Supplementary Table 3 Up-to-date practice of newborn screening programs in different countries: a survey combined a literature review.

	China	America	Germany	UK
Data Source	This study.	• Vernon HJ [**USA1**]. • American College of Medical Genetics Newborn Screening Expert Group [**USA**2]. • NewSTEPs 360 project (2020).	• The "DGNS reports", a German report compiled on the initiative of the relevant professional society (the DGNS) and the laboratories.	• State-organized and -funded reporting issued by the Public Health England (PHE) [**UK**].
Screening IMDs	Two IMDs are nationwide mandatory: PKU, CH.	29 IMDs are in core panel and 25 IMDs secondary panel.	Mandatory expanded NBS for 12 specific target disorders	6 IMDs: PKU, MCADD, MSUD, IVA, GA-1, and HCU.
Scale of NBS agencies	The number of qualified NBS institutions in 2020 in China was 248.	NA.	Eleven laboratories in Germany are currently licensed to perform and bill for NBS service. Eight labs are affiliated with universities, three are private.	16 UK NBS laboratories.
Coverage rate	96.1% in 2016, 98.5% in 2018.	NA.	9,244,411 documented screening examinations in the 9,218,538 children born in the observed period.	96.5%.
Availability of national standards/specifications	Available: "Newborn Disease Screening Technical Specification".	Available: the American College of Medical Genetics (ACMG) outlines a process of	Available: Based on the mandatory regulations for the performance of screening laid	Available: National standards NBS 1-12.

		standardization of outcomes and guidelines for state NBS programs.	out in the pediatric guideline of the Federal Joint Committee (G-BA).	
Qualification requirements for NBS agencies	NBS agencies must have obtained approvals from the health administration of the provinces, autonomous regions, and municipalities directly under the central government, and the annual screening volume should exceed 30,000.	NA.	A minimum volume of 50,000 investigated first-screening samples in 1 year and accreditation by the German national accreditation body DAkks.	The laboratory should be CPA accredited. That is, laboratories undertaking newborn screening shall be accredited by Clinical Pathology Accreditation (UK) Ltd (CPA), now formally part of the United Kingdom Accreditation Service (UKAS).
Before blood sampling takes place	Education about NBS and a written consent are mandatory. Before blood collection, blood collectors should truthfully inform the guardians of the purpose, significance, screening disease types, conditions, methods, sensitivity, and cost of the NBS and obtain signed consent.	Education about NBS is not mandatory. This education can vary from having a hospital staff member discuss the testing to simply handing a parent a pamphlet.	Education about NBS and a written consent are mandatory. the parents must be provided with the information required by the German Genetic Diagnosis Act and give their written consent.	NA.
Sample type	Dried blood spot (DBS)	Dried blood spot (DBS) made	Dried blood spot (DBS) made	Dried blood spot (DBS)

	made by whole blood (e.g., heel blood).	by whole blood.	by whole blood.	made by whole blood.
Age of initial sampling	After 72 hours of birth to 7 days and during this period newborns are required to be full breastfeeding.	Between 24 and 48 hours after birth and no later than 48 hours after birth. Some states require a second newborn screen at 1 to 2 weeks of age.	Between 36 and 72 h after birth of the child. In children who discharge before reach the age of 36 h and in prematurely born babies in whom the first sample is taken before the 32nd week of gestation, follow-up is mandatory at the age of 36 h or at 32 gestational weeks, respectively.	First samples taken on days 5 to 8 (ideally on day 5).
Qualified specimen	Qualified DBS is defined as the following: at least 3 blood spots, and each blood spot is greater than 8 mm in diameter; blood drops naturally penetrate, and the blood spots on the front and back of the filter paper are the same; the blood spots are not contaminated ; there is no bleeding ring in blood spots. (4) Specimens with important information	NA.	NA.	A good quality DBS is one that is taken at the right time, has all data fields completed on the blood spot card, contains sufficient blood to perform all tests, has not been contaminated, and arrives in the laboratory in a timely manner.

	omission are considered as unqualified, too.			
Repeat requests/collection	If blood samples are not collected on time due to special circumstances or unqualified specimens are returned and need to be re-collected, they should make an appointment in time or follow up to collect blood in time.	NA.	A second card is needed when the first sample was obtained before the child was 36 h old or before the 32nd week of gestation.	Recollection when the previous sample was: ● taken when the baby was too young (on or before day 4, where day of birth is day 0). ● insufficient blood. ● unsuitable sample/card (e.g, expired blood spot card, contaminated, in transit for more than 14 days, anti-coagulated sample, sample information is not accurately recorded).
Diagnosis	The initially positive screening sample should be reanalysed and abnormal-results babies are recalled to conduct the confirmation diagnosis test.	A positive screening result (recall) constitutes a preliminary diagnosis and must be urgently verified. Depending on how robust the result and thus the	NA.	Babies with a positive screening result should have a clinical referral initiated within 3 working days of sample receipt by screening

		diagnosis are, either repetition of screening or referral to a specialized center for direct confirmatory investigation is recommended.		laboratory.
Biochemical diagnostic testing	• Fluorescence assay. • Quantitative enzymatic assay. • Tandem mass spectrometry. • Time-resolved fluorescence assay. • Enzyme-linked immunosorbent assay (ELISA).	• Whole-exome sequencing. • Plasma amino acid analysis. • Urine organic acid analysis. • Acylcarnitine profile analysis. • Carnitine analysis. • Acylglycine analysis. • Enzymatic function. • Metabolomics analysis.	MS/MS technology Tandem mass spectrometry (MS/MS). Fluorometry (PE). Quantitative fluorometry. Quantitative colorimetry.	• MS/MS technology, NA. • Second-tier procedure (additional analyses of the same blood sample in a second stage of examination). But no data on confirmatory diagnostic tests.
Timeliness of Screening and Results Reporting	Reports should be issued by the laboratory within **5** working days from the date of receiving qualified DBS samples.	• All NBS tests should be completed within **seven** days of life with results reported to the healthcare provider as soon as possible. • **Five** days for time-critical conditions;	Time between arrival of sample at lab and reporting of result should be within **24** h.	Screen negative results letters are despatched direct to parents by 6 weeks of age.

		• No later than **seven** days of life for all other conditions.		
Timeliness of sample Delivery	The collection of the DBS sample to the receipt of the DBS sample in the laboratory should be finished within a short time (within **5** working days).	NBS specimens should be received at the laboratory as soon as possible; ideally within **24** hours of collection.	Within **2** days.	All samples received within **4** working days of sample collection.
Recall	The screening centres immediately notify the neonatal guardian to take the screening-positive children to the screening center clinic or the designated referral unit to conduct the diagnosis test on the children in a timely manner	Any abnormal result must be communicated to the designated personnel responsible for follow-up.	The recall of screening positive babies is mentioned.	Timely processing of all screen positive samples. 99.7% of babies with a positive screening result have a clinical referral initiated within 4 working days of sample receipt by screening laboratory, and 100% by 17 days of age.
Follow-up	Required but not be regulated.	The pediatrician/ designated follow-up centers is responsible for calling the family and arranging follow-up .	Recommended but not regulated. Whether the parents were not informed/not traced by the institution responsible for screening, or whether they failed to bring their child for	Follow up screening and diagnostic tests shall be undertaken in line with the diagnostic protocols.

			a second blood sample, is not known. It is also unclear how often a positive result was not followed up or whether the treating center or pediatrician failed to communicate the results of the confirmatory diagnostic work-up to the screening laboratory.	
Age at initiation of treatment	NA.	NA.	Within 1 week	NA.
Therapeutic approaches and effects	Not be regulated.	Dietary Modulation; Toxic Metabolite Disposal; Enzyme Replacement Therapy; Novel Compounds.	NA.	NA.
Long-term data collection tracking, and surveillance system	Developing.	NA.	NA.	NA.
Quality assurance programs for screening	External quality assessment (EQA) programs	Newborn Screening Quality Assurance Program issued by the Centers for Disease Control and Prevention.	External quality assessment (EQA) programs.	External quality assessment (EQA) programs.
Quality assurance programs for diagnosis, follow-up, and treatment	Not develop.	NA.	NA.	NA.

Abbreviations: Data were not available, NA; Phenylketonuria, PKU; Medium-chain acyl-CoA dehydrogenase deficiency, MCADD; Maple syrup

urine disease, MSUD; isovaleric acidaemia, IVA; glutaric aciduria type 1, GA-1; homocystinuria (pyridoxine unresponsive), HCU.