genotype and HbH disease severity	Primers for --^{SEA}, --^{Thai} and --^{Fil} were added to the multiplex Gap-PCR if a patients was of Southeast Asian origin. Up until 2010, point mutation analysis was carried out by restriction enzyme analysis, and afterwards by sequencing of the HBA gene. *Funding*: NR

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Study Country	Study design Level of evidence[a] Quality appraisal[b]	Study population characteristics	Eligibility criteria Study objective	Intervention	Comparator	Outcomes assessed Statistical analysis	Comments Funding source
Griswold et al 2002 Canada	A comparative study with concurrent controls Level III-2 Low risk of bias	N = 347 Consecutive patients with α-thal deletions Age 10-17 y: 26 (8%) Age 18-45 y: 240 (69%) Age >45 y: 23 (23%)	*Criteria* Individuals with 1 or 2 gene deletions selected from patients referred for Hb investigation *Objective* Clarification of genotypes	Deletion testing by Multiplex PCR for 6 common mutations (-$\alpha^{3.7}$, -$\alpha^{4.2}$, -$\alpha^{20.5}$, --^{MED}, --^{SEA}, , --^{Fil})	Red cell indices, HPLC for HbA$_2$, HbF and other Hb variants HbH bodies	Concordance	69% of patients were of reproductive age *Funding*: NR
Prior, Bittles & Erber 2004 Australia	Study of diagnostic yield Level III-2 Low risk of bias	N = 920 Samples submitted to Western Australia pathology laboratories in a 12 month period Mean age: 32 y Age range: 5 days -88 y	*Criteria* Samples submitted for Hb investigation from 1. The Migrant Health Service with abnormal indices & 2. Molecular analysis requests from private and public laboratories following HbH screening *Objective* To estimate prevalence of α-thal in WA	Multiplex PCR for 7 common deletions (-$\alpha^{20.5}$, -$\alpha^{3.7}$, -$\alpha^{4.2}$, --^{SEA}, --^{Thai}, --^{Fil}, --^{MED}), PCR/RE for non-deletions (Nco1, Hph1 (-5nt), Mse1 and CS)	Red cell indices, HPLC for HbA$_2$, HbF and other Hb variants HbH bodies	Incremental diagnostic benefit	*Funding*: support from the Genomics Directorate of the Western Australia Department of Health

Test adverse events
Genetic deletion testing is performed on DNA extracted from a blood sample. The assessment did not expect there would be adverse events directly associated with testing, apart from the transient discomfort caused by obtaining the sample. If a DNA sample is insufficient or of too poor quality to provide a result, a second blood sample may be required.

One of the main adverse effects associated with the downstream effects of testing is expected to be the psychological impact of possibly needing to make reproductive choices,using the information obtained. .

Adverse events from change in management
ESC found that there was no evidence meeting the PPICO criteria to determine whether deletion testing changes patient management. Diagnostic accuracy evidence for deletion testing was lacking as there was no independent reference standard in the literature. When deletion testing alone was compared with deletion testing followed by direct sequencing (DS), false positive cases could not be verified, because the tests are sequential and the deletion testing result is assumed to be definitive. Deletion testing is not 100% accurate, although the false positive rate may be very low. In the case of a false positive result, a woman and her partner may undergo unnecessary testing, including PND, and this may lead to unnecessary worry and trauma from prenatal sampling.

ESC noted that for those people found negative by deletion testing but who in actuality do carry a clinically significant deletion or other mutation, there could be consequences for the offspring, if the mutation is inherited with a second significant mutation from the other parent. This scenario is likely because deletions and non-deletion mutations occur that are not included in a common deletion test (GAP-PCR) panel. This is the rationale for having samples that are negative on GAP-PCR deletion testing then undergo MLPA and/or direct (Sanger) sequencing. .

11.   Comparative effectiveness

Direct effectiveness
Two non-comparative studies (Jiang et al 2017, Yamsri et al 2010; Table 2) provided evidence for the proportion of couples that underwent PND following diagnosis of an Hb Bart's affected fetus. One of the studies also reported the proportion of couples that underwent TOP (Table 5). The studies found that couples, in whom both partners tested positive for a clinically significant deletion by gap-PCR or MLPA, were likely to undergo PND when offered (100% and 73%). The lower uptake of PND was influenced by gestational age. Of those who were at 10-13 weeks gestation, 100% underwent TOP (n = 66). When the fetus tested positive for Hb Bart's, 10 out of 10 couples chose to terminate the pregnancy. These studies were performed in countries where α thalassaemia has a high prevalence and maternal screening programs are being trialled.

Table 5 Balance of clinical benefits and harms of deletion testing as measured by the critical patient-relevant outcomes in the key studies

Outcomes (units) Follow-up	Studies (K) Couples (N)	Quality of evidence (GRADE)[a]	Result
Uptake of PND (couples at risk of having an Hb Bart's fetus)	K = 2 N = 42,499	⊕⊙⊙⊙	66/90 (73%) to 304/304 (100%)
Uptake of TOP (couples diagnosed with an Hb Bart's fetus)	K = 1 N = 41,531	⊕⊙⊙⊙	10/10 (100%)

[a] GRADE Working Group grades of evidence (Guyatt et al., 2013)

⊕⊕⊕⊕ **High quality:** We are very confident that the true effect lies close to that of the estimate of effect.

⊕⊕⊕⊙ **Moderate quality:** We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.

⊕⊕⊙⊙ **Low quality:** Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect.

⊕⊙⊙⊙ **Very low quality:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect.

There was no evidence of what the uptake of PND and TOP would be in the absence of deletion testing. It is possible that PND may be performed based on thalassaemia studies or, later in pregnancy based on ultrasound, but it is likely that the rates of PND and TOP would be lower than if deletion testing was being performed. By inference if couples do not know their α thalassaemia status, they cannot make the same reproductive choices.

Linked evidence

Diagnostic performance

The assessment noted that little evidence was identified for the populations specified in the PPICO. The inclusion of broader populations (generally a wider age range) was not expected to impact on the applicability of the results for diagnostic performance due to the condition being heritable and not age related.

It is difficult to determine the sensitivity and specificity, Negative Predictive Value (NPV) and Positive Predictive Value (PPV) for GAP-PCR or MLPA deletion testing alone compared to GAP-PCR or MLPA deletion testing followed by DS, due to the step-wise nature of the testing in the literature. False positive results are unable to be verified properly (partial verification bias, O'Sullivan *et al.* 2018) when testing is performed in this manner, as subjects found positive for a deletion do not continue on to DS (subjects that are negative for a deletion will continue on to DS).

In two studies of participants of broader age range than specified in the PPICO, deletion testing identified 75% to 88% of all mutations identified by step-wise use of both deletion testing and DS. The study by Henderson et al (Table 2), conducted in the UK in a population of mixed ethnicity, is likely to be more relevant to the Australian setting (sensitivity 88%).

Gap-PCR and MLPA are highly concordant with other DNA analysis techniques when they are performed on the same sample and in a population which has undergone Hb analysis (99% – 100%), supporting the notion that deletion testing is an accurate and reliable test. However gap-PCR is targeted to specific deletions and will not detect deletions in regions not tested for or unknown. MLPA is a more flexible technique and can be used to detect a wider range of mutation types.

Diagnostic yield varies according to population prevalence of α thalassaemia, prior screening in the population under investigation, and the number of deletions and non-deletions tested for. The $--^{MED}$ and $--^{SEA}$ are the most common deletions, and occur at high rates within Middle Eastern and Southeast Asian regions respectively. The most commonly occurring

deletion occurring worldwide is -$\alpha^{3.7}$. The most prevalent non-deletion mutations also vary from region to region

Clinical validity
Eight studies (Table 4) compared diagnosis by HbH assay (a component of thalassaemia studies) with diagnosis by GAP-PCR and MLPA deletion testing (Note 7 of the 8 studies used GAP-PCR testing).

In those considered to be at high risk of HbH disease and at reproductive age the tests had a high correlation (100%) for diagnosis of HbH disease (defined as three HBA gene deletions), when HbH was diagnosed by HbH assay. Deletion testing did not add incremental value in this small group. However overall, HbH assays could not easily distinguish between HbH disease, α0 and α+ genotypes, the latter two often being categorised α-trait (two deletion genotypes in general).

For those of reproductive age, deletion testing provided additional diagnosis in 18% (18 cases per 100 tested) and HbH assays gave false positive results in 4.7% (5 cases per 100 tested). For suspected carriers of any age, deletion testing provided an additional diagnosis in 36% (36 cases per 100 tested) and HbH assays gave a false positive results in 1.1% (1 case per 100 tested). (Note 7 of the 8 studies used GAP-PCR testing).

Therapeutic efficacy (change in management)
Three studies identified in the literature search but included broader populations than those specified by the PPICO inclusion criteria ('The first five years of a preventive programme for haemoglobinopathies in Northeastern Iraq' Al-Allawi et al. (2013); 'Prenatal diagnosis of haemoglobinopathies: Our experience of 523 cases' Grosso et al. (2013); and 'Carrier screening for α- and β-thalassemia in pregnancy: The results of an 11-year prospective program in Guangzhou Maternal and Neonatal Hospital' Liao et al. (2005)), reported decisions made by at-risk couples following diagnosis of HbP by DNA analysis. Decisions by way of uptake of PND, TOP or continuation of pregnancy were reported.

Couples for whom both partners tested positive for a clinically significant mutation, were likely to take up PND when offered (74.8%-94.7%). If the pregnant couples were found to carry an Hb Bart's fetus, they were highly likely to undergo TOP. The CA found there was no evidence to indicate what couples would do in the absence of mutation analysis, but it could be assumed that without information about their genetic status couples are less likely to take up PND or TOP, or even be given these options. Almost all who carried an Hb Bart's affected fetus (179/182 affected fetuses) chose to terminate the pregnancy.

Therapeutic effectiveness (health benefit from possible changes in management)
No studies were identified meeting the PPICO inclusion criteria for therapeutic effectiveness (including but not limited to tangible health benefits, improvement of quality of life, pregnancy outcomes).

The primary benefits (or harms) of management changes from deletion testing are expected to be those associated with reproductive choices made available to couples by the genetic information provided by the test. The same level of certainty about a genetic alteration is not available without deletion testing or another form of genetic testing.

Clinical Claim

The clinical evaluation suggested that, relative to a couple's risk of carrying Hb Bart's fetus being determined based on screening tests alone, genetic deletion testing for α thalassaemia has non-inferior safety and superior effectiveness.

12. Economic evaluation

The summary of the CAs economic evaluation is presented inTable 6

Table 6   Summary of the economic evaluation

Perspective	Australian healthcare system (direct health care costs only)
Population	1. Couples that are planning pregnancy 2. Couples that are pregnant
Prior testing	FBC, ferritin and thalassaemia studies
Comparator	No testing [a]
Type of economic evaluation	Cost-effectiveness analysis.

Outcomes	1. In couples planning pregnancy
	    a) Cost per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's (enabling careful family planning)
	    b) Cost per couple with genetically confirmed status (i.e. correctly identifying couples at risk of Hb Bart's and correctly identifying those not at risk)
	2. In couples that are pregnant
	    a) Cost per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's (enabling careful family planning)
	    b) Cost per couple with genetically confirmed status (i.e. correctly identifying couples at risk of Hb Bart's and correctly identifying those not at risk)
	    c) Cost per avoided case of Hb Bart's that is terminated late, stillborn or dies shortly after birth

Sources of evidence	Systematic review
Time horizon	Short-term (less than one year). 1. In couples planning pregnancy: time to identify the couples risk of Hb Bart's 2. In couples that are pregnant: time to reach a diagnosis in the fetus
Methods used to generate results	Decision tree analysis
Software packages used	Microsoft Excel and TreeAge Pro

FBC=full blood count.

[a] this comparison is a hypothetical/historical context for cost-effectiveness assessment purpose. Private and State/Territory funded testing is currently available and utilised in Australian practice

In the base case analysis, only couples that are at risk of Hb Bart's due to common deletions will be identified (i.e. those within the black box denoted in Figure 3). Deletion testing is assumed to perfectly identify people with common deletions.

Figure 3 Matrix to determine the couple's risk of having an affected pregnancy

		Mother's risk	
		αα/αα	αα/α-	α-/α-	αα/--	α-/--	α^{NDM}-/αα	αα^{NDM}/α-	αα^{NDM}/αα	
Father's risk	αα/αα	34.13%	25.23%	8.12%	11.98%	0.86%	0.34%	0.34%	7.28%	88.3%
	αα/α-	2.11%	1.56%	0.50%	0.74%	0.05%	0.02%	0.02%	0.45%	5.5%
	α-/α-	0.68%	0.50%	0.16%	0.24%	0.02%	0.01%	0.01%	0.14%	1.8%
	αα/--	1.00%	0.74%	0.24%	0.35%	0.03%	0.01%	0.01%	0.21%	2.6%
	α-/--	0.07%	0.05%	0.02%	0.03%	0.00%	0.00%	0.00%	0.02%	0.2%
	α^{NDM}-/αα	0.03%	0.02%	0.01%	0.01%	0.00%	0.00%	0.00%	0.01%	0.1%
	αα^{NDM}/α-	0.03%	0.02%	0.01%	0.01%	0.00%	0.00%	0.00%	0.01%	0.1%
	αα^{NDM}/αα	0.61%	0.45%	0.14%	0.21%	0.02%	0.01%	0.01%	0.13%	1.6%
		38.7%	28.6%	9.2%	13.6%	1.0%	0.4%	0.4%	8.2%	

Red cells denote risk of Hb Bart's only; Maroon cells denote risk of Hb Bart's and non-deletion HbH; and Brown cells denote risk of non-deletion HbH only. Black box denotes genotypes identified by common deletion testing only.

NDM = non-deletion mutation.

The overall costs and outcomes, and incremental costs and outcomes as calculated in the presence and absence of genetic deletion testing using GAP-PCR for α thalassaemia, are shown in Table 7 and Table 8 for couples who are planning pregnancy and those that are already pregnant, respectively. The base case analysis used the proposed item fee for α thalassaemia deletion testing using GAP-PCR of $100.

Table 7   Incremental cost-effectiveness in couples planning a pregnancy

	Intervention	Comparator	Increment
Total cost	$585	$139	$445
Couples genetically confirmed as being at risk of having a fetus affected by Hb Bart's	0.0040	0.0000	0.0040
ICER per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's			**$110,266**
Couples identified as being at risk of having a fetus affected by Hb Bart's	0.0040	0.0030	0.0010
ICER per additional couple that is identified as being at risk of having a fetus affected by Hb Bart's			**$426,499**
Couples with genetically confirmed status	0.9998	0.0000	0.9998
ICER per couple with genetically confirmed status			**$446**

ICER = Incremental Cost Effectiveness Ratio

Table 8   Incremental cost-effectiveness in pregnant couples

	Intervention	Comparator	Increment
Total cost	$8,273	$7,856	$417
Couples genetically confirmed as being at risk of having a fetus affected by Hb Bart's	0.0040	0.0000	0.0040
ICER per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's			**$103,179**
Couples identified as being at risk of having a fetus affected by Hb Bart's	0.0040	0.0030	0.0010
ICER per additional couple that is identified as being at risk of having a fetus affected by Hb Bart's			**$399,086**
Couples with genetically confirmed status	0.9998	0.0000	0.9998
ICER per couple with genetically confirmed status			**$417**
Cases of Hb Bart's (i.e. late termination, stillbirth or die shortly after birth)	0.0001	0.0010	-0.0010
ICER per avoided case of Hb Bart's that is terminated late, stillborn or dies shortly after birth			**$419,612**

ICER = Incremental Cost Effectiveness Ratio

The incremental cost-effectiveness ratio (ICER) was most sensitive to changes that affect the prevalence of α^0 and HbH genotypes in the population eligible for testing (i.e. sensitivity analyses that affect overall prevalence, changes to the partner's risk at model entry and the distribution of genotypes within a given total prevalence). Given the differences and fluidity in ethnic make-up in populations across states and over time, there is substantial uncertainty in the estimates presented.

The ICER was also observed to be sensitive to the cost of testing, not only the cost of deletion testing itself, but also the proportion of partners screened in both the intervention and comparator arms of the model.

Two scenario analyses examining different testing pathways were also prepared as part of the assessment. The first assumes that additional testing will be performed to identify all non-deletion mutations, as was conducted in Lau et al. (2009). In this analysis, it was assumed that all α thalassaemia mutations are identified, and additional outcomes have been presented, including cost per couple at risk of either Hb Bart's or non-deletion HbH identified. The results of this scenario analysis are presented in Tables 9 and 10.

Table 9   Incremental cost-effectiveness in couples planning a pregnancy, scenario where further testing is included

	Intervention	Comparator	Increment
Total cost	$909	$139	$770
Couples genetically confirmed as being at risk of having a fetus affected by Hb Bart's	0.0043	0.0000	0.0043
ICER per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's			**$180,784**
Couples identified as being at risk of having a fetus affected by Hb Bart's	0.0043	0.0030	0.0013
ICER per additional couple that is identified as being at risk of having a fetus affected by Hb Bart's			**$609,876**
Couples with genetically confirmed status	0.0097	0.0000	0.0097
ICER per couple with genetically confirmed status			**$79,041**
Couples genetically confirmed as being at risk of having a fetus affected by Hb Bart's	1.0000	0.0000	1.0000
ICER per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's			**$770**

ICER = Incremental Cost Effectiveness Ratio

Table 10 Incremental cost-effectiveness in pregnant couples, scenario where further testing is included

	Intervention	Comparator	Increment
Total cost	$8,600	$7,856	$744
Couples genetically confirmed as being at risk of having a fetus affected by Hb Bart's	0.0043	0.0000	0.0043
ICER per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's			**$174,850**
Couples identified as being at risk of having a fetus affected by Hb Bart's	0.0043	0.0030	0.0013
ICER per additional couple that is identified as being at risk of having a fetus affected by Hb Bart's			**$589,856**
Couples who's risk of Hb Bart's or non-del HbH is genetically confirmed	0.0097	0.0000	0.0097
ICER per couple at risk of Hb Bart's or non-del HbH genetically confirmed			**$76,447**
Couples with genetically confirmed status	1.0000	0.0000	1.0000
ICER per couple with genetically confirmed status			**$744**
Cases of Hb Bart's (i.e. late termination, stillbirth or die shortly after birth)	0.0000	0.0010	–0.0010
ICER per avoided case of Hb Bart's that is terminated late, stillborn or dies shortly after birth			**$711,088**
Cases of Hb Bart's or non-deletion HbH	0.0000	0.0024	–0.0024
ICER per avoided cases of Hb Bart's and non-deletion HbH			**$305,998**

ICER = Incremental Cost Effectiveness Ratio

Further testing is less cost-effective than deletion testing only in identifying couples at risk of Hb Bart's. This is due to the incremental cost of further testing required to identify the additional 4.8% of couples at risk. However, if the outcome of interest is broadened to include identification of couples that are at risk of either Hb Bart's or non-deletion HbH, then further testing appears to be more cost effective than deletion testing only

A cost-effectiveness scenario analysis was conducted which assumed both parents require abnormal screening results before either can receive genetic testing.. Screening results have

not been included in the comparator arm of the model in this scenario as these are required for both parents in both arms, prior to model entry. The results of the scenario analysis are presented in Table 11 and Table 12.

Table 11 Incremental cost-effectiveness in couples planning a pregnancy, scenario where parental testing is conducted simultaneously

	Intervention	Comparator	Increment
Total cost	$602	$0	$602
Couples genetically confirmed as being at risk of having a fetus affected by Hb Bart's	0.0212	0.0000	0.0212
ICER per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's			**$28,443**
Couples identified as being at risk of having a fetus affected by Hb Bart's	0.0212	0.0157	0.0055
ICER per additional couple that is identified as being at risk of having a fetus affected by Hb Bart's			**$110,015**
Couples with genetically confirmed status	0.9989	0.0000	0.9989
ICER per couple with genetically confirmed status			**$603**

ICER = Incremental Cost Effectiveness Ratio

Table 12 Incremental cost-effectiveness in pregnant couples, scenario where parental testing is conducted simultaneously

	Intervention	Comparator	Increment
Total cost	$8,303	$7,742	$561
Couples genetically confirmed as being at risk of having a fetus affected by Hb Bart's	0.0212	0.0000	0.0212
ICER per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's			**$26,517**
Couples identified as being at risk of having a fetus affected by Hb Bart's	0.0212	0.0157	0.0055
ICER per additional couple that is identified as being at risk of having a fetus affected by Hb Bart's			**$102,566**
Couples with genetically confirmed status	0.9989	0.0000	0.9989
ICER per couple with genetically confirmed status			**$562**
Cases of Hb Bart's (i.e. late termination, stillbirth or die shortly after birth)	0.0003	0.0055	-0.0052
ICER per avoided case of Hb Bart's that is terminated late, stillborn or dies shortly after birth			**$107,839**

ICER = Incremental Cost Effectiveness Ratio

By enriching the risk of α thalassaemia in the population eligible for testing (i.e. requiring screening in both parents prior to genetic testing), improvements in the cost-effectiveness of deletion testing are observed.

Revised Economic Evaluation Post-ESC

Additional economic analyses (including sensitivity and scenario analyses) were conducted to reflect an increase in the proposed fee from $100 (for GAP-PCR testing) to $200. This was in response to the comment raised by ESC that the RCPA QAP 2018 Alpha Thalassemia Program indicated indicated that more labs are moving towards using MLPA, which is associated with a higher fee.

No changes were made to the assumptions around the ability of the test to detect deletions (see Figure 3 above).

The respecified incremental cost-effectiveness ratios for couples that are planning pregnancy are presented in Table 13

Table13 Incremental cost-effectiveness in couples planning a pregnancy

	Intervention	Comparator	Increment
Total cost	$695	$139	$555
Couples genetically confirmed as being at risk of having a fetus affected by Hb Bart's	0.0040	0.0000	0.0040
ICER per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's			**$137,492**
Couples identified as being at risk of having a fetus affected by Hb Bart's	0.0040	0.0030	0.0010
ICER per additional couple that is identified as being at risk of having a fetus affected by Hb Bart's			**$531,808**
Couples with genetically confirmed status (risk or not at risk of Hb Barts)	0.9998	0.0000	0.9998
ICER per couple with genetically confirmed status			**$556**

ICER = Incremental Cost Effectiveness Ratio

The respecified incremental cost-effectiveness ratios for pregnant couples are presented in Table 14. For this population, an additional outcome, incremental cost per decrease in a case of Hb Bart's that is terminated late, stillborn or dies shortly after birth, to capture the benefit of allowing for termination before symptoms of hydrops fetalis emerge, is also reported above those presented for couples planning pregnancy.

Table14 Incremental cost-effectiveness in pregnant couples

	Intervention	Comparator	Increment
Total cost	$8,383	$7,856	$527
Couples genetically confirmed as being at risk of having a fetus affected by Hb Bart's	0.0040	0.0000	0.0040
ICER per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's			**$130,405**
Couples identified as being at risk of having a fetus affected by Hb Bart's	0.0040	0.0030	0.0010
ICER per additional couple that is identified as being at risk of having a fetus affected by Hb Bart's			**$504,395**
Couples with genetically confirmed status (risk or not at risk of Hb Barts)	0.9998	0.0000	0.9998
ICER per couple with genetically confirmed status			**$527**
Cases of Hb Bart's (i.e. late termination, stillbirth or die shortly after birth)	0.0001	0.0010	−0.0010
ICER per avoided case of Hb Bart's that is terminated late, stillborn or dies shortly after birth			**$530,337**

ICER = Incremental Cost Effectiveness Ratio

The economic scenario analysis in which both parents require abnormal screening results before either can receive genetic testing was also rerun with the higher test fee. The results are presented in Table 15 and Table 16

Table 15 Incremental cost-effectiveness in couples planning a pregnancy, scenario where parental testing is conducted simultaneously

	Intervention	Comparator	Increment
Total cost	$802	$0	$802
Couples genetically confirmed as being at risk of having a fetus affected by Hb Bart's	0.0212	0.0000	0.0212
ICER per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's			**$37,893**
Couples identified as being at risk of having a fetus affected by Hb Bart's	0.0212	0.0157	0.0055
ICER per additional couple that is identified as being at risk of having a fetus affected by Hb Bart's			**$146,568**
Couples with genetically confirmed status (risk or not at risk of Hb Barts)	0.9989	0.0000	0.9989
ICER per couple with genetically confirmed status			**$803**

ICER = Incremental Cost Effectiveness Ratio

Table16 Incremental cost-effectiveness in pregnant couples, scenario where parental testing is conducted simultaneously

	Intervention	Comparator	Increment
Total cost	$8,503	$7,742	$761
Couples genetically confirmed as being at risk of having a fetus affected by Hb Bart's	0.0212	0.0000	0.0212
ICER per couple that is genetically confirmed as being at risk of having a fetus affected by Hb Bart's			**$35,967**
Couples identified as being at risk of having a fetus affected by Hb Bart's	0.0212	0.0157	0.0055
ICER per additional couple that is identified as being at risk of having a fetus affected by Hb Bart's			**$139,119**
Couples with genetically confirmed status (risk or not at risk of Hb Barts)	0.9989	0.0000	0.9989
ICER per couple with genetically confirmed status			**$762**
Cases of Hb Bart's (i.e. late termination, stillbirth or die shortly after birth)	0.0003	0.0055	−0.0052
ICER per avoided case of Hb Bart's that is terminated late, stillborn or dies shortly after birth			**$146,272**

ICER = Incremental Cost Effectiveness Ratio

13.   Financial/budgetary impacts

An epidemiological approach has been used to estimate the financial implications of listing α thalassaemia deletion testing on the MBS at the applicant's proposed rate of $100 per test (Table 17).

Table 17 Costs to the MBS associated with deletion testing for α of $100

	2019	2020	2021	2022	2023
No. of women of reproductive age	4,963,363	5,031,678	5,100,932	5,171,140	5,242,314
Proportion that are pregnant or plan their first pregnancy each year	2.8%	2.8%	2.8%	2.8%	2.8%
No. pregnant or who plan a pregnancy each year	138,403	140,308	142,239	144,197	146,182
Proportion with an abnormal screening test (abnormal red cell indices in the absence of beta thalassaemia trait)	8.3%	8.3%	8.3%	8.3%	8.3%
No. of women eligible for deletion testing	**11,552**	**11,711**	**11,872**	**12,035**	**12,201**
Yield of α thalassaemia mutations	53.1%	53.1%	53.1%	53.1%	53.1%
No. with α thalassaemia mutations identified	6,133	6,218	6,303	6,390	6,478
No. of partners who uptake screening	6,133	6,218	6,303	6,390	6,478
Proportion of partners with an abnormal screening test	19.1%	19.1%	19.1%	19.1%	19.1%
No. of partners eligible for deletion testing	**1,171**	**1,187**	**1,203**	**1,220**	**1,236**
No. of people eligible for deletion testing	**12,722**	**12,898**	**13,075**	**13,255**	**13,437**
Proportion of couples identified at risk	0.4%	0.4%	0.4%	0.4%	0.4%
No. couples at risk identified	47	47	48	49	49
No. tests required to identify one couple at risk	248	248	248	248	248
Total cost of deletion testing, $100 per test	$1,272,240	$1,289,751	$1,307,503	$1,325,499	$1,343,743
Cost to the MBS, $85 per test [a]	**$1,081,404**	**$1,096,288**	**$1,111,377**	**$1,126,674**	**$1,142,181**
Cost to patients, $15 per test [b]	$190,836	$193,463	$196,125	$198,825	$201,561

[a] Assuming all tests are conducted in the outpatient setting
[b] Assuming that: patients are not bulk-billed; and providers do not charge above the schedule fee

At a proposed schedule fee of $200, the cost per test to the MBS is $170, assuming all tests are conducted in the outpatient setting. The financial implications to the MBS of listing deletion testing are estimated to be $2.16 million in 2019 increasing to $2.28 million in 2023 (Table 18).

Table 18 Revised estimated cost of α thalassaemia deletion testing

	2019	2020	2021	2022	2023
No. of women eligible for deletion testing	11,552	11,711	11,872	12,035	12,201
No. of partners eligible for deletion testing	1,171	1,187	1,203	1,220	1,236
No. of people who uptake deletion testing	12,722	12,898	13,075	13,255	13,437
Total cost of deletion testing, $200 per test	$2,544,480	$2,579,502	$2,615,005	$2,650,997	$2,687,485
Cost to the MBS, $170 per test	**$2,162,808**	**$2,192,577**	**$2,222,755**	**$2,253,348**	**$2,284,362**
Cost to patients, $30 per test	$381,672	$386,925	$392,251	$397,650	$403,123

14.   Key issues from ESC for MSAC

ESC key issue	ESC advice to MSAC
Clinical claim reasonable	Deletion testing is of non-inferior safety and superior effectiveness to no genetic testing (despite limited, poor-quality available evidence). Deletion testing by Gap-PCR is of inferior effectiveness to comprehensive deletion testing +/– sequencing.
Inadequacy of scope to improve equity of funding	Consider broadening scope of funding: • Testing methods – comprehensive deletion testing, gene sequencing • Population groups – prenatal diagnosis, preimplantation genetic diagnosis, cascade testing of relatives.
Inclusion criteria: • equity issues • haem studies prerequisite	Equity issues for limiting male partner testing to when partners have genetic diagnosis of alpha thalassaemia carriage. Perhaps change wording to 'Thalassaemia screening for beta-thalassaemia not conclusive'. Genetic diagnosis required for prenatal diagnosis to occur, so even if HbH bodies are present, genetic testing is of value.
Testing methodology	The MBS item descriptor did not include a genetic testing method. Consider placing a limit on the minimum number of common deletions or % deletions detected in the target population (difficult) to descriptor, or stipulating the technique to be used (corresponding rebate increase required).
Proposed clinical pathway problematic	The CA suggested mean presentation for testing at 15 weeks gestation. Sequential testing would not provide information in a timely manner. The Critique suggested testing both partners by haemoglobin studies then, if both are at risk, proceed with testing.
Limitations on number of tests is impractical	CA suggested 10% repeat testing in practice. Repeat testing may be useful, particularly if the first test had a limited detection spectrum, and if there is limited data sharing between laboratories/treating clinicians.
Meaning of economic evaluation results	Consider in particular the different ways of looking at cost-effectiveness, unclear ICER benchmark or willingness to pay for these outcomes.
Configuration of the base case	Consider the possible input parameter values (e.g. higher fee) and scenarios (e.g. partner pre-screening) to determine which situation is the most adequate base case for MSAC decision-making. Ask CA to reanalyse the costs using multiplex ligation-dependent probe amplification (MLPA) as the base case.
Uncertainty with model inputs and financial inputs	There is uncertainty regarding model parameters versus what will be observed in practice. ICERs and financial impacts have the potential to be considerably different from the base case.
Testing in couples planning pregnancy	Confirming couples who are at risk may inform their reproductive choices, but there is no information on how this translates into actual decisions or health outcomes.
Deletion testing only	Consider if deletion testing only is a reasonable MBS listing, given the substantially higher ICERs for deletion + non-deletion testing, and the extent to which non-deletion testing can be funded by states.

ESC Discussion

Application 1531 is for Medicare Benefits Schedule (MBS) listing of genetic deletion testing for alpha (α) thalassaemia. It was an Application Referral submitted to the MSAC Executive in October 2018, which recommended an expedited pathway; the PASC process was therefore not used for this application. ESC noted that this is a first-time application for α thalassaemia genetic testing.

ESC noted that the incidence and mutation spectra of α thalassaemia vary with ethnic background, with some ethnic groups having a carrier rate as high as 60%. The Contracted Assessment (CA) estimated that up to 8% of these are currently screened by haemoglobin studies in Australia. The incidence of α thalassaemia is set to rise in Australia with increased migration from South-East Asia.

ESC noted that all states and territories currently cover this genetic testing, with variable out-of-pocket costs to patients.

ESC noted that the CA clarified and defined two target populations: 1) females of reproductive age who meet haemoglobinopathy screening criteria, and 2) reproductive partners of females with proven α thalassaemia who meet haemoglobinopathy screening criteria. The CA suggested that α1 and α2 testing will lead to more informed reproductive choices (prenatal diagnosis [PND], preimplantation genetic diagnosis [PGD], in vitro fertilisation [IVF] with egg/sperm donation, adoption) and lower incidence of hydrops fetalis. Also, an early, more certain diagnosis may allow an earlier termination of pregnancy, which may result in a less negative impact on the woman's physical and psychological health than a later termination or a miscarriage or stillbirth.

ESC noted the different deletion detection methods of varying quality and cost. Gap-PCR is an old technique that is prone to errors and can only detect a limited specified panel of deletions; it is being phased out by many laboratories. Multiplex ligation-dependent probe amplification (MLPA) is a more specialised technique that can detect any deletion or duplication, but the proposed fee of $100 does not cover the cost of MLPA ($200/sample). ESC noted that the proposed deletion detection method is unclear. ESC noted that the RCPA QAP 2018 Alpha Thalassemia Program indicates that more labs are moving towards using MLPA. ESC also noted the risk of using a low fee, in that it may allow lower-quality laboratories to enter the market.

ESC noted that the CA presented a clinical algorithm where the woman has first access to genetic testing if not iron deficient (or iron deficient if pregnant, and no historic normal cell indices; note this component from the proposed MBS item descriptor was not specifically included in the clinical pathways of the proposed algorithm for pregnant women (see Figure 2) and with indices and haematological studies not conclusively diagnostic of thalassaemia. If the genetic test is positive, the male partner then receives access to genetic testing. ESC noted the inequities of having male testing depend on the results of the female test results. In addition, sequential testing is a lengthy process that would make it difficult for a couple to terminate a pregnancy within the applicable timeframe if this became an option for them. The Critique presented an alternative pathway where both parents undergo a blood test for red blood cell abnormalities to determine access to genetic testing (economic scenario 2, Tables 9 and 10, (MBS fee $100) and 13 and 14 (MBS fee $200)). ESC noted that even if HbH inclusions were present, genetic testing would be required to enable prenatal testing/PGD to occur. In addition, the HbH test has variable performance and may be unable to distinguish the 0/+ trait.

ESC noted the clinical trial data from two non-comparative level IV studies from large South-East Asian antenatal screening programs, which showed that most double deletion carrier couples chose TOP (73% and 100%). However, the Critique noted that Yamsri's study used a pre-genetic test screening method that is not available in Australia, and queried whether it should be included. There is no evidence on the uptake of PND and TOP without deletion testing; it is assumed to be lower, thus no testing would result in fewer reproductive choices.

ESC noted two diagnostic performance studies. Due to the nature of the stepwise testing, the performance is difficult to assess (false positives do not undergo more testing, and false negatives may not be picked up by sequencing). The CA assessed accuracy of deletion testing by comparing to a reference standard of deletion testing + sequencing. ESC noted that this was inappropriate because deletion testing and sequencing detect different types of mutations. (large deletions and sequence variants, respectively). Specifically, deletion testing with GAP-PCR detects a specific subset of deletions; MLPA can theoretically detect any deletion or duplication (and 1 sequence variant). However, sequencing, in general, cannot detect large deletions (unless via NGS assays, typically whole exome sequencing (WES)/whole genomic sequencing (WGS) panels, that have been validated to do so), but can detect sequence variants. ESC noted that the diagnostic yield depends on population prevalence, prior screening and the number of deletions tested for.

ESC noted that the two diagnostic studies revealed an incremental benefit of using genetic testing compared with HbH inclusion body testing. The absence of HbH inclusion bodies is poorly/moderately predictive of absence of mutation (35% and 43.1%); the presence of HbH inclusions is reasonably predictive of presence of mutation (94.5% and 94%).

ESC noted that the CA assessed three studies ('The first five years of a preventive programme for haemoglobinopathies in Northeastern Iraq' Al-Allawi et al. (2013); 'Prenatal diagnosis of haemoglobinopathies: Our experience of 523 cases' Grosso et al. (2013); and 'Carrier screening for α- and β-thalassemia in pregnancy: The results of an 11-year prospective program in Guangzhou Maternal and Neonatal Hospital' Liao et al. (2005)), looked at reproductive decisions based on reproductive choices for couples at risk of haemoglobinopathy:

- 74.8–95.7% took up PND
- 98.4% chose TOP if fetus was at risk of Hb Bart's.

ESC noted that the Critique provided disaggregated data from three clinical validity studies ('Pre Gestational Thalassaemia Screening in Mainland China: The First Two Years of a Preventive Program, Jiang et al. (2017)' Prenatal diagnosis of haemoglobinopathies: our experience of 523 cases' Grosso et al. (2013) and 'Carrier screening for α-and β-thalassaemia in pregnancy: the results of an 11-year prospective program in Guangzhou Maternal and Neonatal hospital' Liao et al. (2005)). For those indicated for PND, 100%, 90.1% and 95.7% chose PND, and of those found to have an at-risk fetus, 100%, 98.3% and 95.8% chose TOP.

ESC noted that no studies met the inclusion criteria for therapeutic effectiveness.

ESC agreed with the CA that genetic deletion testing had superior effectiveness to no genetic testing, and inferior effectiveness to comprehensive testing (MLPA plus sequencing).

ESC noted the Critique's finding that the CA did not assess regulatory or accreditation requirements for laboratories conducting the testing. However thalassaemia testing is already established in some laboratories, which already must comply with requirements.

ESC suggested that the limitation on the number of tests was impractical. The CA suggested 10% repeat testing in practice. Repeat testing may be useful, particularly if the first test had a limited detection spectrum, and if there is limited data sharing between laboratories or treating clinicians.

ESC considered that the wording of the proposed item descriptor and fee should be altered:

- the item descriptor should specify the type of test (e.g. deletion test; perhaps specifying the method or number of deletions detected)
- 'beta thalassaemia' should be added to (a) (given that the presence/absence of HbH inclusions should not negate need for deletion testing)
- partners be changed to 'reproductive' partners in (b)
- the fee should be $200 to reflect the higher cost of the test actually used by most laboratories

The economic evaluation was a cost-effectiveness analysis (CEA) based on a decision-tree analysis. ESC noted that the type of genetic testing in the economic analysis was unclear. The Assessment Group clarified that the economic analysis was based on GAP-PCR deletion testing followed by sequencing a proportion who had a negative result from the deletion testing. MLPA was not used in the costing because the data came from a laboratory that does not use MLPA. However, ESC noted that it was unclear which testing method was used for the initial deletion testing. ESC considered this to be an important distinction, as the economic model is sensitive to the cost of the genetic testing. ESC noted possible issues with the economic evaluation due to the base case used, as it was based on $100 per test, which may not reflect current laboratory practice. ESC suggested that the CA reanalyse the costs using MLPA as the base case before MSAC considers this application.

ESC noted that the translations, cost inputs, risks and model transitions for the economic evaluation were reasonable. ESC accepted the simplified model assumptions (100% uptake of partner testing, 100% uptake of PND and perfect diagnostic performance).

ESC noted that the sensitivity analysis resulted in several different ICERs based on two scenarios:

- Scenario 1. Deletion testing is done, followed by sequencing to identify all non-deletional mutations. This is less cost-effective than deletion testing alone. However, if the outcomes are broadened to include genetic confirmation of couples that are at risk of either a baby with Hb Bart's or non-deletion HbH, then further testing appears to be more cost-effective than deletion testing alone.
- Scenario 2. Both parents require a confirmed red cell abnormality before either can receive genetic testing. This results in better cost-effectiveness for deletion testing alone.

ESC noted that the economic model was sensitive to three main drivers:

- changes that affect the prevalence of the α^0 and HbH genotypes in the population eligible for testing
- number of partners screened who are eligible for genetic testing
- test cost.

ESC noted that because of the differences in ethnic backgrounds and therefore prevalence across states and over time, the estimates presented are uncertain.

ESC noted that scenario 2 was likely the better clinical pathway, as the population was more targeted. It also produced lower ICERs compared with scenario 1 (e.g. confirmed at-risk status in planning couples: $28,443 vs $180,784; confirmed affected fetus: $26,517 vs $174,820), and higher diagnostic yields (but only if MLPA was used). ESC noted that the next step was unclear if the deletion testing was negative in scenario 2, which would affect ICERs. ESC noted that the applicant was supportive of the Critique's suggested clinical pathway changes – that is, scenario 2, where both parents are screened for red cell abnormalities prior to undergoing genetic testing.

ESC noted the different base case ICERs based on planning couples and pregnant couples. This could be important because it is unclear if cases confirmed at-risk or couples with status genetically confirmed (either at-risk or not at-risk) are the more relevant outcome.

ESC noted that the ICERs are higher in both scenarios compared with the base case. ESC noted that the ICERs are lowest for confirmed status, but questioned the relevance of this in terms of clinical management. The test results affect clinical management in that most couples diagnosed as having an at-risk fetus terminate the pregnancy, suggesting that confirmed at risk is the most relevant testing outcome. ESC noted that, due to the multiple ICERs available and their sensitivity to the drivers, the most relevant testing outcome needs to be clarified before determining total potential cost to the MBS. ESC noted the consumer issues associated with access to testing and genetic counselling for rural/remote people, as well as sensitivities around genetic testing and termination of pregnancies for some cultural groups. Genetic counsellors would need to be aware of such cultural sensitivities when discussing genetic testing.. ESC also noted that, due to the expedited pathway, the application lacked consumer engagement.

15.   Other significant factors

Nil

16.   Applicant's comments on MSAC's Public Summary Document

The applicant had no comment.

17.   Further information on MSAC

MSAC Terms of Reference and other information are available on the MSAC Website:
visit the MSAC website