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Supplementary appendix

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APPENDIX:

Defining global strategies to improve outcomes in sickle cell disease: a *Lancet Haematology* commission

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Laboratory methods for haemoglobinopathy testing

IEF is a very sensitive method and is widely used in LMICs at relatively low cost. IEF separates haemoglobin species according to their isoelectric point on a gel medium. Bands are very sharp compared with classic electrophoresis, and HbF and HbA bands are clearly distinct. The relevant atypical haemoglobin variants can be readily detected. An important disadvantage is that this method is a non-automated technique and therefore time consuming, and the results rely on accurate interpretation by appropriately trained and experienced staff.

HPLC is based on the sequential elution of positively charged haemoglobin species (cations) bound to a negatively charged solid phase in a chromatographic column by buffers, with a gradient of increasing ionic strength. Haemoglobin species eluted from the column are detected by a dual-wavelength detector, generating a chromatogram on which each haemoglobin peak is characterised by its elution time and quantified by integrating its area under the curve. HPLC is a highly resolutive and sensitive technique (detection limit for HbA and HbS is approximately 1% of total haemoglobin). The advantages are that this method is automated, with computer-aided identification and quantification of the haemoglobin species. Disadvantages are the cost of the equipment, the need for technical support and maintenance, the cost of the reagents, and the need for reliable supplies of electricity. It has become the major first-line screening procedure in HICs and the reference control procedure in many reference centres in LMICs.

CE separates different haemoglobins according to their electrophoretic mobility and an electro-osmotic flow generated by a high-voltage electric field in a glass capillary. Haemoglobin species are prompted to migrate toward a 415 nm wavelength detector generating an electropherogram. Haemoglobin species are identified according to their migration zones and relatively quantified. Advantages and disadvantages are similar to HPLC, although the procedure is a bit less costly.

Mass-spectrometry-based techniques allow the identification of haemoglobin species according to the difference in molecular mass of globin or peptide chain fragments. Mass spectrometers are highly sophisticated instruments that can be used in different ways. Two variants of the technique are used for sickle cell disease NBS: tandem mass spectrometry[1] and matrix-assisted laser desorption–ionisation (MALDI) mass spectrometry.[2] In the tandem approach, two mass spectrometers are coupled to separate then identify peptide fragments issued from the tryptic digestion of haemoglobin. The MALDI mass spectrometry approach uses one single mass spectrometry machine to analyse the globin chains as a whole or after fragmentation. An advantage of tandem mass spectrometry is that it is more specific than electrophoretic and chromatographic techniques, allowing the accurate identification of most haemoglobin variants; the disadvantage is that the initial set up is time consuming (similar to IEF). An advantage of the MALDI mass spectrometry is that analysis is extremely rapid and thus the system is amenable to very high throughputs; the disadvantage is that it has only been widely applied to haemoglobin analysis over the last 10 years and currently not all the variants can be identified. Both approaches benefit from a computer-aided identification of the variants, removing operator-dependent variability. The cost of the machines is very high, but tandem mass spectrometry is also used to screen for many other

newborn conditions in HICs, and MALDI mass spectrometry machines routinely equip microbiology laboratories in HICs and an increasing number of reference microbiology laboratories in LMICs.

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Supplementary Tables

Supplementary table S1: Different genotypes which have been shown to cause sickle cell disease, grouped together according to severity. Estimated prevalence as cause of sickle cell disease shown in brackets. Types of HbS/β thalassaemia more common in India, Middle East and southern Europe, and HbSC most common in West Africa.

Sickle cell disease				
Most severe  Least severe				
Sickle cell anaemia	**Severe sickle cell disease**	**Moderately severe sickle cell disease**	**Mild sickle cell disease**	**Very mild sickle cell disease**
HbSS (70 – 90%)	Severe HbS/β^{+}thalassaemia (1-10%)	Moderate HbS/β^{+}thalassaemia (1 – 10%)	Mild HbS/β^{++}thalassaemia (1 – 5%)	HbS/HPFH (1%)
HbS/β^{0}thalassaemia (1-10%)	HbS/O-Arab (1-2%)	HbSC (1 – 30%)	HbSE (<1%)	HbS + other variants (<1%)
	HbS/D-Punjab (1 -2%)	HbA/S-Oman (1 – 2%)	HbA/Jamaica Plain (<0.1%)	
	HbS/C-Harlem (<0.1%)			
	HbC/S-Antilles (<0.1%)			
	HbS/Quebec-CHORI (<0.1%)			

Supplementary Table S2: Examples of current global and regional networks focusing on sickle cell disease.

Network	Scale	Aims
Global Sickle Cell Disease Network (GSCDN)	World	To bring together clinicians and scientists from HICs and LMICs to collaborate on education, screening, management and prevention programmes, and to generate useful data to guide policies.
Sickle Pan African Research Consortium (SPARCO)	Tanzania, Ghana & Nigeria	To develop research capacity for SCD through a multidimensional approach which addresses infrastructure, education & training, provision of longitudinal research data and the translation of research into practise
Réseau d'Etudes de la Drépanocytose en Afrique Centrale (REDAC)	Angola, Cameroon, Congo-Brazzaville, the Democratic Republic of Congo, Gabon, Kenya, Tanzania, Uganda & Zambia	To bring together researchers and clinicians involved in SCD to harmonize the care and offer African patients the possibility of benefiting from quality basic therapies.
Caribbean Association of Researchers in SCD and Thalassemia (CAREST)	Barbados, Cuba, Dominican Republic, French Guyana, Grenada, Guadeloupe, Haïti, Jamaica, Martinique, Saint Lucia, Trinidad & Tobago	To establish collaborations to promote SCD newborn screening programs and early childhood care, to facilitate health worker training and approaches for prevention and treatment of SCD complications, and to carry out inter-Caribbean research studies.

Supplementary Table S3: Estimated birth prevalence of sickle cell disease (SCD) in countries derived from large and pilot newborn screening programme.

Country	State	SCD birth prevalence (per 1,000)	Reference (url/doi)
		LARGE SCREENING PROGRAMMES	
Belgium	Brussels, Liege	0.43	Le et al 2018. https://doi.org/10.1177/0969141317701166
Brazil	Bahia	1.54	Brazilian Ministry of Health, 2013. https://bvsms.saude.gov.br/bvs/publicacoes/doenca_falciforme_saiba_onde_tratamento.pdf
	Rio de Janeiro	0.77	
	Minas Gerais	0.71	
France	/	0.30-0.49	Leleu et al, 2021. https://doi.org/10.1371/journal.pone.0253986
Spain	/	0.03-0.18	García-Morín et al, 2020 https://doi.org/10.1007/s00277-020-04044-z
UK	/	0.47	Streetly et al, 2017 http://dx.doi.org/10.1136/archdischild-2017-313213
USA	/	0.52	Therell et al 2015. https://doi.org/10.1053/j.semperi.2015.03.008
		PILOT SCREENING PROGRAMMES	
Ghana	Accra	18	Segbefia et al, 2021. https://doi.org/10.1002/pbc.29068
Tanzania	/	8	67
Uganda	/	13	Hernandez et al, 2021. https://doi.org/10.1111/tmi.13506

Supplementary Bibliography: List of references (in alphabetical order of the first author's lastname) used to assign countries to one of the categories (national, regional, pilot, no programme, no data) for the availability of a newborn screening programme for sickle cell disease.

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