National Screening Report Germany 2020

German Society for Neonatal Screening (DGNS)

Inken Brockow, Oliver Blankenstein, Uta Ceglarek, Regina Ensenauer, Ralph Fingerhut, Gwendolyn Gramer, Friederike Hörster, Nils Janzen, Jeannette Klein, Erwin Lankes, Martin Lindner, Peter Mirtschink, Simona Murko, Sabine Rönicke, Wulf Röschinger, Olaf Sommerburg, Carsten Speckmann, Theresa Winter, Uta Nennstiel

Date: November 2022

ISSN Number 2199-5494

Corresponding author:

Dr. med. Inken Brockow MPH
Screening Center
Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit
(Bavarian Health and Food Safety Authority)
Veterinärstr. 2
D-85764 Oberschleißheim
Germany
Email: inken.brockow@lgl.bayern.de

Table of Contents

Figures

Abbreviations and Glossary

CAH	Congenital Adrenal Hyperplasia
CACT Deficiency	Carnitine-Acylcarnitine Translocase Deficiency
CF	Cystic Fibrosis (Mucoviscidosis)
CFSPID	Cystic Fibrosis Screen Positive, Inconclusive Diagnosis
CPTI-Deficiency	Carnitine Palmitoyl Transferase I Deficiency
CPTII-Deficiency	Carnitine Palmitoyl Transferase II Deficiency
DB	Dried Blood
ENS	Extended Neonatal Screening
GA I	Glutaric Acidemia Type I
HPA	Hyperphenylalaninemia
IM	Insufficient Material
IRT	Immunoreactive Trypsinogen
IVA	Isovaleric Acidemia
LCHAD Deficiency / TFP Deficiency	Long-Chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency / Mitochondrial Trifunctional Protein Deficiency
MCAD Deficiency	Medium-Chain Acyl-CoA Dehydrogenase Deficiency
MSUD	Maple Syrup Urine Disease
PAP	Pancreatitis-associated Protein
PKU	Phenylketonuria
PPV	Positive Predictive Value
SCID	Severe Combined Immunodeficiency
Second Tier Method	In case of abnormal finding, second examination of additional parameters or alternative method of analysis with the same test card
WoG	Week of Gestation
VLCAD Deficiency	Very-Long-Chain Acyl-CoA Dehydrogenase Deficiency

Screening Laboratories und Screening Centers

The results for screening centers with multiple locations or laboratories which are affiliated with a screening center are broken down by location / affiliation.

(1) Neonatal Screening Lab Berlin
Dr. med. Oliver Blankenstein
Sylter Str. 2, 13353 **Berlin**
030/405 026 391 / Fax: -613
Contact: Dr. Jeannette Klein
Oliver.Blankenstein@charite.de
Jeannette.Klein@charite.de
https://screening.charite.de/

(3/10) Screening Center Saxony
Prof. Dr. med. Berend Isermann
University Clinic Leipzig

(3) Dresden Center
PO Box 160252, 01288 **Dresden**
0351/458 5230 / 5229
Contact: Dr. med. Peter Mirtschink
swscreening@uniklinikum-dresden.de

(10) Leipzig Center
Paul-List-Str. 13-15, 04103 **Leipzig**
0341/9722222 (Control Center ILM)
Contact: Prof. Dr. Uta Ceglarek
mb-sek-ilm@medizin.uni-leipzig.de
uta.ceglarek@medizin.uni-leipzig.de
http://www.screeningzentrum-sachsen.de

(5) Screening Center Hessen
PD Dr. med. Martin Lindner
Theodor-Stern-Kai 7, 60596 **Frankfurt**
069/6301 4594
neugeborenenscreening@kgu.de
www.screening-hessen.de

(6) Neonatal Screening Centre Mecklenburg-Western Pomerania
Prof. Dr. med. Matthias Nauck
Ferdinand-Sauerbruch-Str., 17475 **Greifswald**
Tel. 03834/865501
Contact: Dr. Theresa Winter
matthias.nauck@med.uni-greifswald.de
theresa.winter@med.uni-greifswald.de
http://www.medizin.uni-greifswald.de/klinchem/

(7) Screening Lab, University Children's Hospital
Prof. Dr. med. Gwendolyn Gramer
Martinistr. 52, 20246 **Hamburg**
040/7410 57037
Contact: Dr. Simona Murko
gramer@uke.de, s.murko@uke.de

(8) Screening Lab Hannover
Dr. med. Dr. rer.nat. Nils Janzen
PO Box 911009, 30430 **Hannover**
05108/92163 0
Contact: Dr. Ute Holtkamp
n.janzen@metabscreen.de
u.holtkamp@metabscreen.de
https://www.metabscreen.de

(9) Neonatal Screening Heidelberg
Prof. Dr. med. G.F. Hoffmann
Im Neuenheimer Feld 669, 69120 **Heidelberg**
06221/56 8278 / Fax -4069
Contact: PD. Dr.med. Friederike Hörster
friederike.hoerster@med.uni-heidelberg.de
juergen.guenther.okun@med.uni-heidelberg.de
https://www.neugeborenenscreening.uni-hd.de

(11) Screening Center Saxony Anhalt
University Clinic Magdeburg
Institute for Clinical Chemistry and Pathobiochemistry
Sr. Physician Dr. med. Katrin Borucki
PO Box 140274, 39043 **Magdeburg**
0391/6713986
Contact: Dr. rer. nat Sabine Rönicke
sabine.roenicke@med.ovgu.de
www.stwz.ovgu.de

(12/13) Lab Becker & Colleagues
Neonatal Screening
Prof. Dr.med. Dr. rer. nat. Jürgen Durner
Contact:
Priv.-Doz. Dr.med. Wulf Röschinger
Ottobrunner Str. 6, 81737 **München**
089/544 654 0
w.roeschinger@labor-becker.de
http://www.labor-becker.de/

(14/15) Screening Labor Synlab, Medical Care Center Weiden
Dr. med. Dr. rer nat. Wolfgang Schultis
Zur Kesselschmiede 4, 92637 **Weiden**
0961/309 0
Contact: PD Dr. Ralph Fingerhut
wolfgang.schultis@synlab.com
ralph.fingerhut@synlab.com
https://www.synlab.de/lab/weiden

Screening Center Bavaria (12/14)
Bavarian Health and Food Safety Authority
Dr. med. Uta Nennstiel MPH
Veterinärstr.2
85764 **Oberschleißheim**
09131/6808-5-204
screening@lgl.bayern.de
https://www.lgl.bayern.de/gesundheit/praevention/kindergesundheit/neugeborenenscreening/

1 Introduction

The neonatal screening is a medical population-based preventative measure with the goal of complete and early detection of all newborns affected by any of the targeted diseases of the hormonal, metabolic, immune, hematological and neuromuscular systems as well as cystic fibrosis, so that they can receive early treatment.

The implementation of the "extended newborn screening" (ENS) is regulated in the guideline on the early detection of diseases in children up to the age of 6 years, known as the Paediatric Directive or ("Kinder-Richtlinie") in §§13 - 28 [1]. The 2020 National Screening Report was compiled by the German Society for Neonatal Screening (DGNS e.V.) together with the German screening laboratories. The statistical processing of the screening data was based on the quality criteria defined in the guideline for the implementation of ENS in Germany.

The report refers exclusively to the target diseases defined in the guideline and presents a comprehensive statistical compilation of disease-related screening figures, recall rates (proportion of suspicious [positive] findings), and confirmed diagnoses for the year 2020. Additionally, the report provides process quality data for the whole of Germany.

Process quality describes the process sequences and their evaluation by professional bodies according to predefined indicators. These are as follows for the neonatal screening:

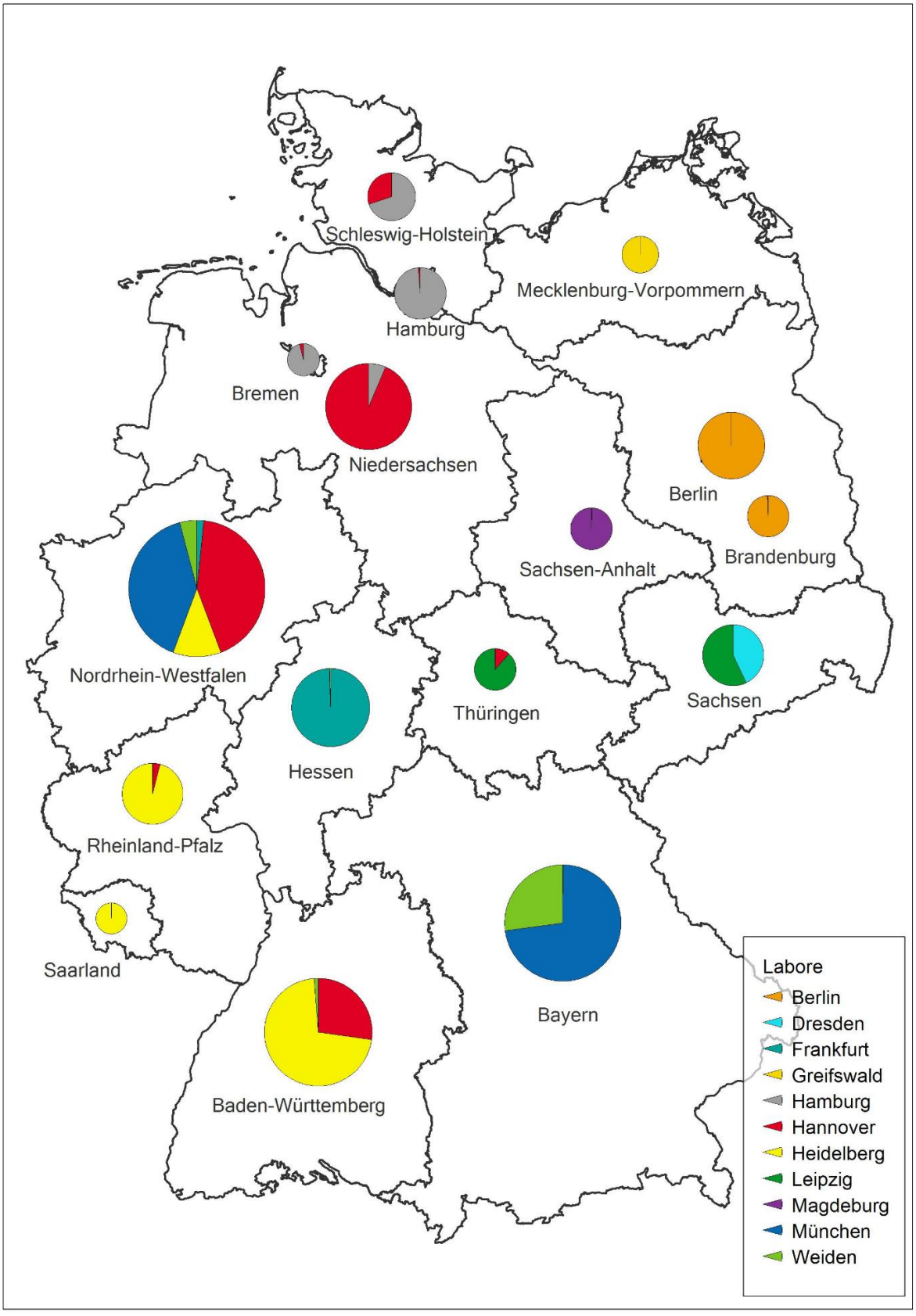
- Total survey of the targeted population
- Completeness of the control (recall) and repeat examinations
- Recording test parameters and cut-offs
- Specificity and sensitivity of diagnostic tests
- Age at blood sample collection, time between blood sample collection and receipt at the laboratory and between receipt of the sample and notification of findings.
- Confirmation diagnostics
    - Type of diagnostics
    - Period of diagnostics
- Final diagnosis
- Age at start of therapy

The laboratories that conducted the screening in Germany in 2020 are listed on the previous page (12 and 13 refer to the same laboratory, once in cooperation with a tracking center and once without; the same is true of 14 and 15). Mentions of sections and subsections in the text refer to the "Paediatrics Directive" (Kinder-Richtlinie) from November 16, 2020. [1] For convenience, the tables have not been numbered sequentially but rather in accordance with the related chapters.

We would like to thank all the laboratories for providing their data. The data have been checked for plausibility. In the cases of remaining inconsistencies, the data submitted by the laboratories were used in the tables.

The screening samples from the individual federal states are distributed among the laboratories ("Labore") as illustrated in Figure 1 and Table 2.2.

Figure 1: Distribution of Screening Samples by State and Laboratory



2 Results

In 2020 a total of 773,144 children were born in Germany according to official statistics. [2] The number of recorded first screenings (769,320) is slightly lower than the number of births. Cumulatively, 99.98% of all newborns were screened. A rejection of the examination was documented for only 486 newborns (0.06%).

Births:	773,144
First screenings:	769,320
Confirmed diagnoses:	826

A reliable statement about the rate of participation in ENS can only be made by reconciling individual data with overall population data. The diseases targeted for the nationwide screening are defined in the Paediatrics Directive. Other diseases screened in individual laboratories as part of studies or state law requirements are not included in this report.

In one in 936newborns, one of the target diseases defined in the guideline was detected during newborn screening. Table 2.1 shows the confirmed cases and prevalence of the target diseases in 2020 in relation to births in Germany.

Table 2.1: Prevalence of diseases detected in 2020 among 773,144 births

Disease	Confirmed cases	Prevalence
Hypothyroidism	265	1: 2,918
Congenital Adrenal Hyperplasia (CAH)	60	1: 12,886
Biotinidase Deficiency	23	1: 33,615
Galactosemia (classic form)	19	1: 40,692
Hyperphenylalaninemia	149	1: 5,189
of which classic phenylketonuria (PKU) n=77 /Cofactor deficiency n=2	79	1: 9,787
Maple Syrup Urine Disease (MSUD)	2	1: 386,572
Medium-Chain Acyl-CoA Dehydrogenase (MCAD) deficiency	84	1: 9,204
Long-chain 3-Hydroxyacyl-CoA Dehydrogenase (LCHAD) / TFP deficiency	11	1: 70,286
Very Long-Chain Acyl-CoA-Dehydrogenase (VLCAD) deficiency	12	1: 64,429
Carnitine Palmitoyl Transferase I (CPT I) deficiency	3	1: 257,715
Carnitine Palmitoyl Transferase II (CPT II) deficiency	0	
Carnitine-Acylcarnitine Translocase (CACT) deficiency	0	
Glutaric Acidemia (GA) Type I	7	1: 110,449
Isovaleric Acidemia (IVA)	6	1: 128,857
Tyrosinemia	7	1: 110,449
Cystic Fibrosis (CF)	146	1: 5,296
Severe Combined Immunodeficiency (SCID / Leaky-SCID / Syndrome)	32	1: 24,161
of which SCID	5	1: 154,629
Total	**826**	**1: 936**

2.1 Total Initial Screenings

The proportion of laboratories in the initial screening and in the confirmed diagnoses are shown in Table 2.2. Confirmed cases also include those with negative initial screening or conspicuous follow-up screening cards. The proportion of confirmed cases per laboratory roughly corresponds to the proportion of the total number of initial screening examinations.

Table 2.2: Distribution of initial screening and all confirmed cases among laboratories

Lab	Initial Screening	Proportion of total population (%)	Number of confirmed cases	Proportion of confirmed cases (%)
1	60,828	7.91	70	8.47
3	13,790	1.79	16	1.94
5	59,118	7.68	65	7.87
6	12,005	1.56	12	1.45
7	47,165	6.13	50	6.05
8	182,396	23.71	212	25.67
9	140,955	18.32	157	19.01
10	34,075	4.43	37	4.48
11	15,951	2.07	13	1.33
12/13	163,267	21.22	156	18.89
14/15	39,770	5.17	38	4.60
Total	**769,320**	**100**	**826**	**100**

According to the Paediatrics Directive, screening should be arranged for every newborn before discharge from the maternity facility. If the first screening is carried out before 36 hours of life or before a corrected gestational age of 32 weeks (WoG) a second screening should be carried out.

The following table shows the number of first screening examinations stratified by age and gestational age. This is generally defined as follows:

- **"<32 WoG"**: all samples collected from children born before a corrected gestational age of 32 WoG, regardless of age at the time the sample was collected.
- **"<36h"**: all samples in children over 32 WoG taken before 36 hours of life.

The proportion of initial screenings <36h increased significantly in 2020, likely pandemic-related with many outpatient births.

Table 2.3: Age at time of initial screening

		≥36h and ≥32WoG		<36h and ≥32WoG		<32WoG	
Lab	Total	n	%	n	%	n	%
1	60,828	59,816	98.34	448	0.74	564	0.93
3	13,790	13,185	95.61	357	2.59	248	1.80
5	59,118	58,185	98.42	402	0.68	531	0.90
6	12,005	11,582	96.48	267	2.22	156	1.30
7	47,165	46,227	98.01	640	1.36	298	0.63
8	182,396	178,140	97.67	2,320	1.27	1,936	1.06
9	140,955	137,302	97.41	1,580	1.12	2,073	1.47
10	34,075	33,466	98.21	290	0.85	319	0.94
11	15,951	15,444	96.82	330	2.07	177	1.11
12	95,589	93,048	97.34	1,592	1.67	949	0.99
13	67,678	65,646	97.00	1,251	1.85	781	1.15
14	32,648	31,677	97.03	630	1.93	341	1.04
15	7,122	6,974	97.92	41	0.58	107	1.50
Total	769,320	750,692	97.58	10,148	1.32	8,480	1.10

2.2 Ratio of requested to received second screening examinations and stratified recall rates by laboratory

Table 2.4 shows the total second screening examinations requested and performed as reported by the laboratories. The reason for the request has not been inquired about since 2018. These numbers often do not match the sum of the data on required second screening examinations in Tables 2.3, 2.5, and 2.7. This question was evidently interpreted in differing ways in the laboratories e.g., by additionally indicating abnormal findings in early admissions before 36h or <32SSW as recall.

In Table 2.5, the control examinations due to an abnormal initial screening (recall) are shown stratified by laboratory and by age at life or gestational age.

Table 2.4: Received second screenings

Lab	Second screenings requested	Second screenings received	%
1	1,476	1,375	93.16
3	272	272	100
5	1,025	962	93.85
6	423	385	91.02
7	1,033	854	82.67
8	6,425	5,911	92.00
9 [a]	4,957	4,105	82.81
10 [a]	859	790	91.97
11	507	485	95.66
12	3,362	3,302	98.22
13	2,361	2,166	91.74
14	968	925	95.56
15	157	152	96.82
Total	**23,825**	**21,684**	**91.09**

[a] External findings from other screening laboratories are not recorded

Table 2.5: Requested repeat examinations due to abnormal findings (recall)[a]

		Recall total		Recall >=36h[b]		Recall <36h		Recall <32 WoG	
Lab	Initial Screening	n	%	n	%	n	%	n	%
1	60,828	286	0.47	198	0.33	8	1.79	80	14.18
3	13,790	48	0.35	45	0.34	0	0.00	3	1.21
5	59,118	337	0.57	336	0.58	0	0.00	1	0.19
6	12,005	95	0.79	83	0.72	4	1.50	8	5.13
7	47,165	707	1.50	565	1.22	109	17.03	33	11.07
8	182,396	1,324	0.73	847	0.47	286	12.32	198	10.23
9	140,955	794	0.56	766	0.56	8	0.51	20	0.96
10	34,075	293	0.86	184	0.55	80	27.59	29	9.09
11	15,951	134	0.84	70	0.45	57	17.27	7	3.95
12	95,589	277	0.28	247	0.26	17	0.82	13	0.74
13	67,678	237	0.35	209	0.32	5	0.40	23	2.94
14	32,648	163	0.46	157	0.46	5	0.79	1	0.29
15	7,122	65	0.91	43	0.62	6	14.63	16	14.95
Total	**769,320**	**4,760**	**0.62**	**3,748**	**0.50**	**585**	**5.76**	**427**	**5.04**

[a] Excluding recall „MS/ MS abnormal finding for uncertain target disease", as some labs report recalls for projects and the data are not comparable [b] incl. recall unassigned due to missing information

As a public health measure, the newborn screening is intended to benefit all children born in Germany. This requires tracking for completeness. For children born as inpatients, this can be done by checking the consecutive birth register numbers in the screening laboratory or, if permitted by state legislation, by a personal comparison with the registration registers of the residents' registration offices. A comparison of the screening reports with a unique screening ID assigned at birth for each child or with hearing screening reports is also useful for ensuring completeness.

At present these options are not being implemented nationwide in Germany. With the aim of nevertheless monitoring the completeness of the screening examinations, in accordance with the Paediatrics Directive [§ 21 Paragraph 6], blank filter paper cards are to be sent to the screening laboratory in the event of refusal of screening or death of the newborn before a possible first blood sample is taken. The laboratories receive these blank cards in widely varying numbers. In addition, blank cards are often sent in for declined early collections. The total number of blank cards sent increased significantly in 2020 relative to the total number of initial screening reports, likely pandemic-related with many outpatient deliveries. In contrast, the number of blank cards sent in due to refusal to participate in the screening remained about the same.

The blank card system seems to work primarily for refused screening examinations. Based on the data from the perinatal survey, considerably higher numbers would be expected both for children who died before screening and for those who were transferred.

Table 2.6: Blank cards received by the laboratory

		Reason for blank card						
	Initial Screening Total	Deceased	Screening refused	Trans-ferred	Early screening rejected	Not differen tiable	Total	
Lab	n	n	n	n	n	n	n	%
1	60,828	312	99	379	345	4,772	5,907	9.71
3	13,790	26	29	72		489	616	4.47
5	59,118	28	65	1,148	323	2,694	4,258	7.20
6	12,005	16	29	19		706	770	6.41
7	47,165				708		708	1.50
8	182,396					5,189 [a]	5,189	2.84
9	140,955	8	207	239	1,872		2,326	1.65
10	34,075	172	54		2,153		2,379	6.98
11	15,951	45	11	25	57	349	487	3.05
12	95,589			197	319	1,868	2,384	2.49
13 [b]	67,678							
14	32,648			25	28	177	230	0.70
15 [b]	7,122							
Total	**769,320**	**607**	**494**	**2,104**	**10,994**	**11,055**	**25,254**	**3.28**

[a] Total number, differentiation not possible

[b] Lab does not track blank cards

Table 2.7: Secondary screening card due to inferior sample quality

Lab	Initial screening Total	Control requested	Control received	received/ requested (%)	Proportion of samples / Initial screening (%)	IM [a]
1	60,828	293	259	88.40	0.48	529
3	13,790	n/a	138			n/a
5	59,118	518	490	94.59	0.88	n/a
6	12,005	4	4	100	0.03	37
7	47,165	262	n/a		0.56	n/a
8	182,396	395	387	97.97	0.22	246
9	140,955	101	86	85.15	0.07	827
10	34,075	7	7	100	0.02	186
11	15,951	15	15	100	0.09	n/a
12	95,589	835	823	98.56	0.87	5
13	67,678	619	558	90.15	0.91	n/a
14	32,648	54	52	96.30	0.17	3
15	7,122	17	16	94.12	0.24	4
Total	**769,320**	**3,120**	**2,835**	**94.37** [a]	**0.37** [a]	**1,837**

[a] Calculated without data for lab 3 and 7

The definition of a screening card with poor sample quality has not been clear so far, which is why the proportion of control cards requested for this reason in relation to the initial screening varies greatly. For example, some laboratories count test cards with highly scattered IRT values from different stamps here, as this indicates contamination of the card, while others count these as CF recalls.

Insufficient material (IM) includes samples for which the number of blood-soaked circles on the screening card was not sufficient to perform the full screening (including samples for which the CF algorithm could not be fully run). This number of samples was listed separately in Table 2.7. and was not included in the requested control cards.

3 Processing Time

3.1 Age at the time of blood sample collection

According to the Paediatrics Directive (§ 20 paragraph 1) blood samples should be collected between 36 and 72 hours after birth. In 96.2% of cases in which the time of blood sampling was provided, collection took place in the designated time frame, in 3.8% not until after 72 hours and in 1.39% before 36 hours (Table 3.1). The proportion of samples which were collected after 72 hours - i.e., outside the designated time frame - was reduced from 22.3% in 2006 to 3.8% in 2020 (Figure 2).

This means a marked improvement in process quality, as adherence to the optimal time frame is of great importance for the effectiveness of the screening. Potentially life-threatening metabolic or electrolyte crises can be avoided through very early diagnosis and initiation of therapy in affected children.

Table 3.1: Age at blood sample collection - Initial screening

	Total	<36h		36h-<=48h		48h-<=72h		≥72h	
Lab	n	n	%	n	%	n	%	n	%
1	60,825	497	0.82	23,106	37.99	34,459	56.65	2,763	4.54
3	13,790	99	0.72	4,183	30.33	9,161	66.43	347	2.52
5	59,118	467	0.79	44,594	75.43	12,288	20.79	1,769	2.99
6	12,005	279	2.32	5,760	47.98	5,607	46.71	359	2.99
7	47,165	737	1.56	23,905	50.68	19,650	41.66	2,873	6.09
8	182,050	2,397	1.32	91,089	50.04	81,213	44.61	7,351	4.04
9	140,955	1,738	1.23	76,739	54.44	57,008	40.44	5,470	3.88
10	34,075	337	0.99	12,635	37.08	19,878	58.34	1,225	3.60
11	15,951	408	2.56	6,346	39.78	8,344	52.31	853	5.35
12	94,766	1,716	1.81	61,340	64.73	28,970	30.57	2,740	2.89
13	67,678	1,315	1.94	39,645	58.58	24,165	35.71	2,553	3.77
14	32,644	654	2.00	18,208	55.78	12,949	39.67	833	2.55
15	7,122	50	0.70	4,058	56.98	2,929	41.13	85	1.19
Total	**768,144** [a]	**10,694**	**1.39**	**411,608**	**53.58**	**316,621**	**41.22**	**29,221**	**3.80**

[a] The number of samples for which times are known is below the total number of initial screening samples in some laboratories due to missing data

3.2 Period between sample collection and receipt by the lab

The time interval between taking blood samples and reporting abnormal results should not exceed 72 hours (Paediatrics Directive § 18 paragraph 3), However, in 30.71% of cases in which the shipping times were provided, the sample did not reach the lab until more than 72 hours after the blood sample was taken, In another 23.18% of cases, the time period ranged from 48 to 72 hours.

The proportion of dispatch times greater than 72 hours varies greatly between the laboratories and has increased over the years. Overall, efforts must be made work with the submitting parties to shorten the time span for sample shipment, particularly on weekends, so as not to jeopardize the success of screening for target diseases at risk of early decompensation. (Table 3.2, Figure 3).

Table 3.2: Period between sample collection and receipt by the lab

	Total	≤24h		>24h-48h		>48h-72h		>72h	
Lab	n	n	%	n	%	n	%	n	%
1	60,765	13,466	22,16	20,396	33.57	12,074	19.87	14,829	24.40
3	13,790	5,085	36,87	5,804	42.09	2,086	15.13	815	5.91
5	59,110	4,779	8,08	20,019	33.87	15,834	26.79	18,478	31.26
6	12,005	385	3,21	3,257	27.13	3,512	29.25	4,851	40.41
7	47,165	10,795	22,89	12,062	25.57	9,009	19.10	15,299	32.44
8	182,050	14,171	7,78	47,037	25.84	48,303	26.53	72,539	39.85
9	140,955	9,281	6,58	32,436	23.01	33,296	23.62	65,942	46.78
10	34,075	4,088	12,00	12,801	37.57	9,827	28.84	7,359	21.60
11	15,951	2,149	13,47	5,980	37.49	4,507	28.26	3,315	20.78
12	94,765	24,968	26,35	36,487	38.50	19,363	20.43	13,947	14.72
13	67,678	16,494	24,37	22,067	32.61	14,919	22.04	14,198	20.98
14	32,644	18,018	55,20	8,822	27.02	3,664	11.22	2,140	6.56
15	7,122	932	13,09	2,314	32.49	1,681	23.60	2,195	30.82
Total	**768,075** [a]	**124,611**	**16,22**	**229,482**	**29.88**	**178,075**	**23.18**	**235,907**	**30.71**

[a] The number of samples for which times are known is below the total number of initial screening samples in some laboratories due to missing data

3.3 Period between receipt by the lab and reporting the results

In accordance with the Paediatrics Directive § 26 Paragraph 3, examinations must be performed and pathological findings reported on the day the specimen is received, 73.66% of the results are reported within 24 hours, whereby no distinction is made between pathological and inconspicuous findings. In the case of marginally elevated findings, the time in the laboratory can be extended due to internal repeat examinations.

In 2017 the proportion of findings that were not reported until two to three days after receipt by the laboratory rose and has remained roughly the same since then. This may be related to the new CF screening introduced at the end of 2016. Delays in notification apply primarily to unremarkable findings, as abnormal findings are usually reported immediately (Table 3.3, Figure 4).

Table 3.3: Period between receipt by the lab and reporting the results

	Total	≤24h		>24h-48h		>48h-72h		>72h	
Lab	n	n	%	n	%	n	%	n	%
1	60,578	17,119	28.26	31,886	52.64	8,191	13.52	3,382	5.58
3	13,790	6,019	43.65	5,274	38.25	2,010	14.58	487	3.53
5	59,120	48,986	82.86	10,110	17.10	19	0.03	5	0.01
6	12,005	8,320	69.30	227	1.89	1,514	12.61	1,944	16.19
7	47,165	19,337	41.00	20,894	44.30	4,763	10.10	2,171	4.60
8	182,396	168,272	92.26	11,002	6.03	1,067	0.58	2,055	1.13
9	140,955	102,394	72.64	33,919	24.06	3,770	2.67	872	0.62
10	34,075	30,123	88.40	3,519	10.33	399	1.17	34	0.10
11	15,951	8,984	56.32	4,091	25.65	2,220	13.92	656	4.11
12	95,589	74,693	78.14	15,568	16.29	2,837	2.97	2,491	2.61
13	67,678	53,722	79.38	10,323	15.25	2,201	3.25	1,432	2.12
14	32,648	23,833	73.00	6,951	21.29	1,317	4.03	547	1.68
15	7,122	4,720	66.27	2,355	33.07	42	0.59	5	0.07
Total	**769,072** [a]	**566,522**	**73.66**	**156,119**	**20.30**	**30,350**	**3.95**	**16,081**	**2.09**

[a] The number of samples for which times are known is below the total number of initial screening samples in some laboratories due to missing data

Figure 2: Age at the time of blood sample collection 2006 to 2020

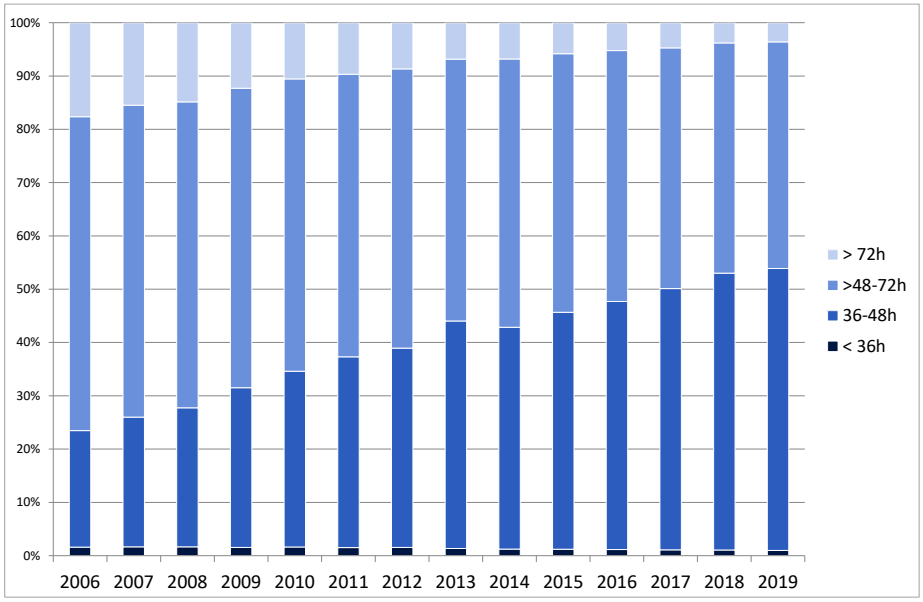


Figure 3: Time between blood sample collection and receipt by the lab 2006 to 2020

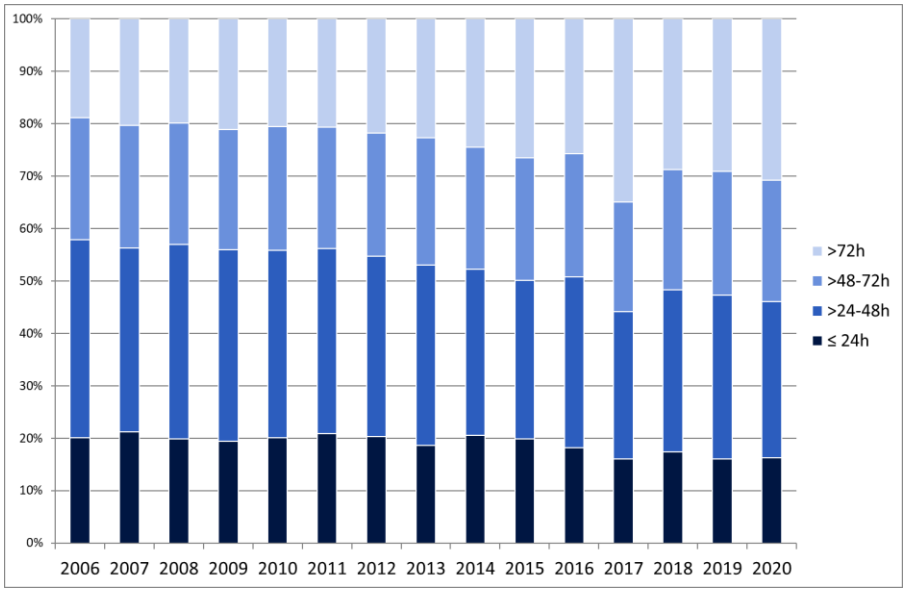
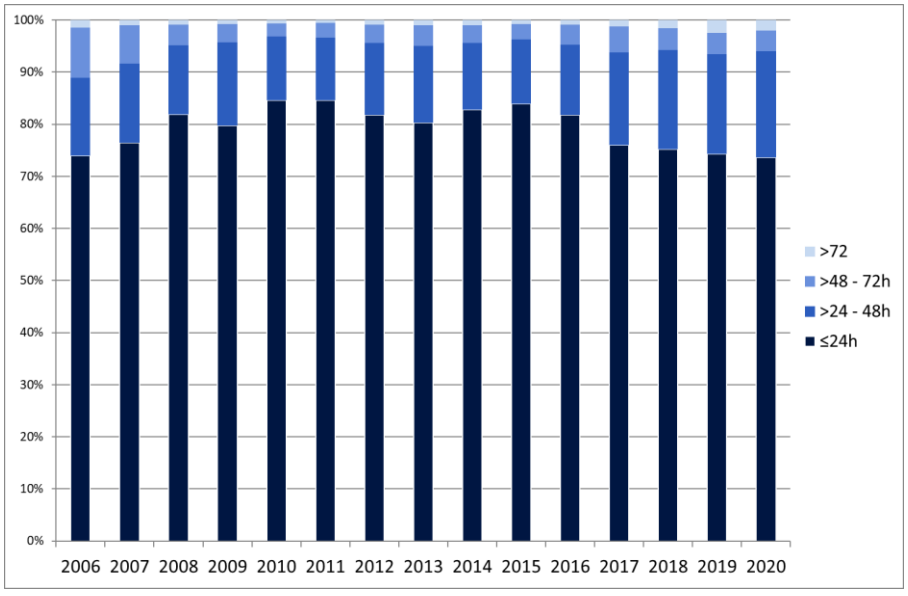


Figure 4: Time between receipt by the lab and reporting the results 2006 to 2020



4 Quality parameters of screening analysis

The quality of a test procedure is determined by sensitivity, specificity and positive predictive value (PPV). In a screening procedure, the sensitivity (sick people with a positive test) but also the specificity (proportion of healthy people with a negative test) should be high in order to identify all those affected on the one hand and to cause as little unnecessary worry and subsequent expense as possible on the other. The recall rate for the ENS was 0.52% in 2020. In the CF screening, the positivity rate was 0.1%. This means that out of 1,000 screening examinations, approximately 6 results requiring a control examination can be expected. If the blood sample is taken before 36 hours of life or 32 weeks of pregnancy, a second screening must be carried out, irrespective of the result of the analysis. When taking only screening samples into account that were collected after 36 hours of life from babies born at term, the recall rate for the entire screening (ENS and CF) is 0.5%. The classification of abnormal findings in blood collection <36h or before 32 weeks as recall also has a negative effect on the PPV in CAH and hypothyroidism. For example, the PPV for hypothyroidism would be 35.67% if only the recall at collection >36h is taken into account.

The overall specificity for newborn screening was 99.44%. Sensitivity cannot be given because the number of children missed in screening is not systematically recorded. Here, registries for the target diseases of the screening would be very helpful, combined with an obligation for the treating centers to report diagnosed cases.

Table 4: Recall rates and cases found through screening for Germany 2020
(Initial screening N= 769,320)

Disease	Recall	Recall rate (%)	Confirmed Cases	PPV	Specificity
Hypothyroidism	1,173	0.152	255 [b]	21.74	99.88
CAH	923	0.119	60	6.50	99.86
Biotinidase Deficiency	281	0.037	23	8.19	99.97
Galactosemia [a]	217	0.028	19	8.76	99.97
PKU/HPA	248	0.032	149	60.08	99.99
MSUD	42	0.005	2	4.76	99.99
MCAD	181	0.024	84	46.41	99.99
LCHAD	30	0.004	11	36.67	99.99
VLCAD	142	0.018	12	8.45	99.98
CPT-I Deficiency	6	0.001	3	50.00	99.99
CPT-II Deficiency [d]	9	0.001	0		
GA I	144	0.019	7	4.86	99.98
IVA	109	0.014	6	5.50	99.99
Tyrosinemia	125	0.016	7	5.60	99.97
CF	750	0.097	140 b	18.67	99.92
SCID [c]	380	0.049	32	8.42	99.95
Total ENS	**4,760**	**0.619**	**810** [b]	**17.02**	**99.44**

[a] Only classic galactosemia
[b] Excluding 10 hypothyroid and 6 CF cases with unremarkable screening
[C] Initial screening from 8/2020 [d] Can include recalls for CACT

4.1 Time of Initial screening in confirmed cases

The success of the screening depends on the reliability of the results and the speed with which, in suspected cases, confirmatory diagnostics are carried out and therapeutic measures initiated. According to the guideline, the blood sample should not be taken less than 36 hours before or more than 72 hours after birth except in the case of early discharge. Any delay represents a potential risk for the children concerned.

Table 4.1 shows the age at Initial screening for children with one of the targeted diseases. For better clarity, ages of more than 72 hours are given in days, calculated from the number of hours of life,

Table 4.1: Time of Initial screening in confirmed cases

Disease	36-72h	4-7d	>7d	<36h	<32WoG[a]	Incomplete information [b]	Total
Hypothyroidism	221	8	0	8	26	2	265
CAH	49	1	1	5	1	3	60
Biotinidase Deficiency	23	0	0	0	0	0	23
Galactosemia	17	2	0	0	0	0	19
PKU/HPA	135	3	1	7	3	0	149
MSUD	1	0	0	1	0	0	2
MCAD	79	1	0	2	2	0	84
LCHAD	6	0	0	2	3	0	11
VLCAD	12	0	0	0	0	0	12
CPT I	3	0	0	0	0	0	3
CPT II	0	0	0	0	0	0	0
GA I	7	0	0	0	0	0	7
IVA	5	1	0	0	0	0	6
Tyrosinemia	7	0	0	0	0	0	7
CF	136	5	2	1	0	2	146
SCID	31	0	0	1	0	0	32
Total	**732**	**21**	**4**	**27**	**35**	**7**	**826**

[a] Data independent of age in days at the time the blood sample was collected

[b] Exact age at the time of blood collection and/or week of gestation not provided

5 Recall rate, confirmed cases and confirmation stratified by disease

The following chapter presents recall rates and confirmed cases for the target diseases as well as the diagnostic measures taken to confirm the diagnosis, stratified by laboratory. For hypothyroidism and CAH, the recall is also reported separately for recall ≥ 36h, recall <36h and recall <32 WoG. For the other diseases, this stratified presentation was omitted due to the low number of cases <36h and <32 WoG,

Diagnostic measures can only be reported if the laboratories are informed of them. Knowledge of the individual results of confirmation diagnostics is important for quality assurance in the laboratory but they are not always communicated to the laboratories by the attending physicians. In particular, molecular genetic examinations are often only initiated during the course of the disease and therefore are not included in the findings of the confirmation diagnostics sent to the laboratory. In 2017, for instance, in 180 (24,42%) cases of cystic fibrosis, so little information was available that the diagnosis of "cystic fibrosis" could neither be confirmed nor ruled out. Since 2018, only confirmed CF cases, rather than all positive CF screening results, are requested. The number of non-confirmed abnormal CF screening results is therefore not known from all laboratories. As a rule, it is not possible to draw conclusions from CF screening figures about the probability of a CF diagnosis, unless 2 mutations in the CFTR gene were found in the last step of the screening algorithm (see Fig. 5).

The figures were reported as of November 16, 2022. Cases from birth year 2020 which were found at a later date are not included in this report. Cases reported twice (e.g., from different laboratories) were only counted once. The plausibility check of the cases reported as confirmed was carried out by Prof. Dr. Regina Ensenauer and Prof. Dr. Gwendolyn Gramer for metabolic diseases by Dr. Oliver Blankenstein and Dr. Erwin Lankes for endocrinological diseases, by PD Dr. Olaf Sommerburg for cystic fibrosis and by PD Dr. Carsten Speckmann for Severe Combined Immunodeficiency,

A total of 33 cases were considered despite missing information on confirmation diagnosis. In 27 cases, the validators judged a diagnosis to be probable based on the screening values or only "diagnosis confirmed" was noted in the data set (13 metabolic screening, 9 hypothyroidism, 2 AGS and 3 SCID cases). Also in 6 CF cases only "diagnosis confirmed" was available (see Table 6.1.1.1). In 48 cases with abnormal ENS, the information on the confirmation diagnostics was not sufficient to confirm the diagnosis (see section 6,1,2).

As a result, the true prevalence of some diseases may be higher than reported here. Also, diagnosed cases with unremarkable screening results are not systematically recorded. In 2020, 10 cases of hypothyroidism and 6 CF cases were clinically diagnosed following unremarkable screening reported to the laboratories. In the interest of quality assurance of the laboratory analysis and evaluation of the quality of the results, the most comprehensive feedback possible must be sought from the attending physicians.

In the following tables, recall rates <0,01% and for n < 5 are not calculated, because for smaller values the random fluctuations would have a disproportionately large impact,

Some laboratories count abnormal findings before 36 hours or 32 weeks of gestation as recall, although the findings must be checked in any case. The differences in the following tables are partly due to this.

5.1 Congenital Hypothyroidism

Table 5.1.1: Hypothyroidism confirmed cases / recall rate

		Total			≥ 36h		
Lab	Initial screening	Recall (n)	Recall rate (%)	Confirmed cases (n)	Recall (n)	Recall rate (%)	Confirmed cases (n)
1	60,828	80	0.13	25	75	0.13	24
3	13,790	16	0.12	2	14	0.11	2
5	59,118	62	0.10	18	62	0.11	18
6	12,005	7	0.06	2	6	0.05	2
7	47,165	100	0.21	14	53	0.11	13
8	182,396	489	0.27	67	239	0.13	61
9	140,955	93	0.07	53	90	0.07	42
10	34,075	83	0.24	9	30	0.09	9
11	15,951	51	0.32	4	7	0.05	3
12	95,589	78	0.08	39	64	0.07	31
13	67,678	60	0.09	22	54	0.08	16
14	32,648	40	0.12	8	37	0.12	7
15	7,122	14	0.20	2	12	0.17	2
Total	**769,320**	**1,173**	**0.15**	**265** [a]	**743**	**0.10**	**231** [b]

		<36h			<32 WoG		
Lab	Initial screening	Recall (n)	Recall rate (%)[c]	Confirmed cases (n)	Recall (n)	Recall rate (%)[c]	Confirmed cases (n)
1	60,828	4		1	1		
3	13,790	0			2		
5	59,118	0			0		
6	12,005	1			0		
7	47,165	44	6.88	1	3		
8	182,396	244	10.52	3	6	0.31	3
9	140,955	0		1	3		10
10	34,075	53	18.28		0		
11	15,951	44	13.33		0		1
12	95,589	7	0.44	1	7	0.74	7
13	67,678	1		1	5	0.64	5
14	32,648	2			1		
15	7,122	2			0		
Total	**769,320**	**402**	**3.96**	**8**	**28**	**0.33**	**26**

[a] including 10 cases with an unremarkable initial screening

[b] including 2 cases without indication of the time of the initial screening

[c] recall rates only provided if recall rate ≥ 0,01% and n ≥ 5

Of the 265 congenital hypothyroidism cases validated as confirmed, ten cases were unremarkable in the initial screening or additionally in the control screening at 32 SSW. In one pair of twins (SSW 33), the regular 1st test card at 47 hours was unremarkable (TSH 2.69 and 2.20 mU/l, respectively), the TSH value at control before discharge on the 27th day of life was then conspicuous (TSH 19.5 mU/l and 11.78 mU/l, respectively). Only after several follow-up letters the confirmation diagnosis was performed on the 50th day of life and the therapy was started with decreased fT4 values of 0.23 ng/dl and 0.58 ng/dl, respectively. Another twin (27 WoG) with unremarkable TSH values on the 1st test card at 37 hours and the 2nd test card at 841 hours (0.4 mU/l and 9.3 mU/l, respectively) was checked again on the 43rd day of life due to abnormal values of the second twin, and therapy was started at a decreased fT4 of 10 pmol/l. For the other children, no information is available on possible causes of the false negative screening.

In addition, n= 40 hyperthyrotropinemia were reported and validated as confirmed. These were not included in the calculation of prevalence,

Table 5.1.2: Hypothyroidism Confirmation

Lab	Confirmed cases	TSH	fT3	fT4	Sonography	SD Antibodies	Confirmed cases without verification details
1	25	(Serum)	5	25	23	15	
3	2	25	1	2	2	2	
5	18	2	13	17	14	14	
6	2	17	2	2	2	1	
7	14	2	5	7	1	2	6
8	67	7	55	64	62	50	
9	53	65	35	48	21	8	2
10	9	51	7	8	5	7	
11	4	9	3	3	3	3	
12	39	3	28	39	4	6	
13	22	39	15	22			
14	8	22	7	8		3	
15	2	8	1	1	1		1
Total	**265**	**1**	**177**	**246**	**138**	**111**	**19**

5.2 Congenital Adrenal Hyperplasia (CAH)

Table 5.2.1: CAH Confirmed cases / Recall rate

		Total			≥ 36h		
Lab	**Initial screening**	Recall (n)	Recall rate (%)[c]	Confirmed cases (n)	Recall (n)	Recall rate (%)[c]	Confirmed cases (n)
1 [a]	60,828	11	0.02	8	6	0.01	8
3	13,790	2		1	2		1
5	59,118	136	0.23	2	135	0.23	2
6	12,005	15	0.12	0	10	0.09	0
7	47,165	307	0.65	4	251	0.54	3
8 [b]	182,396	97	0.05	13	59	0.03	12
9	140,955	141	0.10	13	139	0.10	13
10	34,075	103	0.30	2	55	0.16	2
11	15,951	51	0.32	2	33	0.21	1
12 [b]	95,589	30	0.03	7	23	0.02	4
13 [b]	67,678	21	0.03	5	15	0.02	5
14 [a]	32,648	5	0.02	2	5	0.02	2
15 [a]	7,122	4		1	2		1
Total	**769,320**	**923**	**0.12**	**60**	**735**	**0.10**	**54 [d]**

		<36h			<32 WoG		
Lab	**Initial screening**	Recall (n)	Recall rate (%)[c]	Confirmed cases (n)	Recall (n)	Recall rate (%)[c]	Confirmed cases (n)
1 [a]	60,828	0		0	5	0.89	0
3	13,790	0		0	0		0
5	59,118	0		0	1		0
6	12,005	1		0	4		0
7	47,165	44	6.88	0	12	4.03	1
8 [b]	182,396	1		1	37	1.91	0
9	140,955	1		0	1		0
10	34,075	25	8.62	0	23	7.21	0
11	15,951	13	3.94	1	5	2.82	0
12 [b]	95,589	6	0.38	3	1		0
13 [b]	67,678	0		0	6	0.77	0
14 [a]	32,648	0		0	0		0
15 [a]	7,122	2		0	0		0
Total	**769,320**	**93**	**0.92**	**5**	**95**	**1.12**	**1**

[a] Lab uses 2nd tier method [b] Lab uses 2nd tier method only in cases of blood collection > 36 hours

[c] Recall rates only provided if recall rate ≥ 0,01% and n ≥ 5

[d] Includes 3 cases with incomplete data at the time of the first screening

Table 5.2.2: CAH Confirmation

Lab	Confirmed cases	17-OHP (Serum)	Steroids (Serum/DB)	Urinary steroids	Molecular genetics	Confirmed cases without confirmation details
1	8	6	8		8	
3	1	1	1			
5	2	2	2			
6	0					
7	4	1			3	1
8	13	9	11	1	11	
9	13	12	8		3	
10	2	1	1			
11	2	1	1	1	1	1
12	7	7	7	1	5	
13	5	3			5	
14	2	2	2		1	
15	1	1				
Total	**60**	**46**	**41**	**3**	**37**	**2**

A second-tier procedure, previously performed in only four laboratories, significantly reduces the recall rate of AGS screening.

5.3 Biotinidase Deficiency

Table 5.3.1: Biotinidase Deficiency - Confirmed cases / Recall rate

Lab	Initial screening	Recall	Recall rate (%) [a]	Confirmed cases
1	60,828	34	0.06	4
3	13,790	1		1
5	59,118	6	0.01	3
6	12,005	8	0.07	0
7	47,165	53	0.11	1
8	182,396	79	0.04	6
9	140,955	41	0.03	3
10	34,075	1		0
11	15,951	2		1
12	95,589	26	0.03	1
13	67,678	25	0.04	2
14	32,648	3		1
15	7,122	2		0
Total	**769,320**	281	0.04	23

[a] Recall rates only provided if recall rate ≥ 0,01% and n ≥ 5

Of n= 23 confirmed cases, a partial biotinidase deficiency was diagnosed in n=13 cases.

Table 5.3.2: Biotinidase Deficiency Confirmation

Lab	Confirmed cases	Biotinidase (Serum/TB)	Molecular genetics	Confirmed cases without confirmation details
1	4	3	2	
3	1			1
5	3	3		
7	1	1	1	
8	6	5	1	1
9	3	3	2	
11	1	1		
12	1	1	1	
13	2	2		
14	1	1		
Total	**23**	**20**	**7**	**2**

5.4 Classic Galactosemia

Table 5.4.1: Classic Galactosemia Confirmed cases / Recall rate

Lab	Initial screening	Recall	Recall rate (%) [b]	Confirmed cases [a]
1	60,828	25	0.04	2
3	13,790	1		0
5	59,118	7	0.01	1
6	12,005	3		0
7	47,165	41	0.09	2
8	182,396	101	0.06	6
9	140,955	14	0.01	3
10	34,075	2		0
11	15,951	2		0
12	95,589	15	0.02	3
13	67,678	2		2
14	32,648	2		0
15	7,122	2		0
Total	**769,320**	**217**	**0.03**	**19**

[a] Only classic galactosemia

[b] Recall rates only provided if recall rate ≥ 0,01% and n ≥ 5

Table 5.4.2: Classic Galactosemia Confirmation

Lab	Confirmed cases	Enzymatics	Galactose, Gal1P	Molecular genetics	Confirmed cases without confirmation details
1	2	2	2	2	
5	1	1		1	
7	2	1	1	2	
8	6	5	6	5	
9	3	3	3	2	
12	3			2	1
13	2		2		
Total	**19**	**12**	**14**	**14**	**1**

In addition, n=30 cases with a galactosemia variant, n=4 with a kinase deficiency, and n=2 with an epimerase deficiency were reported.

5.5 Phenylketonuria (PKU) / Hyperphenylalaninemia (HPA)

Table 5.5.1: PKU/HPA Confirmed cases / Recall rate

Lab	Initial screening	Recall	Recall rate %) [a]	Confirmed cases
1	60,828	16	0.03	9
3	13,790	6	0.04	6
5	59,118	17	0.03	11
6	12,005	7	0.06	4
7	47,165	25	0.05	8
8	182,396	49	0.03	45
9	140,955	37	0.03	28
10	34,075	9	0.03	7
11	15,951	4		3
12	95,589	14	0.01	11
13	67,678	19	0.03	14
14	32,648	42	0.13	3
15	7,122	3		0
Total	**769,320**	**248**	**0.03**	**149**

[a] Recall rates only provided if recall rate ≥ 0,01% and n ≥ 5

Of n=149 confirmed cases, 77 were diagnosed with PKU, 70 with HPA and 2 with cofactor deficiency.

Table 5.5.2: PKU/HPA Confirmation

Lab	Confirmed cases	Phe (Serum/DB)	Phe/Tyr	Molecular genetics	Pterins (Urine/DB)	DHPR (DB)	Confirmed cases without confirmation details
1	9	8	7	5	9	8	
3	6	6	6				
5	11	9	5	4	9	9	1
6	4	4	1	4	4	4	
7	8	8	7	6	7	8	
8	45	41	21	14	28	28	1
9	28	22	20	9	26	26	
10	7	6	6	4	5	4	
11	3	2	2		2	2	1
12	11	10	2	7	10	10	1
13	14	14	14		12	12	
14	3	3	1		3	3	
15	0						
Total	149	**133**	**92**	**53**	**115**	**114**	**4**

Table 5.5.3: PKU BH4-Test / BH4 Sensitivity

Lab	Confirmed cases	BH4-Test	BH4 sensitive
1	9	6	1
3	6	3	1
5	11	1	
6	4	1	
7	8	4	
8	45	20	8
9	28	9	2
10	7	4	3
11	3	2	
12	11		
13	14	1	
14	3		
15	0		
Total	**149**	**51**	**15**

5.6 Maple Syrup Urine Disease (MSUD)

The overall recall rate is very low at 0.005%.

Table 5.6.1: MSUD - Confirmed cases / Recall rate

Lab	Initial screening	Recall	Confirmed cases
1	60,828	4	1
3	13,790	0	0
5	59,118	1	0
6	12,005	1	0
7	47,165	15	0
8	182,396	1	0
9	140,955	8	0
10	34,075	0	0
11	15,951	0	0
12	95,589	0	0
13	67,678	2	0
14	32,648	7	1
15	7,122	3	0
Total	**769,320**	**42**	**2**

Table 5.6.2: MSUD Confirmation

Lab	Confirmed cases	Confirmation (Serum)	Organic acids (urine)	Enzyme activity	Molecular genetics	Confirmed cases without confirmation details
1	1	1	1			
14	1				1	
Total	**2**	**1**	**1**	**0**	**1**	**0**

5.7 Medium-Chain Acyl-CoA Dehydrogenase (MCAD) Deficiency

Table 5.7.1: MCAD deficiency- Confirmed Cases/Recall rate

Lab	Initial screening	Recall	Recall rate (%) [a]	Confirmed cases
1	60,828	10	0.02	7
3	13,790	1		1
5	59,118	3		3
6	12,005	4		2
7	47,165	37	0.08	5
8	182,396	34	0.02	27
9	140,955	59	0.04	15
10	34,075	10	0.03	5
11	15,951	1		1
12	95,589	2		2
13	67,678	9	0.01	8
14	32,648	11	0.03	8
15	7,122	0		0
Total	**769,320**	**181**	**0.02**	**84**

[a] Recall rates only provided if recall rate ≥ 0,01% and n ≥ 5

Table 5.7.2: MCAD Deficiency Confirmation

Lab	Confirmed cases	Confirmation (Serum/DB)	Organic Acids (urine)	Enzyme activity	Molecular genetics	Confirmed cases without confirmation details
1	7	2	6	5	7	
3	1	1	1		1	
5	3			1	1	1
6	2	2	1		1	
7	5		3	3	5	
8	27	17	11	8	22	
9	15	1	6	7	7	1
10	5	5	4	1	5	
11	1	1	1	1		
12	2			1	1	
13	8	6	1	1	3	1
14	8			2	5	2
15	0					
Total	**84**	**35**	**34**	**30**	**58**	**5**

5.8 Long-Chain-3-Hydroxyacyl-CoA Dehydrogenase (LCHAD) Deficiency

The overall recall rate is very low at 0.004%. Of the 11 confirmed cases, 2 were classified as mitochondrial trifunctional protein deficiency.

Table 5.8.1: LCHAD Deficiency - Confirmed cases / Recall rate

Lab	Initial screening	Recall	Confirmed cases
1	60,828	2	1
3	13,790	0	0
5	59,118	6	0
6	12,005	5	1
7	47,165	1	1
8	182,396	1	1
9	140,955	9	1
10	34,075	1	1
11	15,951	0	0
12	95,589	3	3
13	67,678	2	2
14	32,648	0	0
15	7,122	0	0
Total	**769,320**	**30**	**11**

Table 5.8.2: LCHAD Deficiency Confirmation

Lab	Confirmed cases	Confirmation (Serum)	Organic Acids (urine)	Enzyme activity	Molecular genetics	Confirmed cases without confirmation details
1	1		1		1	
6	1	1	1		1	
7	1		1		1	
8	1				1	
9	1				1	
10	1	1	1		1	
12	3	1		1	3	
13	2	1			1	
Total	**11**	**4**	**4**	**1**	**10**	

5.9 Very-Long-Chain Acyl-CoA Dehydrogenase Deficiency

Table 5.9.1: VLCAD Deficiency- Confirmed cases / Recall rate

Lab	Initial screening	Recall	Recall rate (%) [a]	Confirmed cases
1	60,828	2		1
3	13,790	0		0
5	59,118	0		0
6	12,005	4		0
7	47,165	11	0.02	3
8	182,396	6		2
9	140,955	104	0.07	0
10	34,075	0		0
11	15,951	5	0.03	0
12	95,589	0		0
13	67,678	4		4
14	32,648	3		2
15	7,122	3		0
Total	**769,320**	**142**	**0.02**	**12**

[a] Recall rates only provided if recall rate ≥ 0,01% and n ≥ 5

Table 5.9.2: VLCAD Confirmation

Lab	Confirmed cases	Confirmation (Serum)	Organic Acids (urine)	Enzyme activity	Molecular genetics	Confirmed cases without confirmation details
1	1		1	1	1	
7	3	1	3	2	3	
8	2	1	1	1	2	
13	4	2		3	4	
14	2			1	1	
Total	**12**	**4**	**5**	**8**	**11**	

5.10 CPT I / CPT II / CACT Deficiency

The overall recall rate is very low at 0.001%. Recall CACT deficiency may be recorded in Recall CPT II deficiency.

Table 5.10.1: CPT I / CPT II / Deficiency Recall

	Initial screening	Recall	Confirmed Cases
CPT I Deficiency	769,320	6	3
CPT II Deficiency / CACT Deficiency	769,320	9	0

Table 5.10.2: CPT I / II Deficiency Confirmation

Lab	Confirmed Cases	Confirmation (Serum/TB)	Enzyme activity	Molecular genetics	Confirmed cases without details of confirmation
1	1	1		1	
8	1	1			
13	1	1		1	
Total	**3**	**3**		**2**	

5.11 Glutaric Aciduria Type I (GA I)

Table 5.11.1: GA I - Confirmed Cases / Recall rate

Lab	Initial screening	Recall	Recall rate (%) [a]	Confirmed cases
1	60,828	6	0.01	0
3	13,790	0		0
5	59,118	3		1
6	12,005	3		1
7	47,165	7	0.01	0
8	182,396	1		1
9	140,955	112	0.08	3
10	34,075	6	0.02	0
11	15,951	1		0
12	95,589	2		0
13	67,678	0		0
14	32,648	3		1
15	7,122	0		0
Total	**769,320**	**144**	**0.02**	**7**

[a] Recall rates only provided if recall rate ≥ 0,01% and n ≥ 5

Table 5.11.2: GA I Confirmation

Lab	Confirmed cases	Confirmation (Serum/TB)	Organic Acids (urine)	Enzyme activity	Molecular genetics	Confirmed cases without confirmation details
5	1	1	1		1	
6	1	1	1	1	1	
8	1	1	1		1	
9	3		3		1	
14	1	1	1			
Total	**7**	**4**	**7**	**1**	**4**	

5.12 Isovaleric Acidemia (IVA)

Table 5.12.1: IVA - Confirmed Cases / Recall rate

Lab	Initial screening	Recall	Recall rate (%)[a]	Confirmed cases
1	60,828	6	0.01	2
3	13,790	1		1
5	59,118	5	0.01	0
6	12,005	10	0.08	0
7	47,165	6	0.01	0
8	182,396	3		1
9	140,955	23	0.02	0
10	34,075	10	0.03	0
11	15,951	5	0.03	0
12	95,589	9	0.01	0
13	67,678	11	0.02	1
14	32,648	13	0.04	0
15	7,122	7	0.10	1
Total	**769,320**	**109**	**0.01**	**6**

[a] Recall rates only provided if recall rate ≥ 0,01% and n ≥ 5

The IVA recall rate increased significantly in 2018 compared to 2017 (from n=68 to n=109) and has remained about the same since then. A frequent explanation is the administration of Pivmecillinam for urinary tract infections in the mother shortly before birth, which leads to false positive screening results.

Table 5.12.2: IVA Confirmation

Lab	Confirmed cases	Confirmation (Serum)	Organic Acids (urine)	Enzyme activity	Molecular genetics	Confirmed cases without confirmation details
1	2	1	2		1	
3	1	1	1		1	
8	1				1	
13	1				1	
15	1	1			1	
Total	**6**	**3**	**3**		**5**	

5.13 Tyrosinemia

Table 5.13.1: Tyrosinemia – Confirmed Cases

Lab	Initial Screening	Recall	Recall Rate (%) [a]	Confirmed Cases
1	60,828	3		0
3	13,790	0		0
5	59,118	0		0
6	12,005	2		0
7	47,165	1		1
8	182,396	87	0.05	3
9	140,955	10	0.01	1
10	34,075	13	0.04	0
11	15,951	2		0
12	95,589	2		1
13	67,678	1		1
14	32,648	1		0
15	7,122	3		0
Total	**769,320**	**125**	**0.02**	**7**

[a] Recall rates only provided if recall rate ≥ 0,01% and n ≥ 5

Table 5.13.2: Tyrosinemia Confirmation

Lab	Confirmed Cases	Confirmation (Serum/TB)	Confirmation Organic Acids	Enzyme activity	Molecular genetics	Confirmed cases without confirmation information
7	1	1		1	1	
8	3	2		2	1	1
9	1	1		1		
12	1	1			1	
13	1	1		1		
Total	**7**	**6**		**5**	**3**	**1**

5.14 Severe Combined Immunodeficiency (SCID)

Severe combined immunodeficiency (SCID) was added to ENS as a new target disease in 8/2019.

Table 5.14.1: SCID - Confirmed Cases / Recall rate

Labor	Initial Screening	Recall	Recall rate (%) [a]	Confirmed cases
1	60,828	22	0.04	1
3	13,790	4		1
5	59,118	20	0.03	11
6	12,005	4		0
7	47,165	80	0.17	5
8	182,396	166	0.09	7
9	140,955	29	0.02	5
10	34,075	20	0.06	1
11	15,951	2		0
12	95,589	2		0
13	67,678	14	0.02	0
14	32,648	10	0.03	0
15	7,122	7	0.10	1
Total	**769,320**	**380**	**0.05**	**32**

[a] Recall rates only provided if recall rate ≥ 0,01% and n ≥ 5

Table 5.14.2: SCID Confirmed Cases

Lab	Confirmed Cases	Genetics	Cytology	Without information about the confirmation diagnostics
1	1	0	1	
3	1	1	1	
5	11	10	10	
7	5	5	4	
8	7	3	1	3
9	5	5	4	
10	1	1	1	
15	1	1	1	
Total	**32**	**26**	**23**	**3**

Of the 32 cases, 5 were classified as SCID, 4 as leaky SCID/Omenn syndrome, and 23 as part of syndromes. One child was diagnosed with SCID at 10 months of age after screening findings were erroneously reported as unremarkable.

5.15 Cystic Fibrosis (CF)

Since September 2016, screening for cystic fibrosis has been performed in three stages as a serial combination of two biochemical tests. First, the concentration of immunoreactive trypsin (IRT) is determined, and in the case of elevated values, the concentration of pancreatitis-associated protein (PAP) is measured as a second step. In the case of pathological PAP, a molecular genetic examination is performed in a third step.

Here, the 31 most common pathogenic mutations of the cystic fibrosis transmembrane regulator gene (CFTR gene) in Germany are searched for (Figure 5). The screening is considered conspicuous (positive) if an IRT value is above the 99.9th percentile ("failsafe" method or "safety net") or if one of the 31 examined mutations of the CFTR gene is detected on at least one allele in the third stage. In all other constellations, the screening is considered unremarkable (negative).

This screening algorithm results in "failsafe" (IRT >99.9th percentile) conditions in 79.9% of the 690 positive screening findings (see Fig. 5). The diagnosis of CF was confirmed in only 140 children (20.3%); in addition, 6 children were diagnosed with cystic fibrosis after an unremarkable CF screening (Table 5.14.4).

According to the Paediatrics Directive, CF screening requires both a separate declaration of consent and a consultation with a physician; screening cannot be performed by a midwife alone with the option to consult with a physician, as is the case with ENS in exceptional cases. The proportion of newborns without CF screening was 1% in 2020 (Table 5.14.1).

Table 5.14.1: Number of Cases without CF Screening

Lab	Initial screening ENS	Without CF Screening	Proportion without CF Screening (%)
1	60,828	99	0.16
3	13,790	11	0.08
5	59,118	1,624	2.75
6	12,005	25	0.21
7	47,165	2,433	5.16
8	182,396	1,357	0.74
9	140,955	146	0.10
10	34,075	492	1.44
11	15,951	47	0.29
12	95,589	863	0.90
13	67,678	363	0.54
14	32,648	199	0.61
15	7,122	10	0.14
Total	**769,320**	**7,669**	**1.00**

Table 5.14.2: CF – Confirmed cases and abnormal screening findings

Lab	Initial screening with CF Screening	Recall	Recall Rate (%)	Confirmed cases
1	60,729	64	0.11	8
3	13,779	16	0.12	3
5	57,494	70	0.12	15
6	11,980	19	0.16	2
7	44,732	23	0.05	6
8	181,039	208	0.11	32
9	140,809	111	0.08	32
10	33,583	33	0.10	12
11	15,904	8	0.05	2
12	94,726	94	0.10	17
13	67,315	65	0.10	10
14	32,449	23	0.07	5
15	7,112	16	0.22	2
Total	**761,651**	**750**[b]	**0.10**	**146**[a]

[a] of which 6 cases with unremarkable CF screening [b] Some laboratories regard abnormal IRT values at initial screening <36h or <32 SSW or highly scattering IRT values as recall. As a result, CF recall is higher than the number of positive screening cases in Figure 5.

Table 5.14.3: CF – Validation of confirmed cases

Lab	Confirmed Cases	One Sweat Test	Two Sweat Tests	Conductivity	2 Mutations in confirmation or screening	Meconium ileus
1	8	6	2		3	1
3	3	1	2	3	3	
5	15	8	5	1	5	
6	2	1	1		1	
7	6	3	2		4	
8	32	8	20		29	5
9	32	10	16	10	15	2
10	12	6	1		10	
11	2	2			2	1
12	17	10	6	10	9	6
13	10	4	5		6	
14	5	3	1	1	5	2
15	2					
Total	**146**	**62**	**61**	**25**	**92**	**17**

In 11 reported cases, the information was not sufficient to confirm the diagnosis. Of n=146 confirmed cases, 141 cases were diagnosed with cystic fibrosis and 2 cases were diagnosed with Cystic Fibrosis Screen Positive, Inconclusive Diagnosis (CFSPID); 3 cases did not have sufficient information (genetics) to differentiate between CF and CFSPID.

Screening was positive in 111 (76%) of CF cases via fail safe, 29 (19.9%) cases had one or 2 mutations detected from the screening panel (31 mutations), and 6 children (4.0%) had an unremarkable CF screening.

In n=97 of the confirmed cases, information on genetics was available from screening or from confirmation. Consequently, 71 cases had two mutations from the panel of 31, in 25 cases one mutation was present, and only 1 child had 2 other mutations and was also not found via fail safe with an IRT of 38 ng/ml (unremarkable screening). Overall, meconium ileus was reported in 17 children.

For confirmation diagnostics, information on one (n=62) or two (n=61) sweat tests was available for 123 cases, for 15 cases information on only 2 mutations present was available, 2 cases were validated as probable solely on the basis of a conspicuous conductivity, and for 6 cases only the remark "diagnosis confirmed" was provided.

Of the confirmed CF cases, six were not found via the pre-specified screening algorithm and were unremarkable on screening. One of these children was diagnosed due to meconium ileus, 5 children were identified due to failure to thrive (see Table 5.14.4). It is not known if there were any other children with cystic fibrosis who were not identified in the screening.

Table 5.14.4: Confirmed Cases with unremarkable CF Screening

Screening Parameter	Found via	Count (n)
IRT unremarkable	Meconium ileus (n=1) Failure to thrive (n=3)	**4**
PAP unremarkable	Failure to thrive (n=2)	**2**

6 Lost to follow-up

Of a total of 23,825 second cards requested, 21,684 (91.09%) were sent in, meaning that no further information was available for 8.91% of the cards requested (Table 2.4.). The breakdown of the response rate according to the reasons for requesting the second card (recall/early collection) has no longer been requested since 2018.

6.1 Cases without confirmation data

Of 81 children with positive screening results in the ENS, it is not known whether confirmation diagnostics took place or were completed. 33 of these cases, for which no information on confirmation was available but for which there were clearly pathological screening values or the remark "diagnosis confirmed", were validated as "probable case" (Tab. 6.1.1.1) and included in the calculation of prevalence. This was not possible for 48 children (Tab. 6.1.2.1).

6.1.1 Confirmed cases without information about validation diagnostics

33 cases were validated as probable cases without confirmation information.

Table 6.1.1.1: Confirmed Cases without information about validation

		Reason no confirmation provided			
Disease	Confirmed cases without validation	No feedback from clinic / pediatrician	Clinic did not request confirmation	Only the remark "diagnosis confirmed"	Unclear
Hypothyroidism	9			2	7
CAH	2	1		1	
Biotinidase Deficiency	2				2
Galactosemia	1			1	
PKU/HPA	4	2	1		1
MCAD	5	2			3
Tyrosinemia	1			1	
CF	6			6	
SCID	3			2	1
Total	**33**	**5**	**1**	**13**	**14**

6.1.2 Unconfirmed cases from the ENS (lost to follow up)

Table 6.1.2.1: Cases with implausible or missing confirmation information

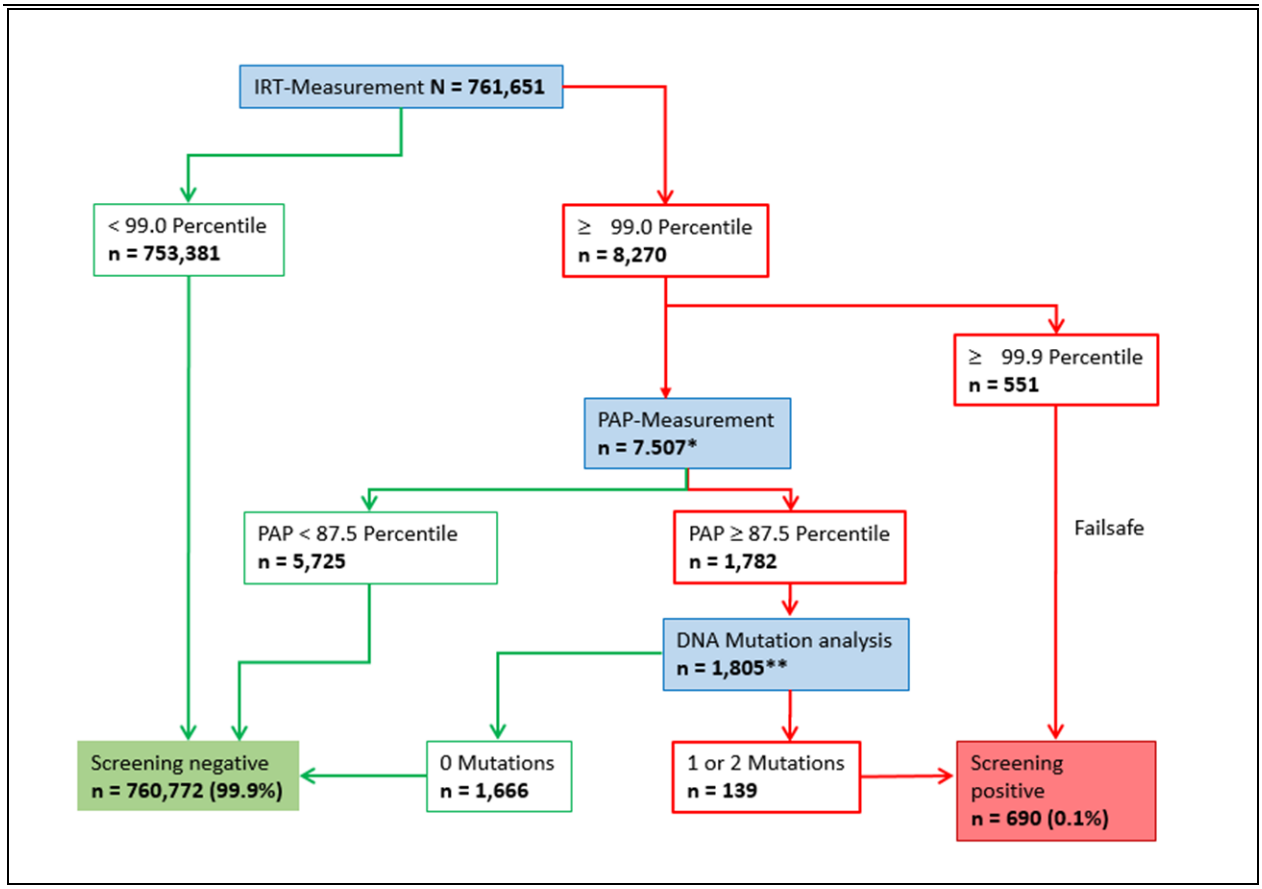
	Number of Cases
Disease	n
Congenital Hypothyroidism	18
CAH	2
Biotinidase Deficiency	2
Galactosemia	2
MCAD	3
Tyrosinemia	1
CF	11
SCID	9
Total	**48**

Table 6.1.2.2: Proportion of cases by lab with implausible or missing confirmation data

Lab	Number of reported cases	Number of verified cases	Of which verified cases without information about confirmation	Number of cases identified as unclear/open due to lack of confirmation	Proportion of reported cases without confirmation (%)
1	71	70	0	1	1.41
3	16	16	1	0	6.25
5	68	65	3	3	8.82
6	12	12	0	0	
7	65	50	8	15	35.38
8	218	212	6	6	5.50
9	162	157	5	5	6.17
10	45	37	0	8	17.78
11	14	13	2	1	21.43
12	85	84	2	1	3.53
13	73	72	1	1	2.74
14	31	31	2	0	6.45
15	14	7	3	7	71.43
Total	**874**	**826**	**33**	**48**	**9.27**

7 Screening Algorithm Cystic Fibrosis (CF)

Figure 5: Screening Algorithm Cystic Fibrosis Germany 2020



* PAP measurement was not performed for all abnormal IRT values >99.0% but <99.9% (no failsafe), because some were early collections or there was not enough material for examination.

** Mutation analysis also in children with the product of IRT and PAP value above laboratory cut-off.

An additional 6 children with a confirmed diagnosis had an unremarkable screening result, i.e., these children were not detected by the screening algorithm (see Table 5.14.4).

8 Methods and Cutoff-Values used in Screening

Table 8.1: Filter paper

Lab	Filter paper
1	ID Biological (Ahlstrom 226)
3	ID Biological (Ahlstrom 226)
5	Munktell
6	ID Biological (Ahlstrom 226)
7	ID Biological (Ahlstrom 226)
8	Ahlstrom Munksjö
9	ID Biological (Ahlstrom 226)
10	ID Biological (Ahlstrom 226)
11	Perkin Elmer 226
12/13	ID Biological (Ahlstrom 226)
14/15	ID Biological (Ahlstrom 226)

Table 8.2 Hypothyroidism

Lab	Parameter	Cutoff	Method
1	TSH	<15 mU/l	AutoDELFIA
3	TSH	15 mU/l	AutoDELFIA
5	TSH	15 mU/l	AutoDELFIA
6	TSH	15 mU/l	DELFIA
7	TSH	15 µU/ ml	GSP
8	TSH	15 mU/l (≤ 8 days of life) 10 mU/l (>8 days of life)	DELFIA
9	TSH	15 µU/ml	GSP
10	TSH	15 mU/l	AutoDELFIA
11	TSH	15 mU/l	DELFIA
12 /13	TSH	<20 mU/l	AutoDELFIA
14 /15	TSH	<20 mU/l (1st day of life) <15 mU/l (2nd-4th day of life) <10 mU/l (> 4th day of life)	AutoDELFIA

Table 8.3: Congenital Adrenal Hyperplasia (CAH)

Lab	Parameter	Method
1*	17 OHP	AutoDELFIA
3	17 OHP	AutoDELFIA Kit B024
5	17 OHP	AutoDELFIA
6	17 OHP	DELFIA
7	17 OHP	GSP
8*	17 OHP	DELFIA
9	17 OHP	GSP
10*	17 OHP	AutoDELFIA
11	17 OHP	DELFIA
12/13*	17 OHP	AutoDELFIA
14/15*	17 OHP	AutoDELFIA

*Lab uses 2nd tier method (steroid profile using LC-MS/MS)

Table 8.4: Biotinidase Deficiency

Lab	Parameter	Cutoff	Methods
1	Biotinidase	>30%	Qualitative colorimetry
3	Biotinidase	>30%	Qualitative colorimetry
5	Biotinidase	30% of panel mean	Qualitative colorimetry
6	Biotinidase	55 U	Fluorometry (PE)
7	Biotinidase	85,7 U/g Hb	GSP
8	Biotinidase	<30% daily mean	Quantitative colorimetry
9	Biotinidase	< 0.2	Qualitative colorimetry
10	Biotinidase	<30%	Qualitative colorimetry
11	Biotinidase	<30%	Quantitative colorimetry
12/13	Biotinidase	<30%	Quantitative fluorometry
14/15	Biotinidase	>30%	Quantitative colorimetry

Table 8.5: Galactosemia

Lab	Parameter	Normal range	Method
1	GALT Galactose	>3.5 U/g Hb <13 mg/dl	Quantitative fluorometry Fluorometry (PE)
3	GALT Galactose	>3.5 U/g Hb <15 mg/dl	Fluorometry (PE)
5	GALT Galactose	>3.5 U/g Hb 20 mg/dl	Quantitative fluorometry Quantitative colorimetry
6	GALT	3.5 U/g Hb	Fluorometry (PE)
7	GALT	3.5 U/g Hb	Quantitative fluorometry
8	GALT Galactose	<20% daily mean 30 mg/dl (until 28th day of life, after that 18mg/dl)	Quantitative fluorometry Quantitative colorimetry
9	GALT Galactose	5.3 U/g Hb 20 mg/dl	Fluorometry (PE) BIORAD Quantase
10	GALT Galactose	>3.5 U/gHb Until 13 Oct 2020 <1111 nmol/l From 13 Oct 2020: 461 µmol/l	Fluorometry (PE) BIORAD Quantase Fluorometry (PE)
11	GALT	3.5 U/g Hb	Fluorometry (PE)
12/13	GALT Galactose	>20% < 30 mg/dl	Colorimetry non-kit Quant. fluoro, (non-kit)
14/15	GALT Galactose	<3.5 U/g Hb <7.4 mg/dl	Quantitative fluorometry BIORAD Quantase kit from Zentech

Table 8.6: Tandem mass spectrometry (MS/MS)

Lab	Method
1	non-derivatized PE kit
3	non-derivat. Chromsystems
5	non-derivatized PE kit
6	non-derivatized PE kit
7	non-derivatized PE kit
8	non-derivitized non Kit
9	non-derivatized Chromsystems kit
10	deriv. Chromsystems Kit
11	non-derivat. Chromsystems Kit
12/13	derivatized non-kit
14/15	non-derivat. Chromsystems Kit

9 Literature

[1] Paediatric Guideline Effective: 14 May, 2020 of the Federal Joint Committee on the Early Detection of Diseases in Children (Paediatrics Directive – "Kinder-Richtlinie"); https://www.g-ba.de/downloads/62-492-2156/Kinder-RL_2020-05-14_iK-2020-03-25.pdf

[2] Destatis, Federal Statistical Office, Births 2020 https://www.destatis.de/DE/Themen/Gesellschaft-Umwelt/Bevoelkerung/Geburten/_inhalt.html (accessed 9 September, 2022)