

Appendix 3. Evaluation of phenotypes in heterozygotes (PS4)

Note: When evaluating cases be aware of potential complex genotype interactions.

	Evidence	Parameter	Impact threshold	Alternative terms	Points per case	Max	Comment
beta-thalassemia HBB/LCRB [AR]	Reduced MCV	MCV (fL)	<79	Microcytosis	1.5	16	For beta-thal trait only. Very specific phenotype. Caution needed for the presence of another pathogenic variant in the beta globin locus or in HIV-positive patients on antiretroviral therapy (ART)
	Reduced MCH	MCH (pg)	<27	Hypochromia			
	Increased HbA2	HbA2 (%)	>3.5				
	Reduced MCV	MCV (fL)	<79	Microcytosis	0.15	1.5	Primarily for thalassaemias and thalassaemic Hb variants. Do not use if the RBC count is decreased or iron deficiency is present
	Reduced MCH	MCH (pg)	<27	Hypochromia			
	Reduced MCV	MCV (fL)	<79	Microcytosis	0.2	1.6	Primarily for thalassaemias and thalassaemic Hb variants. Do not use if there is an indication of recent correction of iron deficiency.
	Reduced MCH	MCH (pg)	<27	Hypochromia			
	Normal or increased RBC count	RBC (10 ¹² /L)	4.7–6.1 for men (normal); 4.2–5.4 for women (normal)				

	Reduced MCV Reduced MCH excluding iron deficiency (i.e. normal serum ferritin, transferrin saturation, TIBC)	MCV (fL) MCH (pg)	<79 <27	Microcytosis Hypochromia	0.3	3	Primarily for thalassaemias and thalassaemic Hb variants

*only consider independent (unrelated) cases. Multiple cases in a family are counted as one

** do NOT use multiple point levels for the same case

Point sum and evidence strength

Supporting	Moderate	Strong	Very Strong
0.5-1.49	1.5-3.49	3.5-15.99	≥16