National Screening Report Germany 2019

German Society for Neonatal Screening (DGNS)

Inken Brockow, Oliver Blankenstein, Sarah Breuer, Uta Ceglarek, Regina Ensenauer, Gwendolyn Gramer, Nils Janzen, Jeannette Klein, Erwin Lankes, Martin Lindner, Peter Mirtschink, Cornelia Müller, Simona Murko**,** Sabine Rönicke, Wulf Röschinger, Olaf Sommerburg, Carsten Speckmann, Uta Nennstiel

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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

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Abbreviations and Glossary

CAH	Congenital Adrenal Hyperplasia
CACT Deficiency	Carnitine-Acylcarnitine Translocase Deficiency
CF	Cystic Fibrosis (Mucoviscidosis)
CF-SPID	Cystic Fibrosis Screen Positive, Inconclusive Diagnosis
CPT-I Deficiency	Carnitine Palmitoyl Transferase I Deficiency
CPT-II 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 / 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/

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. René Santer
Martinistr. 52, 20246 **Hamburg**
040/7410 57037
Contact: Dr. Simona Murko
r.santer@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
juergen.guenther.okun@med.uni-heidelberg.de
friederike.hoerster@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 aim of early and complete detection coupled with quality assured therapy for all newborns with treatable hormonal, metabolic and immune system diseases as well as cystic fibrosis.

In the policies for early detection of diseases in children up to 6 years of age, known as the Paediatric Directive ("Kinder-Richtlinie"), the regulations for implementing the extended newborn screening program (ENS) are defined in §13 - §28. The 2019 National Screening Report was compiled by the German Society for Neonatal Screening (DGNS e.V.) together with the German screening laboratories. The statistical analysis of the screening data was performed in accordance with the guideline and quality criteria of the NBS implementation. This report pertains only to the diseases which are defined in this guideline. After publication in the Federal Gazette on February 8, 2019, screening for severe combined immunodeficiency (SCID) was introduced as a new target disease of the NBS.

The report provides a comprehensive statistical summary of disease-related screening figures, recall rates (proportion of abnormal [positive] findings), and confirmed diagnoses for the year 2019. Additionally, the report provides process quality data for all 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:

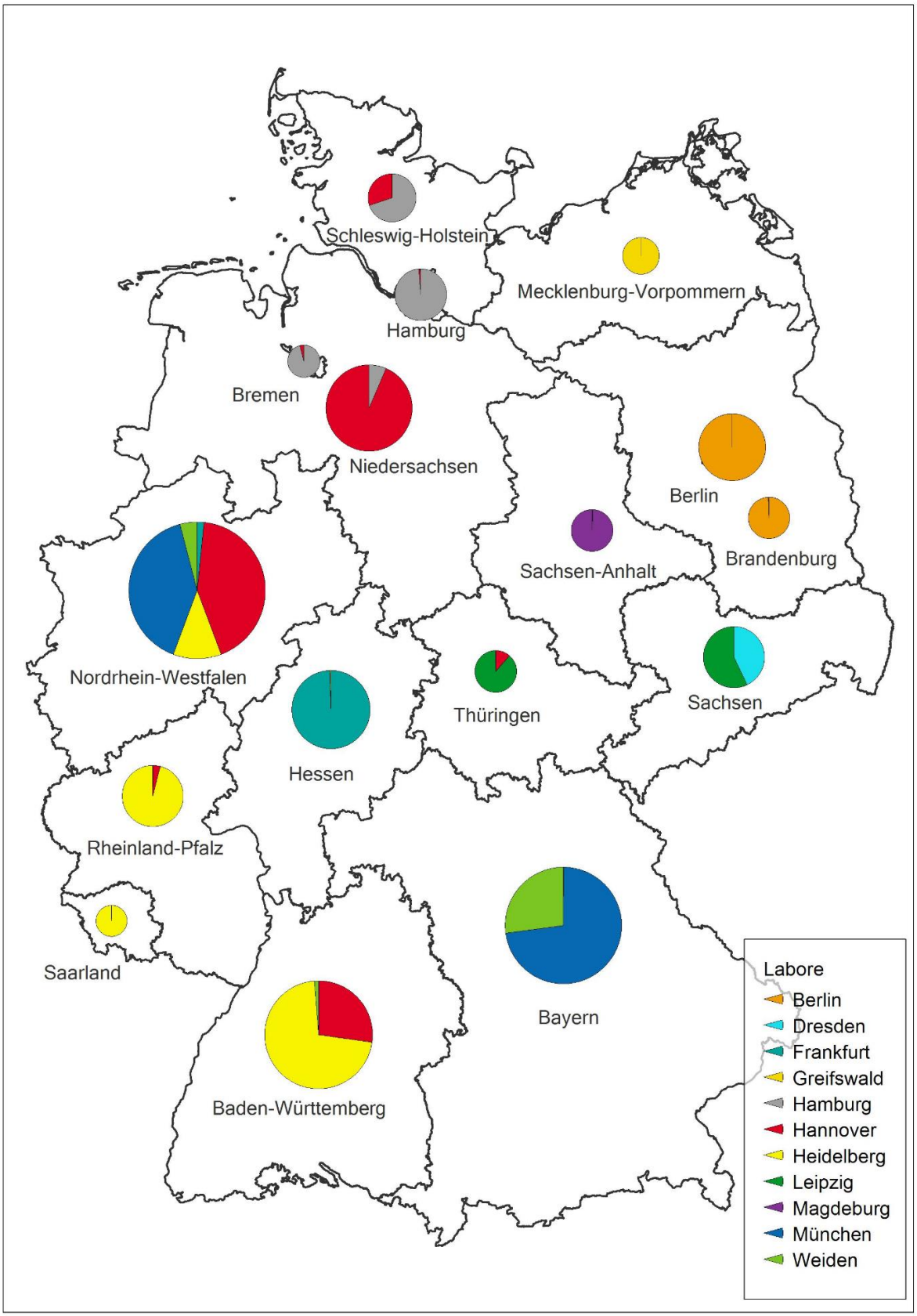
- Total survey of the targeted population
- Completeness of the control (recall) and repeat examinations
- Recording test parameters and cut-offs
- Specificity and sensitivity of diagnostic tests
- Age at blood sample collection, time between blood sample collection and receipt at the laboratory and 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 2019 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 "Paediatric Directive" from November 16, 2019. [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 2019



2 Results

In 2019 a total of 778,090 children were born in Germany according to official statistics. [2] The number of recorded first screenings (777,922) 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:	778090
First screenings:	777922
Confirmed diagnoses:	768

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 “Paediatric Directive”. Other diseases screened in individual laboratories as part of studies or state law requirements are not included in this report.

In one in 1,013 newborns, 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 2019 in relation to births in Germany.

Table 2.1: Prevalence of diseases detected in 2019 among 778,090 births

Disease	Confirmed cases	Prevalence	
Hypothyroidism	258	1:	3016
Congenital Adrenal Hyperplasia (CAH)	46	1:	16915
Biotinidase Deficiency	21	1:	37052
Galactosemia (classic form)	10	1:	77809
Hyperphenylalaninemia	151	1:	5153
Of which classic phenylketonuria (PKU)	59	1:	13188
Maple Syrup Urine Disease (MSUD)	4	1:	194523
Medium-Chain Acyl-CoA Dehydrogenase (MCAD) deficiency	79	1:	9849
Long-chain 3-Hydroxyacyl-CoA Dehydrogenase (LCHAD) / TFP deficiency	3	1:	259363
Very Long-Chain Acyl-CoA-Dehydrogenase (VLCAD) deficiency	8	1:	97261
Carnitine Palmitoyl Transferase I (CPT I) deficiency	0		
Carnitine Palmitoyl Transferase II (CPT II) deficiency	2	1:	389045
Carnitine-Acylcarnitine Translocase (CACT) deficiency	0		
Glutaric Acidemia (GA) Type I	4	1:	194523
Isovaleric Acidemia (IVA)	7	1:	111156
Tyrosinemia	6	1:	129682
Cystic Fibrosis (CF)	151	1:	5153
Severe Combined Immunodeficiency (SCID)*	18		
Total	**768**	**1:**	**1013**

* SCID: screening starting 08/2019 ➔ no calculation of prevalence

2.1 Total Initial Screening Figures

The proportion of laboratories in the initial screening and all confirmed cases per lab are shown in Table 2.2. Confirmed cases also include those with negative initial screening or conspicuous second screening cards.

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	58059	7.46	74	9.64
3	15067	1.94	19	2.47
5	60081	7.72	60	7.81
6	12655	1.63	13	1.69
7	53816	6.92	46	5.99
8	180296	23.18	173	22.53
9	140812	18.10	145	18.88
10	34529	4.44	32	4.17
11	16312	2.10	11	1.43
12/13	163027	20.96	141	18.36
14/15	43268	5.56	54	7.03
Total	**777922**	**100**	**768**	**100**

According to the Paediatric Directive, every newborn should be screened before discharge from the maternity facility. If the first screening is carried out before 36 hours of life or before 32 weeks of gestation (WoG), a second screening should be carried out.

The following table shows the number of first screening examinations stratified by age and gestational age, defined as follows:

- < 32 WoG: all samples from children born before 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.

Table 2.3: Age at time of initial screening

		≥36h and ≥32WoG		<36h and ≥32WoG		<32WoG	
Lab	Total	n	%	n	%	n	%
1	58059	57093	98.34	424	0.73	542	0.93
3	15067	14729	97.76	81	0.54	257	1.71
5	60081	59090	98.35	402	0.67	589	0.98
6	12655	12208	96.47	267	2.11	180	1.42
7	53816	52519	97.59	545	1.01	752	1.40
8	180296	176647	97.98	1690	0.94	1959	1.09
9	140812	137685	97.78	1082	0.77	2045	1.45
10	34529	33891	98.15	231	0.67	407	1.18
11	16312	15900	97.47	254	1.56	158	0.97
12	92573	90502	97.76	968	1.05	1103	1.19
13	70454	68793	97.64	855	1.21	806	1.14
14	34450	33704	97.83	439	1.27	307	0.89
15	8818	8520	96.62	71	0.81	227	2.57
Total	**777922**	**761281**	**97.86**	**7309**	**0.94**	**9332**	**1.20**

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 differ from the sum of the data on necessary second screening examinations in Tables 2.3, 2.5, and 2.7. This question was apparently interpreted by the laboratories in differing ways.

Table 2.5 shows the necessary follow-up examinations due to an abnormal initial screening (recall) stratified by laboratory and by age or gestational age.

Table 2.4: Received second screenings

Lab	Second screenings requested	Second screenings received	%
1	1923	1827	95.01
3	210	210	100
5	994	847	85.21
6	447	428	95.75
7	709	n/a	n/a
8	5525	4851	87.80
9	3739	2939[b]	78.60
10	878	797	90.77
11	412	393	95.39
12	2954	2922	98.92
13	2026	1838	90.72
14	897	880	98.10
15	298	281	94.30
Total	**21012**	**18213**	**89.71[a]**

[a] Calculation without laboratory 7, as no information was provided.
[b] External findings from other screening laboratories are not recorded

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

Lab	Initial Screening	Recall total		Recall >=36h[b]		Recall <36h		Recall <32 WoG	
		n	%	n	%	n	%	n	%
1	58059	276	0.46	237	0.42	18	4.25	21	3.87
3	15067	79	0.52	70	0.48	4	4.94	5	1.95
5	60081	330	0.53	247	0.42	3	0.75	12	2.04
6	12655	89	0.61	83	0.68	1	0.37	5	2.78
7	53816	779	1.45	477	0.91	65	11.93	237	31.52
8	180296	1427	0.79	933	0.53	300	17.75	194	9.90
9	140812	720	0.51	681	0.49	0	0.00	39	1.91
10	34529	326	0.93	216	0.64	61	26.41	49	12.04
11	16312	104	0.64	57	0.36	31	12.20	16	10.13
12	92573	308	0.33	276	0.30	18	1.86	14	1.27
13	70454	187	0.26	170	0.25	5	0.58	12	1.49
14	34450	138	0.40	116	0.34	7	1.59	15	4.89
15	8818	69	0.66	45	0.53	6	8.45	18	7.93
Total	**777922**	**4832**	**0.62**	**3608**	**0.47**	**519**	**7.10**	**637**	**6.83**

[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 without temporal classification

As a public health measure, the newborn screening is intended to benefit all children born in Germany. To guarantee that the screening is offered to all newborns, it is necessary to track completeness. For children delivered in obstetric units, this can be done in the screening center using the birth registry records, or when permitted by law, by cross-checking the data with the records from residents' registration office.

At present, neither of these options is being implemented nationwide in Germany. With the aim of nevertheless monitoring the integrity of the screening, the following regulation was included in the "Paediatric Directive" [§ 21 Paragraph 6]:

Refusal of screening or the death of the newborn prior to a possible first blood sampling must be documented on blank filter paper cards in accordance with §20 and sent to the screening laboratory. The laboratories receive blank test cards in widely varying quantities. The number of the blank cards sent in due to refusal to participate has remained approximately the same relative to the total number of Initial screening cards submitted.

This system seems to work primarily in cases of refusal to take part in the screening. In addition, blank cards are frequently sent in due to rejected early screening. For both death prior to screening and for transfer of the newborn, considerably higher numbers would be expected based on the data from the perinatal survey.

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	58059	412	137	365	2863	269	4046	6.97
3	15067	36	17				53	0.35
5	60081	24	56	1294	1367	268	3009	5.01
6	12655	50	22		381		453	3.58
7	53816		7				7	0.01
8	180296					3145[a]	3145	1.74
9	140812	13	181	191		881	1266	0.90
10	34529	175	51			1673	1899	5.50
11	16312	69	15	16	199	40	339	2.08
12	92573			197	1009	235	1441	1.56
13[b]	70454							
14	34450			21	98	28	147	0.43
15[b]	8818							
Total	**777922**	**779**	**486**	**2084**	**5917**	**6539**	**15805**	**2.03**

[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	Control requested	Control received	received/ requested (%)	Proportion of samples / Initial screening (%)	IM[a]
1	58059	355	328	92.39	0.61	524
3	15067	10	10	100	0.07	10
5	60081	488	452	92.62	0.81	n/a
6	12655	2	2	100	0.02	21
7	53816	169	169	100	0.31	608
8	180296	543	533	98.16	0.30	181
9	140812	8	8	100	0.01	791
10	34529	18	16	88.89	0.05	186
11	16312	13	13	100	0.08	2
12	92573	583	571	97.94	0.63	14
13	70454	422	399	94.55	0.60	n/a
14	34450	39	38	97.44	0.11	3
15	8818	20	19	95.00	0.23	20
Total	**777922**	**2851**	**2730**	**95.76**	**0.37**	**2360**

[a] IM (Insufficient Material) includes samples for which the number of circles saturated with blood on the screening card was not sufficient to perform the full screening (including samples for which the CF algorithm could not be completely executed).

3 Processing Time

3.1 Age at the time of blood sample collection

According to the "Paediatric Directive" (§ 20 paragraph 1) blood samples should be collected between 36 and 72 hours after birth. In 95.4% of cases in which the time of blood sampling was provided, collection took place in the designated time frame, in 3.6% not until after 72 hours and in 0.99% 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 2005 to 3.6% in 2019 (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	58054	509	0.88	21398	36.86	33757	58.15	2390	4.12
3	15067	94	0.62	4354	28.90	10227	67.88	392	2.60
5	60070	437	0.73	45992	76.56	12178	20.27	1463	2.44
6	12655	293	2.32	5787	45.73	6281	49.63	294	2.32
7	53819	643	1.19	26413	49.08	23622	43.89	3138	5.83
8	179861	1571	0.87	86741	48.23	84604	47.04	6945	3.86
9	140812	1221	0.87	73959	52.52	60849	43.21	4783	3.40
10	34529	297	0.86	12539	36.31	20404	59.09	1289	3.73
11	16312	250	1.53	6065	37.18	9243	56.66	754	4.62
12	91836	1096	1.19	57757	62.89	30483	33.19	2500	2.72
13	70454	673	0.96	46489	65.98	20426	28.99	2866	4.07
14	34445	479	1.39	18159	52.72	14793	42.95	1014	2.94
15	8818	90	1.02	5309	60.21	3279	37.19	140	1.59
Total	**776732** [a]	**7653**	**0.99**	**410962**	**52.91**	**330146**	**42.50**	**27968**	**3.60**

[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 (§ 18 paragraph 3). However, in 29.1% 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 23.6% 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. Overall, efforts must be made work with submitters 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	58006	12695	21.89	19670	33.91	11310	19.50	14331	24.71
3	15067	4991	33.13	6780	45.00	2507	16.64	789	5.24
5	60069	5041	8.39	21278	35.42	16394	27.29	17356	28.89
6	12655	413	3.26	4051	32.01	3776	29.84	4415	34.89
7	53623	10597	19.76	15072	28.11	11007	20.53	16947	31.60
8	179861	13201	7.34	48790	27.13	49307	27.41	68563	38.12
9	140812	8851	6.29	30710	21.81	33869	24.05	67382	47.85
10	34529	4412	12.78	13546	39.23	9969	28.87	6602	19.12
11	16312	2044	12.53	6666	40.87	4563	27.97	3039	18.63
12	91825	24450	26.63	37959	41.34	19158	20.86	10258	11.17
13	70454	17463	24.79	25721	36.51	15136	21.48	12134	17.22
14	34445	19361	56.21	9006	26.15	4058	11.78	2020	5.86
15	8818	967	10.97	3436	38.97	2272	25.77	2143	24.30
Total	**776476** [a]	**124486**	**16.03**	**242685**	**31.25**	**183326**	**23.61**	**225979**	**29.10**

[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 Paediatric Directive § 26 Paragraph 3, examinations must be performed and pathological findings reported on the day the specimen is received. 74.2% 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.

From 2016 to 2017 the proportion of findings that were not reported until two to three days after receipt by the laboratory rose. 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	57748	19829	34.34	24181	41.87	5271	9.13	8467	14.66
3	15067	12031	79.85	1864	12.37	874	5.80	298	1.98
5	59980	43844	73.10	15120	25.21	1000	1.67	16	0.03
6	12653	8342	65.93	121	0.96	2071	16.37	2119	16.75
7	53816	22207	41.26	23719	44.07	5412	10.06	2478	4.60
8	180296	167873	93.11	9476	5.26	966	0.54	1981	1.10
9	140792	112634	80.00	24411	17.34	3345	2.38	402	0.29
10	34529	31436	91.04	2855	8.27	210	0.61	28	0.08
11	16312	9770	59.89	4458	27.33	1529	9.37	555	3.40
12	92573	68023	73.48	17612	19.02	5710	6.17	1228	1.33
13	70454	51032	72.43	13542	19.22	5012	7.11	868	1.23
14	34450	26901	78.09	6273	18.21	731	2.12	545	1.58
15	8818	3282	37.22	5387	61.09	140	1.59	9	0.10
Total	**777488**[a]	**577204**	**74.24**	**149019**	**19.17**	**32271**	**4.15**	**18994**	**2.44**

[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 2005 to 2019

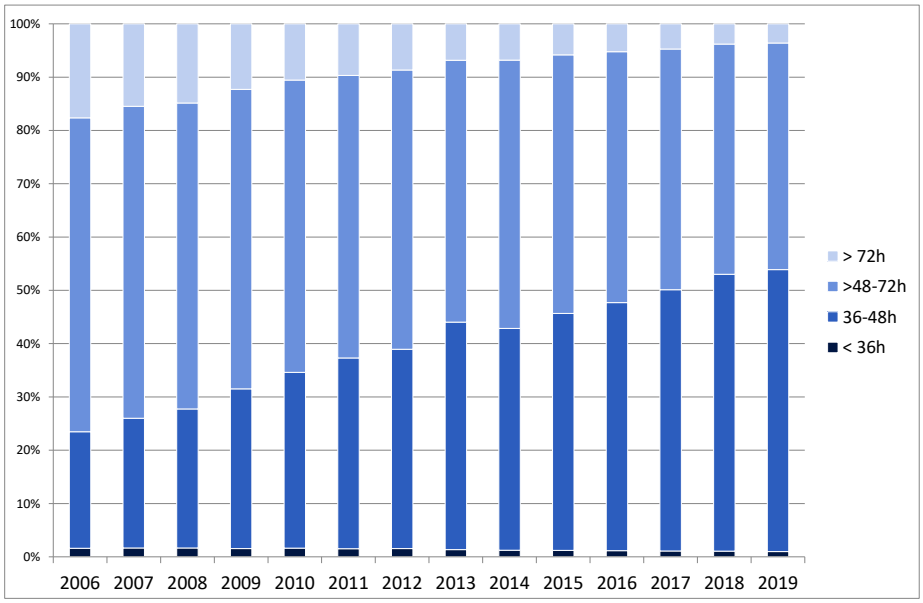


Figure 3: Time between blood sample collection and receipt by the lab 2005 to 2019

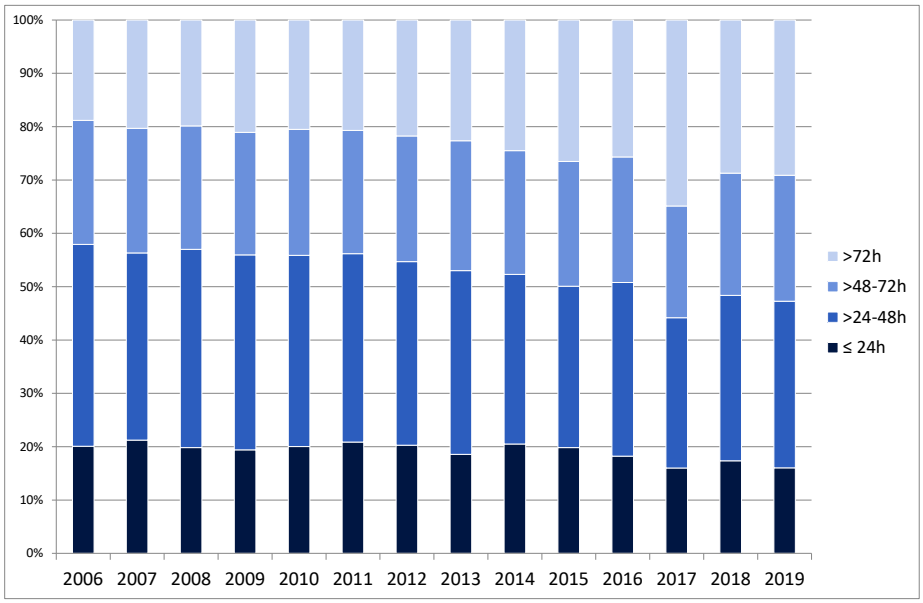
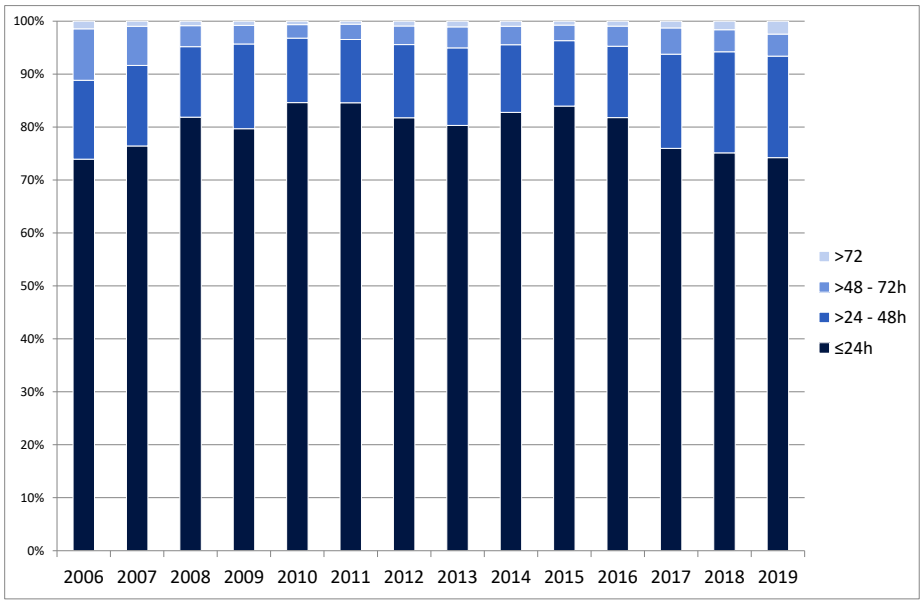


Figure 4: Time between receipt by the lab and reporting the results 2005 to 2019



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) and especially 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 0.5% in 2019. In the CF screening, the positivity rate was 0.14%. 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 0.48%. The increased recall rate for blood collection <36h or before 32 WoG also has a negative impact on the PPV in CAH and hypothyroidism.

The overall specificity for newborn screening was 99.48%. The sensitivity cannot be determined, as the number of children missed in the screening has not been systematically recorded. Here, registers of the target diseases in the screening would be very helpful.

Table 4: Recall rates and cases found through screening for Germany 2019 (Initial screening N= 777922)

Disease	Recall	Recall rate (%)	Confirmed Cases	PPV	Specificity
Hypothyroidism	887	0.11	251[b]	28.30	99.89
CAH	1276	0.16	45 [b]	3.53	99.84
Biotinidase Deficiency	241	0.03	21	8.71	99.97
Galactosemia [a]	191	0.02	10	5.24	99.98
PKU/HPA	319	0.04	151	47.34	99.98
MSUD	30	0.004	4	13.33	99.99
MCAD	185	0.02	79	42.70	99.99
LCHAD	11	0.001	3	27.27	99.99
VLCAD	101	0.01	7 [b]	6.93	99.99
CPT-I Deficiency	12	0.002	0		
CPT-II Deficiency [d]	14	0.002	2	14.29	99.99
GA I	43	0.01	4	9.30	99.99
IVA	105	0.01	7	6.67	99.99
Tyrosinemia	118	0.02	6	5.08	99.99
CF	1104	0.14	145 [b]	12.68	99.88
SCID [c]	236		17 [b]	7.20	
Total ENS	**4832**	**0.62**	**752** [b]	**15.56**	**99.48**

[a] Only classic galactosemia [b] Excluding cases with unremarkable screening: 1 CAH, 7 hypothyroidism, 1 VCLAD, 1 SCID and 6 CF [C] Initial screening from 8/2019 [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	220	6	1	5	25	1	258
CAH	40	1	0	2	3	0	46
Biotinidase Deficiency	19	0	0	1	0	1	21
Galactosemia	10	0	0	0	0	0	10
PKU/HPA	145	1	0	5	0	0	151
MSUD	4	0	0	0	0	0	4
MCAD	72	2	0	4	0	1	79
LCHAD	2	0	0	1	0	0	3
VLCAD	7	0	0	1	0	0	8
CPT I	0	0	0	0	0	0	0
CPT II	2	0	0	0	0	0	2
GA I	4	0	0	0	0	0	4
IVA	7	0	0	0	0	0	7
Tyrosinemia	5	0	0	1	0	0	6
CF	138	1	3	6	3	0	151
SCID	14	1	1	2	0	0	18
Total	688	13	5	28	31	3	768

[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 September 24, 2021. Cases from birth year 2019 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. Martin Lindner 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.

Cases with missing information on confirmation diagnostics were only taken into account if the validators judged a diagnosis to be probable based on the screening results. This occurred in a total of 42 cases in 2019 (22 metabolic screening, 19 hypothyroid and 1 CAH). For 37 cases with abnormal ENS, the information on the confirmation diagnostics was not sufficient to confirm the diagnosis (see section 6).

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 2019, 1 case of CAH, 7 cases of hypothyroidism, 1 VCLAD, 1 SCID 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. The DGNS provides the appropriate paperwork and parental consent forms.

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	58059	87	0.15	21	74	0.13	16
3	15067	13	0.09	4	13	0.09	4
5	60081	68	0.11	18	66	0.11	16
6	12655	8	0.06	5	8	0.07	5
7	53816	71	0.13	16	51	0.1	16
8	180296	299	0.17	54	187	0.11	47
9	140812	99	0.07	57	91	0.07	53
10	34529	49	0.14	12	17	0.05	11
11	16312	32	0.20	2	9	0.06	1
12	92573	72	0.08	34	56	0.06	31
13	70454	40	0.06	14	38	0.06	12
14	34450	38	0.11	16	33	0.1	12
15	8818	11	0.12	5	9	0.11	4
Total	**777922**	**887**	**0.11**	**258** [a]	**652**	**0.08**	**227**

		<36h			<32 WoG		
Lab	**Initial screening**	Recall (n)	Recall rate (%)[b]	Confirmed cases (n)	Recall (n)	Recall rate (%)[b]	Confirmed cases (n)
1	58059	8	1.89	1	5	0.92	4
3	15067	0			0		0
5	60081	0		1	2		1
6	12655	0		0	0		0
7	53816	18	3.30	0	2		0
8	180296	102	6.04	1	10	0.51	6
9	140812	0		0	8	0.39	4
10	34529	30	12.99	0	2		1
11	16312	21	8.27	0	2		1
12	92573	9	0.93	1	7	0.63	2
13	70454	2		0	0		2
14	34450	1		1	4		3
15	8818	1		0	1		1
Total	**777922**	**192**	**2.63**	**5**	**43**	**0.46**	**25**

[a] including 7 cases with an unremarkable initial screening and 1 case without indication of the time of the initial screening.

[b] recall rates only provided if recall rate ≥ 0.01% and n ≥ 5

Of the 258 confirmed and validated congenital hypothyroidism cases, seven were unremarkable in the initial screening or in the control card at 32 WoG. This could be explained by the administration of catecholamine in one of these children. In 2 premature infants, after an abnormal initial screening, the required 2nd TC was unremarkable (TSH <10 or 14.8mU/l at a cut-off of 15mU/l) but the serum findings taken at the same time (TSH 17.4 mU/l, fT4 0.69 ng/dl or TSH 26mU/l, fT4 13.76 pmol/l decreasing to 12.12 pmol/l) were abnormal, so that therapy was started promptly.

In addition, n= 30 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 (Serum)	fT3	fT4	Sonography	SD Antibodies	Confirmed cases without verification details
1	21	21		19	18	6	
3	4	4	4	4	4	3	
5	18	15	4	13	14	11	3
6	4	4	3	4	3	3	
7	16	1	1	1			15
8	54	52	44	50	48	30	
9	57	57	31	56	8	9	
10	12	12	9	11	6	8	
11	2	2	2	2	1	2	
12	35	35	29	35	4	1	
13	14	13	11	14			
14	16	16	11	16	10	3	
15	5	2	2	2	3	1	1
Total	**258**	**234**	**151**	**227**	**119**	**77**	**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 (%)[d]	Confirmed cases (n)	Recall (n)	Recall rate (%)[d]	Confirmed cases (n)
1[b]	58059	20	0.03	5	10	0.02	5
3	15067	4	0.03	2	3	0.02	2
5	60081	136	0.23	2	127	0.21	2
6	12655	19	0.15	1	14	0.11	1
7	53816	361	0.67	0	173	0.33	0
8[c]	180296	205	0.11	8	52	0.03	8
9	140812	269	0.19	12	266	0.19	10
10	34529	174	0.50	5	110	0.32	5
11	16312	37	0.23	0	19	0.12	0
12[c]	92573	28	0.03	7	24	0.03	6
13[c]	70454	19	0.03	2	11	0.02	1
14[b]	34450	2		2	1		1
15[b]	8818	2		0	1		0
Total	**777922**	**1276**	**0.16**	**46** [a]	**811**	**0.11**	**41**

		<36h			<32 WoG		
Lab	**Initial screening**	Recall (n)	Recall rate (%)[d]	Confirmed cases (n)	Recall (n)	Recall rate (%)[d]	Confirmed cases (n)
1[b]	58059	3		0	7	1.29	0
3	15067	0		0	1	0.39	0
5	60081	2		0	7	1.19	0
6	12655	0		0	5	2.78	0
7	53816	36	6.61	0	152	20.21	0
8[c]	180296	134	7.93	0	19	0.97	0
9	140812	0		0	3		2
10	34529	29	12.55	0	35	8.60	0
11	16312	7	2.76	0	11	6.96	0
12[c]	92573	4		1	0		0
13[c]	70454	2		1	6	0.74	0
14[b]	34450	0		0	1		1
15[b]	8818	0		0	1		0
1[b]	**777922**	**217**	**2.97**	**2**	**248**	**2.66**	**3**

[a] including 1 case with an inconspicuous initial screening

[b] Lab uses 2nd tier method

[c] Lab uses 2nd tier method for screening >36h

[d] Recall rates only provided if recall rate ≥ 0.01% and n ≥ 5

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	5	1	2		5	
3	2	2	2		2	
5	2				1	1
6	1	1	1		1	
7						
8	8	6	7		7	
9	12	11	7	1	3	
10	5	4	5		2	
11						
12	7	7	6	1	4	
13	2	2			1	
14	2	2	2	1	2	
15						
Total	**46**	**36**	**32**	**3**	**28**	**1**

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

Of the 46 confirmed AGS cases, one case was not found in the initial screening: 24 WoG, first card taken at 49h (17-OHP 92nmol/l) 2 days after transfusion; in two subsequent cards at 394h and 1317h (= control at 32 WoG) 17OHP normal. Hydrocortisone treatment in bronchopulmonary dysplasia; in case of abnormal genitals and electrolyte shift selective genetic diagnostics: compound-heterozygous CYP21A2 gene (c.955C>T and c.1069C>T).

5.3 Biotinidase Deficiency

Table 5.3.1: Biotinidase Deficiency - Confirmed cases / Recall rate

Lab	Initial screening	Recall	Recall rate (%)[a]	Confirmed cases
1	58059	18	0.03	1
3	15067	2		0
5	60081	2		0
6	12655	5	0.04	0
7	53816	58	0.11	4
8	180296	63	0.03	6
9	140812	29	0.02	3
10	34529	2		0
11	16312	4		0
12	92573	30	0.03	2
13	70454	16	0.02	2
14	34450	7	0.02	2
15	8818	5	0.06	1
Total	**777922**	**241**	**0.03**	**21**

[a] Recall rates only provided if recall rate ≥ 0.01% and n ≥ 5

Of n= 21 confirmed cases, a partial biotinidase deficiency was diagnosed in n=12 cases.

Table 5.3.2: Biotinidase Deficiency Confirmation

Lab	Confirmed cases	Biotinidase (Serum/DB)	Molecular genetics	Confirmed cases without confirmation details
1	1	1	1	
7	4	4	3	
8	6	5		1
9	3	2	1	1
12	2	1	1	
13	2	2	1	
14	2	2		
15	1	1		
Total	**21**	**18**	**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	58059	18	0.03	2
3	15067	2		0
5	60081	12	0.02	0
6	12655	2		0
7	53816	15	0.03	2
8	180296	116	0.06	2
9	140812	6		0
10	34529	0		0
11	16312	1		0
12	92573	10	0.01	2
13	70454	7	0.01	2
14	34450	0		0
15	8818	2		0
Total	**777922**	**191**	**0.02**	**10**

[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	
7	2	1	2	2	
8	2	1	1	1	
12	2			1	1
13	2	2	1	1	
Total	**10**	**6**	**6**	**7**	**1**

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	58059	32	0.06	20
3	15067	9	0.06	5
5	60081	26	0.04	16
6	12655	8	0.06	5
7	53816	89	0.17	12
8	180296	35	0.02	33
9	140812	37	0.03	25
10	34529	21	0.06	6
11	16312	4		3
12	92573	11	0.01	8
13	70454	15	0.02	8
14	34450	25	0.07	7
15	8818	7	0.08	3
Total	**777922**	**319**	**0.04**	**151**

[a] Recall rates only provided if recall rate ≥ 0.01% and n ≥ 5

Of n=151 confirmed cases, 59 were diagnosed with PKU, 89 with HPA and 3 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	20	20	19	13	3	20	
3	5	5	5	1	2	2	
5	16	13	4	5	13	13	2
6	5	5	1	3	4	4	
7	12	9	8	2	5	7	3
8	33	28	16	10	19	20	3
9	25	21	15	4	22	22	1
10	6	5	5	5	5	4	1
11	3	2	1	2	2	2	
12	8	8	4	4	7	7	
13	8	7	6		4	5	1
14	7	6	2	3	7	7	
15	3	2	2		1	2	1
Total	**151**	**131**	**88**	**52**	**94**	**115**	**12**

Table 5.5.3: PKU BH4-Test / BH4 Sensitivity

Lab	Confirmed cases	BH4-Test	BH4 sensitive
1	20	8	2
3	5	5	2
5	16		3
6	5		
7	12		
8	33	15	5
9	25	6	
10	6	3	2
11	3	3	1
12	8	3	1
13	8	1	
14	7	2	1
15	3		
Total	**151**	**46**	**17**

5.6 Maple Syrup Urine Disease (MSUD)

The overall recall rate is very low at 0.0039%.

Table 5.6.1: MSUD - Confirmed cases / Recall rate

Lab	Initial screening	Recall	Confirmed cases
1	58059	2	1
3	15067	3	0
5	60081	0	0
6	12655	3	0
7	53816	5	0
8	180296	1	1
9	140812	12	1
10	34529	0	0
11	16312	0	0
12	92573	1	0
13	70454	1	1
14	34450	2	0
15	8818	0	0
Total	**777922**	30	4

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		1	
8	1	1				
9	1	1			1	
13	1	1			1	
Total	**4**	**4**	**1**	**0**	**3**	**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	58059	11	0.02	8
3	15067	11	0.07	4
5	60081	3		3
6	12655	2		1
7	53816	36	0.07	5
8	180296	30	0.02	26
9	140812	60	0.04	15
10	34529	9	0.03	1
11	16312	2		1
12	92573	7	0.01	6
13	70454	8	0.01	8
14	34450	5	0.01	1
15	8818	1		0
Total	**777922**	**185**	**0.02**	**79**

[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	8	2	8	7	7	
3	4				3	1
5	3				2	1
6	1	1	1		1	
7	5		1	1	5	
8	26	13	15	5	17	2
9	15	7	10	7	12	
10	1	1	1	1	1	
11	1		1			
12	6	4			5	
13	8	8	1		3	
14	1			1		
Total	**79**	**36**	**38**	**22**	**56**	**4**

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

The overall recall rate is very low at 0.0014%.

Table 5.8.1: LCHAD Deficiency - Confirmed cases / Recall rate

Lab	Initial screening	Recall	Confirmed cases
1	58059	1	0
3	15067	0	0
5	60081	1	0
6	12655	2	0
7	53816	0	0
8	180296	1	1
9	140812	1	0
10	34529	1	0
11	16312	2	1
12	92573	1	1
13	70454	0	0
14	34450	1	0
15	8818	0	0
Total	**777922**	**11**	**3**

Table 5.8.2: LCHAD Deficiency Confirmation

Lab	Confirmed cases	Confirmation (Serum)	Organic Acids (urine)	Enzyme activity	Molecular genetics	Confirmed cases without confirmation details
8	1	1				
11	1	1	1		1	
12	1			1	1	
Total	**3**	**2**	**1**	**1**	**2**	**0**

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 (%)[b]	Confirmed cases
1	58059	2		0
3	15067	0		0
5	60081	2		1
6	12655	6	0.05	0
7	53816	21	0.04	0
8	180296	16	0.01	0
9	140812	29	0.02	3
10	34529	3		0
11	16312	2		0
12	92573	1		0
13	70454	1		1
14	34450	7	0.02	3
15	8818	0		0
Total	**777922**	**90**	**0.01**	**8**[a]

[a] Includes 1 case with an inconspicuous first screening [b] 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
5	1			1		
9	3	1		2	2	
13	1				1	
14	3			1	2	1
Total	**8**	**1**	**0**	**4**	**5**	**1**

In one VLCAD case, the initial screening values (C14:1 and C14:1/C4) at 37 hours of life, gestation 40 weeks were unremarkable. Diagnosis was made at 5 months of age with clinical suspicion of a metabolic disorder. Palmitoyl-CoA oxidation 3.05 +/-0.12 mU/mg protein (corresponding to 25% residual activity), human genetics ACADVL: c.205-8_205-7 delinsGC / 1829C>A.

Another case was genetically diagnosed prenatally.

5.10 CPT I / CPT II / CACT Deficiency

For the CACT deficiency, neither recalls nor confirmed cases were reported. Recalls may have been reported for CPT II deficiency. The overall recall rate is very low at 0.0012%.

Table 5.10.1: CPT I /II / CACT Deficiency Recall

	Initial screening	Recall	Confirmed Cases
CPT I Deficiency	777922	5	0
CACT Deficiency	777922	9	2

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
8	1			1	
13	1			1	
Total	**2**			**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	58059	7	0.01	1
3	15067	1		0
5	60081	4		1
6	12655	1		0
7	53816	6	0.01	0
8	180296	1		1
9	140812	20	0.01	1
10	34529	0		0
11	16312	0		0
12	92573	2		0
13	70454	0		0
14	34450	1		0
15	8818	0		0
Total	**777922**	**43**	**0.01**	**4**

[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
1	1		1		1	
5	1	1	1		1	
8	1					1
9	1		1		1	
Total	**4**	**1**	**3**		**3**	**1**

5.12 Isovaleric Acidemia (IVA)

Table 5.13.1: IVA - Confirmed Cases / Recall rate

Lab	Initial screening	Recall	Recall rate (%)[a]	Confirmed cases
1	58059	5	0.01	0
3	15067	7	0.05	1
5	60081	7	0.01	1
6	12655	5	0.04	0
7	53816	12	0.02	0
8	180296	6		3
9	140812	10	0.01	0
10	34529	10	0.03	0
11	16312	2		0
12	92573	10	0.01	1
13	70454	0		0
14	34450	9	0.03	0
15	8818	4		1
Total	**777922**	**87**	**0.01**	**7**

[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 remained about the same in 2019. 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
3	1				1	
5	1		1			
8	3	2	2		3	
12	1		1		1	
15	1	1	1			
Total	**7**	**3**	**5**		**5**	

5.13 Tyrosinemia

Table 5.13.1: Tyrosinemia – Confirmed Cases

Lab	Initial Screening	Recall	Recall Rate (%)[a]	Confirmed Cases
1	58059	3		2
3	15067	2		0
5	60081	0		0
6	12655	1		0
7	53816	1		0
8	180296	65	0.04	0
9	140812	12	0.01	0
10	34529	12	0.03	0
11	16312	0		0
12	92573	19	0.02	1
13	70454	0		0
14	34450	1		1
15	8818	2		2
Total	**777922**	**118**	**0.02**	**6**

[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
1	2	2	2		2	
12	1				1	
14	1	1			1	
15	2	1			2	
Total	**6**	**4**	**2**		**6**	

5.14 Severe Combined Immunodeficiency (SCID)

Severe combined immunodeficiency (SCID) was added to ENS as a new target disease in 8/2019. Since the number of initial screenings per laboratory are only known for the full year 2019, no recall rate can be calculated for SCID for 2019.

Table 5.14.1: SCID Confirmed Cases

Lab	Recall	Confirmed Cases
1	9	1
3	2	1
5	0	5
6	0	0
7	54	1
8	89	3
9	36	6
10	11	0
11	1	0
12	10	0
13	10	1
14	7	0
15	7	0
Total	**236**	**18**

Table 5.14.2: SCID Confirmed Cases

Lab	Confirmed Cases	Genetics	Without information about the confirmation diagnostics
1	1	0	
3	1	0	
5	5	5	
7	1	1	
8	3	2	2
9	6	5	
13	1	0	
Total	**18**	**13**	**2**

One child with confirmed SCID diagnosis had a false-negative initial screening (WoG 36, BA 72h: TREC 9, cut-off 5) and was diagnosed at 3 months of age due to chronic diarrhea and recurrent infections.

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, initially for immunoreactive trypsin (IRT). If this is elevated, pancreatitis-associated protein (PAP) is assessed as a second step and, in the case of pathological PAP, a molecular genetic test is performed in a third step. Here, the 31 most common pathogenic mutations of the cystic fibrosis trans-membrane regulator gene (CFTR gene) in Germany are screened for (Figure 5). The screening is considered conspicuous (positive) if the 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) accounting for 76% of the 799 positive screening findings (see Fig. 5). The diagnosis of CF was confirmed in only 151 children (18.9%), of which 107 (17.59%) were confirmed after positive screening by failsafe and 38 (19.89%) upon detection of one or two of the 31 mutations. In addition, 6 children were diagnosed with CF after unremarkable CF screening (Table 5.14.4).

According to the Paediatric Directive, a separate consent form is required for CF screening, and screening cannot be performed by a midwife alone, as is the case with ENS in exceptional situations, but only with the opportunity to consult a physician. The proportion of newborns without CF screening was 1.09% in 2019 (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	58059	704	1.21
3	15067	6	0.04
5	60081	1409	2.35
6	12655	10	0.08
7	53816	2574	4.78
8	180296	1536	0.85
9	140812	148	0.11
10	34529	694	2.01
11	16312	39	0.24
12	92573	771	0.83
13	70454	478	0.68
14	34450	117	0.34
15	8818	15	0.17
Total	**777922**	**8501**	**1.09**

Table 5.14.2: CF – Confirmed cases / Recall Rate

Lab	Initial screening with CF Screening	Recall	Recall Rate (%)	Confirmed cases
1	57355	59	0.10	12
3	15061	22	0.15	2
5	58672	68	0.11	13
6	12645	26	0.21	2
7	51242	50	0.09	6
8	178760	206	0.12	34
9	140664	94	0.07	22
10	33835	34	0.10	8
11	16273	17	0.10	4
12	91802	106	0.11	32
13	69976	69	0.10	6
14	34333	33	0.10	8
15	8803	28	0.32	2
Total	**769421**	**1104**	**0.14**	**151**[a]

[a] of which 6 cases with unremarkable CF screening

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	12	8	3		5	1
3	2		2	2	1	
5	13	5	6	1	9	4
6	2		2		2	
7	6	5	1		4	
8	34	6	18		33	5
9	22	3	16	6	8	3
10	8	5		3	6	1
11	4	2	2		2	
12	32	20	6	22	18	6
13	6	4			4	1
14	8	7		5	2	1
15	2	1	1		1	
Total	**151**	**66**	**57**	**39**	**95**	**22**

In 27 cases reported by the laboratories, the information was not sufficient to confirm the diagnosis. Out of n=151 confirmed cases, 135 were diagnosed with cystic fibrosis and 7 with Cystic Fibrosis Screen Positive, Inconclusive Diagnosis (CFSPID), in 9 cases there was insufficient information (genetics) to distinguish between CF and CFSPID. Screening via failsafe was positive in 70.9% of the cases, one or 2 mutations from the panel were detected in 25.2% of them, and the CF screening was unremarkable in 4.0% of the cases.

In n=100 of the confirmed cases, genetic data from screening or confirmation were available. Two mutations from the panel of 31 were present in 72 cases, one mutation in 27 cases, and only 1 child with an IRT of 98ng/ml (not failsafe) had 2 other mutations. In total, 22 children were reported to have meconium ileus. Information on one sweat test (n=67) or two sweat tests (n=52) was available in 123 cases. Information on 2 existing mutations was available in only 26 cases, and 1 case was validated as probable only on the basis of a twofold abnormal conductance, and full sequencing was pending in another case with extreme hypotrophy and pancreatic insufficiency at birth.

Of the confirmed diagnoses 6 were not found via the predefined screening algorithm for cystic fibrosis and were unremarkable in the screening. Three of these children were diagnosed due to meconium ileus, 3 children were diagnosed based on failure to thrive (see Table 5.14.4). It is not known whether other children with cystic fibrosis were not found at screening.

Table 5.14.4: Confirmed Cases with unremarkable CF Screening

Screening Parameter	Found via	Count (n)
IRT unremarkable	Meconium ileus (n=3) Failure to thrive (n=1)	**4**
PAP unremarkable	Failure to thrive (n=1)	**1**
None of the 31 Mutations	Failure to thrive (n=1)	**1**

6 Lost to follow-up

Of a total of 21,012 second cards requested, 18,213 (86.68%) were sent in, meaning that no further information was available for 10.29% of the cards requested (Table 2.4). These calculations exclude the 709 cases from lab 7 as the number of second screenings received is not known. 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 79 children with positive screening results in the ENS, it is not known whether the validation diagnostics took place or were completed. 42 of these cases, for which no confirmation information was available but with unambiguous screening results, were validated as 'probable cases' on the basis of the screening results (Table 6.1.1.1) and included in the calculation of prevalence. This was not possible for 37 children (Tab. 6.1.2.1).

6.1.1 Confirmed cases without information about validation diagnostics

42 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 confirmation	No feedback from clinic / pediatrician	Clinic did not request confirmation	Only diagnosis with no information on the diagnostics performed	Without parental consent	Unclear
Hypothyroidism	19	1				18
CAH	1					1
Biotinidase Deficiency	2	1				1
Galactosemia	1			1		
PKU/HPA	1	1				
MCAD	11	2	1		1	7
IVA	4				1	3
VLCAD	1			1		
SCID	2					2
Total	**42**	**5**	**1**	**2**	**2**	**32**

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

Table 6.1.2.1: Cases with implausible or missing confirmation information

Disease	Number of Cases n
Congenital Hypothyroidism	13
CAH	11
Biotinidase Deficiency	1
PKU / HPA	3
MCAD	1
VLCAD	2
IVA	1
Tyrosinemia	1
SCID	4
Total	**37**

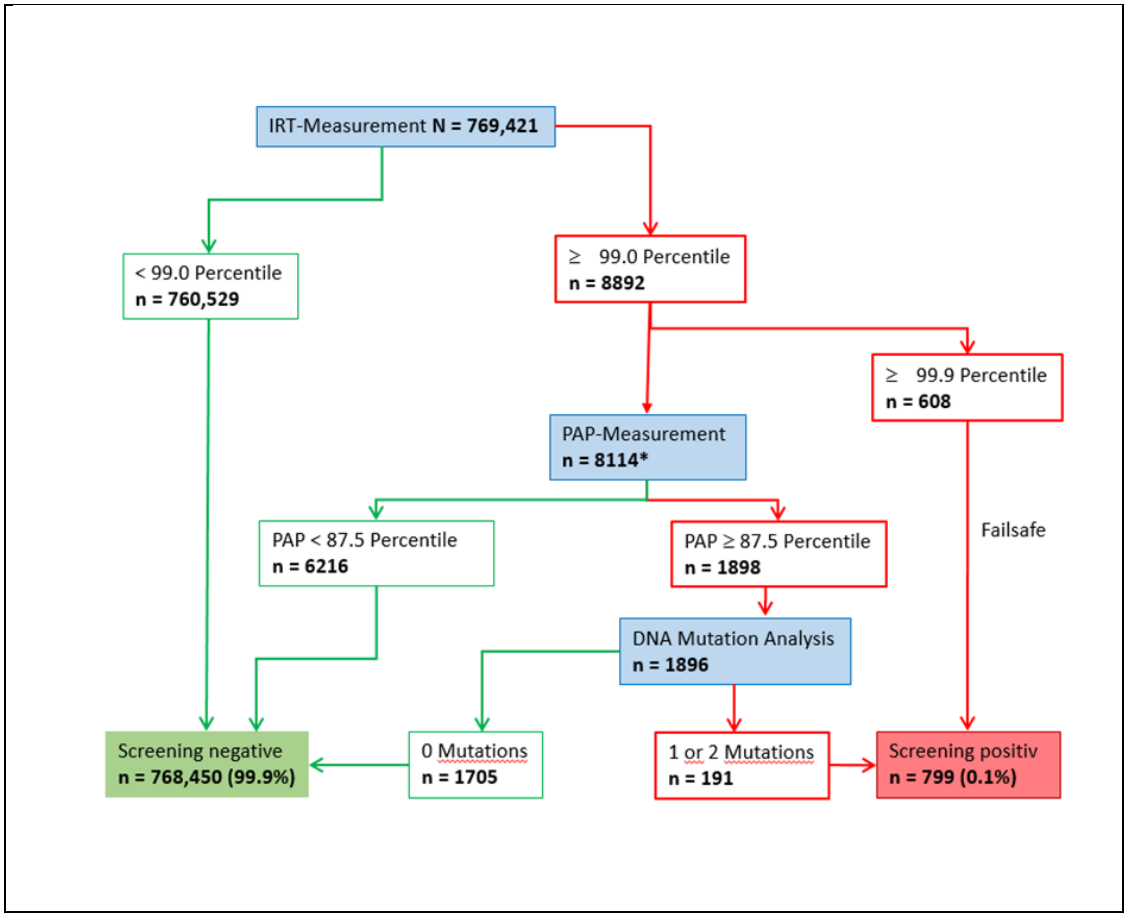
Table 6.1.2.2: Proportion of cases by lab with implausible or missing confirmation data

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	74	74			
3	23	19	1	4	21.74
5	61	60	7	1	13.11
6	13	13			
7	56	46	18	10	50.0
8	181	173	9	8	9.39
9	147	145	2	2	2.72
10	42	32		10	23.81
11	11	11			
12	95	95	1		1.05
13	46	46	1		2.13
14	40	40	1		2.43
15	16	14	2	2	25.0
Total	**805**	**768**	**42**	**37**	**9.81**

7 Screening Algorithm Cystic Fibrosis (CF)

7.1 Screening Algorithm Germany

Figure 5: Screening Algorithm Cystic Fibrosis Germany 2019



* 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.

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 Cutoffs used in Screening

Table 8.1: Filter paper

Lab	Filter paper
1	ID Biological (Ahlstrom 226)
3	ID Biological (Ahlstrom 226)
5	ID Biological (Ahlstrom 226)
6	ID Biological (Ahlstrom 226)
7	ID Biological (Ahlstrom 226)
8	Ahlstrom Munksjö
9	ID Biological (Ahlstrom 226)
10	ID Biological (Ahlstrom 226)
11	ID Biological (Ahlstrom 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 mU/l	GSP
8	TSH	15 mU/l (≤ 8 days) 10 mU/l (>8 days)	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 (1 day) 15 mU/l (2-4 days) 10 mU/l (≥ 5 days)	AutoDELFIA
14 /15	TSH	20 mU/l (3 days) 15 mU/l (4-5 days) 10 mU/l (> 5 days)	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	AutoDELFIA
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

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	Extinction < 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	>3.5 U/g Hb	Quantitative fluorometry
	Galactose	<20 mg/dl	Fluorometry (PE)
3	GALT	>2.3 U/g Hb	Fluorometry (PE)
	Galactose	<15 mg/dl	
5	GALT	>3.5 U/g Hb	Quantitative fluorometry
	Galactose	20 mg/dl	Quantitative colorimetry
6	GALT	>3.5 U/g Hb	Fluorometry (PE)
7	GALT	>3.5 U/g Hb	Quantitative fluorometry
8	GALT	>20% daily mean	Quantitative fluorometry
	Galactose	<30 mg/dl	Quantitative colorimetry
9	GALT	>5.3 U/g Hb	Fluorometry (PE)
	Galactose	<20 mg/dl	BIORAD Quantase
10	GALT	>3.5 U/gHb	Fluorometry (PE)
	Galactose	1111 µmol/l	BIORAD Quantase
11	GALT	>3.5 U/g Hb	Fluorometry (PE)
12/13	GALT	>20%	Colorimetry non-kit / Quant. fluoro.
	Galactose	< 15 mg/dl	(non-kit)
14/15	GALT	>3.5 U/g Hb	Quantitative fluorometry
	Galactose	<15 mg/dl	Quantitative colorimetry

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	derivatized PE kit
8	non-derivitized non Kit
9	derivatized non-kit
10	deriv. Chromsystems Kit
11	non-derivat. Chromsystems Kit
12/13	derivatized non-kit
14/15	non-derivat. Chromsystems Kit

9 Literature

[1] Pediatric Guideline Effective: November 26, 2021 of the Federal Joint Committee on the Early Detection of Diseases in Children (Pediatric Guideline – “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 2019 https://www.destatis.de/DE/Themen/Gesellschaft-Umwelt/Bevoelkerung/Geburten/_inhalt.html (accessed June 6, 2021)