National

Screening Report

Germany 2014

German Society for Neonatal Screening (DGNS)

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Abbreviations and Glossary:

CAH	Congenital adrenal hyperplasia
CACT - Deficiency	Carnitine-Acylcarnitine-Translocase-Deficiency
CPTI - Deficiency	Carnitine-Palmitoyl-CoA-Transferase I-Deficiency
CPTII - Deficiency	Carnitine-Palmitoyl-CoA-Transferase II-Deficiency
PT	Preterm < 32 WoG
GA I	Glutaric acidaemia Type I
BW	Birth weight
HPA	Hyperphenylalaninemia
IVA	Isovaleric acidaemia
LCHAD - Deficiency	Long-Chain-3-Hydroxy-Acyl-CoA-Dehydrogenase-Deficiency
DoL	Day of life
GV 1 - 3	Guide value 1 - 3
MCAD - Deficiency	Medium-Chain-Acyl-CoA-Dehydrogenase-Deficiency
MSUD	Maple syrup urine disease
NGS	Newborn screening
SV	Secondary value
PKU	Phenylketonuria
PPV	Positive predictive value
Second-tier Process	In case of abnormal finding, second examination of additional parameters or alternative analysis procedure with the same test card
WoG	Week of gestation
VLCAD - Deficiency	Very-Long-Chain-Acyl-CoA-Dehydrogenase-Deficiency

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1 Introduction

The newborn 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 endocrine and metabolic diseases.

In the policies for early detection of diseases in children up to 6 years of age, known as the „Children's Guidelines" (Kinder-Richtlinien) [1], the regulations for implementing the newborn screening program (NBS) are defined in appendices 2-4. The 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 guidelines and quality criteria of the NBS implementation. This report pertains only to the metabolic and endocrine diseases which are defined in these guidelines. It provides a comprehensive statistical summary of disease-related screening figures, recall rates and confirmed diagnoses for the year 2014. 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 newborn screening:

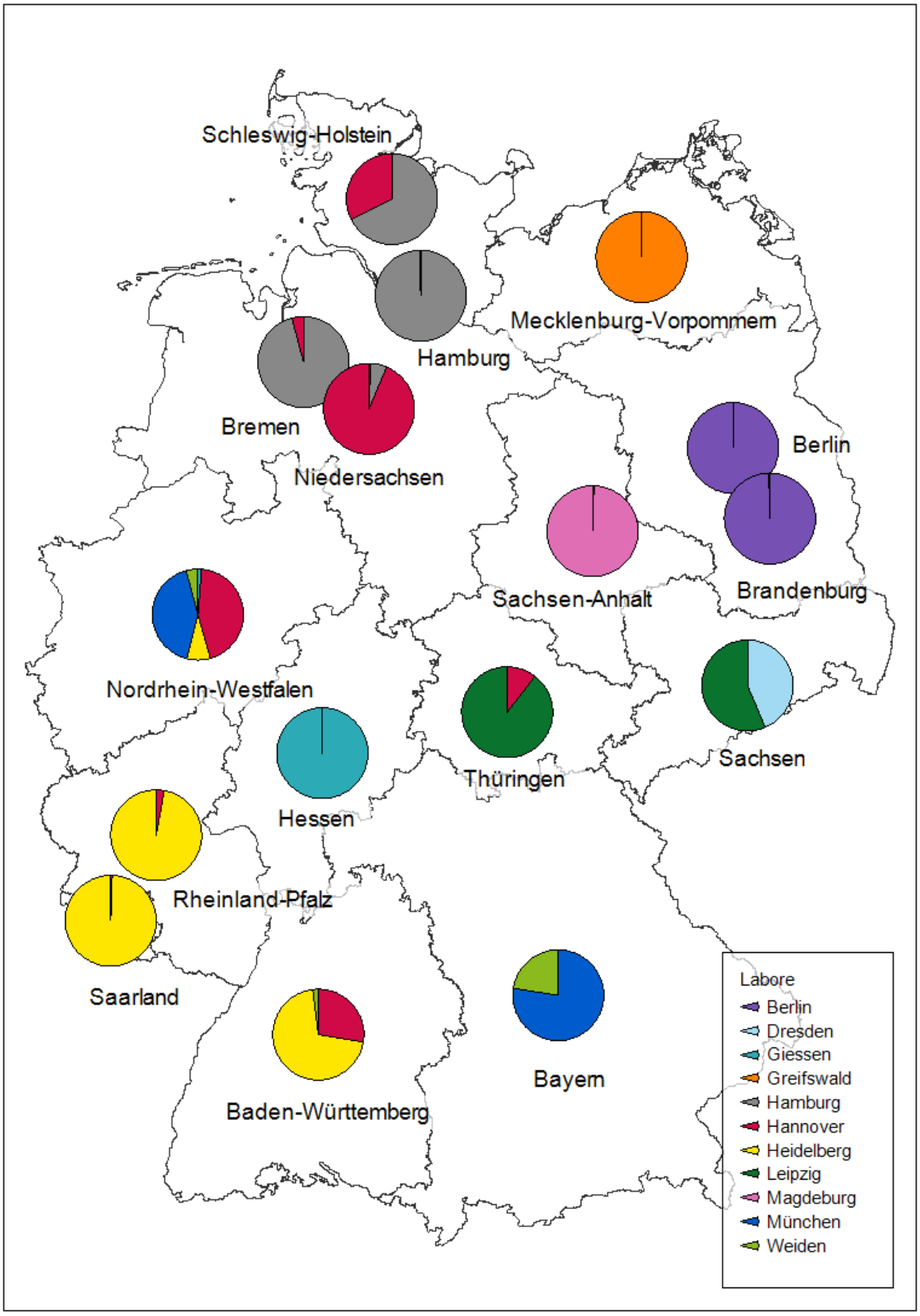
- Total survey of the targeted population
    - Collection method and rate
    - Blank card system
- Completeness of the control (recall) and follow-up examinations
- Recording test parameters and cutoffs
- Stratified recall rates, positive predictive values and prevalence by illness, laboratory, age and gestational age
- Specificity and sensitivity of diagnostic tests
- Processing times (pre-analytic and laboratory only), age at time the blood sample was taken, time between blood collection, arrival in the laboratory and communication of the result
- Individual screening values of newborns for whom further testing is recommended
- Confirmation diagnostics
    - Type of diagnostics
    - Period of diagnostics
- Final diagnosis
- Start of therapy

The laboratories that conducted the screening in 2014 in Germany are listed on the previous page (12 and 13 refer to the same laboratory, once in cooperation with the screening facility and once without; the same is true of 14 and 15). In the tables the laboratories are encrypted. Mentions of sections and subsections in the text refer to the "Children's Guidelines" of December 16, 2010 [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 has been checked for plausibility. In the cases of remaining inconsistencies, the data reported by the laboratories were used in the tables (inconsistencies can sometimes be systemic).

The screening samples from the individual federal states are distributed among the laboratories (“Labore”) as illustrated in Figure1.

Figure 1: Distribution of screening samples by state and laboratory



2 Results

In 2014, a total of 714,927 children were born in Germany [2]. The number of recorded screenings (716,496) exceeds the number of births. Reasons for the surplus screening samples could be control cards that were not declared as such and were received by another laboratory, or samples from newborns whose births were not registered in Germany. This cannot be further clarified due to the genetic diagnostics law, which prohibits the exchange of data between screening labs.

Births [2]:	714,927
First screenings:	716,496
Confirmed diagnoses (see Table 3):	519

A reliable statement about the rate of participation in NBS can only be made by reconciling individual data with overall population data. However, due to legal restrictions this is only possible in the state of Bavaria.

The diseases targeted for the nationwide screening are defined in the “Children’s Guidelines”. In some laboratories, screenings for additional illnesses are carried out for scientific studies or based on state-level regulations; the results of those screenings are not covered in this report.

One of the targeted disease was found in 1 out of every 1,378 newborns, Table 2 shows the prevalence of the targeted diseases in Germany in 2014.

Table 2: Frequency of diseases detected by screening 2014

Disease	Confirmed cases	Prevalence
Hypothyroidism	**213**	1: 3,356
Congenital adrenal hyperplasia (CAH)	**45**	1: 15,887
Biotinidase deficiency (incl. partial defect)	**27**	1: 26,479
Galactosaemia (classic)	**8**	1: 89,366
Phenylketonuria (PKU) n=66 / Hyperphenylalaninemia (HPA) n=66		
Cofactor-Deficiency n=1	**137**	1: 5,218
Maple syrup urine disease (MSUD)	**0**	
Medium-Chain-Acyl-CoA-Dehydrogenase (MCAD)-Deficiency	**65**	1: 10,999
Long-Chain-3-OH-Acyl-CoA-Dehydrogenase (LCHAD)-Deficiency	**3**	1: 238,309
(Very-)Long-Chain-Acyl-CoA-Dehydrogenase (VLCAD)-Deficiency	**6**	1: 119,155
Carnitine-Palmitoyl-CoA-Transferase I (CPTI)-Deficiency	**0**	
Carnitine-Palmitoyl-CoA-Transferase II (CPTII)-Deficiency	**0**	
Carnitine-Acylcarnitine-Translocase (CACT)-Deficiency	**0**	
Glutaric aciduria Type I (GA I)	**6**	1: 119,155
Isovalerianacidaemia (IVA)	**9**	1: 79,436
Total	**519**	**1: 1,378**

2.1 Primary screening totals

According to the ""Children's Guidelines", a screening sample should be taken from every newborn before leaving the birth facility. For reliable screening results, blood samples must be collected after the end of 32nd week of gestation (WoG) and 36th hour of life. If the first screening occurs before the 36th hour of life or before the completed 32nd gestational week, it should be followed by a repeat screening. The following table shows the results of the primary screening stratified by age and gestational age.

Table 2.1: Age at primary screening

		≥36h and ≥32WoG		<36h and ≥32WoG		<32WoG	
Lab	Total	n	%	n	%	n	%
1	56065	54619	97.42	783	1.40	663	1.18
3	15911	15591	97.99	142	0.89	178	1.12
5	54074	52728	97.51	646	1.19	700	1.29
6	12898	12449	96.52	292	2.26	157	1.22
7	49772	48360	97.16	752	1.51	660	1.33
8	165561	162180	97.96	1436	0.87	1945	1.17
9	119373	116482	97.58	1303	1.09	1588	1.33
10	35957	35302	98.18	360	1.00	295	0.82
11	16858	16335	96.90	318	1.89	205	1.22
12	88611	86628	97.76	843	0.95	1140	1.29
13	67020	65418	97.61	814	1.21	788	1.18
14	25830	25335	98.08	277	1.07	218	0.84
15	8566	8282	96.68	92	1.07	192	2.24
Total	**716496**	**699709**	**97.66**	**8058**	**1.12**	**8729**	**1.22**

2.2 Ratio of requested to received repeat screenings

Table 2.2 lists the repeat screenings in total and split by reason, defined as follows:

- **„<32WoG":** all samples of newborns before 32 WoG, independent of age and primary screening result
- **„<36h":** all samples of newborns after 32 WoG, but less than 36 hours old, independent of the primary screening result
- **Recall:** essential repeat testing due to abnormal primary screening at a gestational age ≥ 32 WoG and age ≥ 36h

Table 2.2: Requested and received repeat screenings

Lab	Total requested [a]	Total received	%	Recall requested [a]	Recall received	%
1	1602	1478	92.26	182	173	95.05
3	430	430	100.00	126	126	100.00
5	1662	1650	99.28	314	311	99.04
6	533	524	98.31	94	94	100.00
7[b]	1889	0		496	n.s.	
8	4153	3903	93.98	822	811	98.66
9	3579	2994	83.65	703	652	92.75
10	996	972	97.59	341	341	100.00
11	561	551	98.22	44	44	100.00
12	2607	2587	99.23	671	667	99.40
13	2016	1934	95.93	367	363	98.91
14	608	605	99.51	119	119	100.00
15	364	289	79.40	78	78	100.00
Total	**21000**	**17917**	**93.75[b]**	**4357**	**3779**	**97.88[b]**

Lab	<36h requested [a]	<36h received	%	<32WoG requested [a]	<32WoG received	%
1	780	685	87.82	640	620	96.88
3	139	139	100.00	165	165	100.00
5	704	698	99.15	644	641	99.53
6	292	283	96.92	147	147	100.00
7[b]	747	n.s.		646	n.s.	
8	1435	1267	88.29	1896	1825	96.26
9	1297	841	64.84	1579	1501	95.06
10	360	341	94.72	295	290	98.31
11	318	308	96.86	199	199	100.00
12	839	823	98.09	1097	1097	100.00
13	861	797	92.57	788	774	98.22
14	275	272	98.91	214	214	100.00
15	94	33	35.11	192	178	92.71
Total	**8141**	**6487**	**87.73[b]**	**8502**	**7651**	**97.39[b]**

[a] Deaths are not included in the number of requested samples
[b] Calculation excludes laboratories with undifferentiated or implausible results

2.3 Ensuring completeness of the screening

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 born 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.

Currently neither option is available nationwide. With the goal of monitoring the integrity of the screening, the following regulation was added to the ""Children's Guidelines" ("Kinderrichtlinie"):

The obstetric unit should use a blank test card to document refusal to participate in the screening or death of a neonate. This test card should then be sent to the screening centre. The laboratories receive blank test cards in varying numbers. The number of the blank cards due to refusal relative to the total number of screening cards has remained approximately constant.

This system seems to work primarily in cases of refusal to either participate in the screening or to have blood samples taken before the 36th hour of life. In cases of death prior to screening or transfer of the newborn, higher numbers would be expected based on the data from the perinatal survey.

Table 2.3: Blank cards received by the laboratory

		Reason for blank card			
	Primary screening total	Deceased	Screening declined	Blank cards to due transfer, refusal to provide blood sample < 36h and undetermined reasons	Total
Lab	n	n	n	n	n
1	56065	66	34	4222	4322
3	15911	74	36	1980	2090
5	54074	22	115	3037	3174
6	12898	42	15	292	349
7[b]	49772	n.s.	n.s.	n.s..	n.s.
8	165561	n.s.	n.s..	2198[a]	2198
9	119373	3	154	655	812
10	35957	155	44	1940	2139
11	16858	59	12	284	355
12	88611	n.s.	n.s.	1740[a]	1740
13[b]	67020	n.s.	n.s.	n.s..	n.s..
14	25830	n.s.	n.s.	128[a]	128
15[b]	8566	n.s.	n.s.	n.s.	n.s..
Total	**716496**	**421**	**410**	**16476**	**17307**

[a] Total number, differentiation not possible
[b] Lab does not track blank cards

Table 2.4: Secondary screening card due to inferior sample quality

Lab	Primary screening	Control requested	Control received	received/ requested (%)	Proportion of/ Primary screening (%)
1	56065	470	439	93.40	0.84
3	15911	37	37	100.00	0.23
5	54074	433	430	99.31	0.80
6	12898	2	2	100.00	0.02
7	49772	86	k.A.		0.17
8	165561	501	475	94.81	0.30
9	119373	494	443	89.68	0.41
10	35957	123	122	99.19	0.34
11	16858	5	5	100.00	0.03
12	88611	583	568	97.43	0.66
13	67020	519	k.A.		0.77
14	25830	30	29	96.67	0.12
15	8566	15	15	100.00	0.18
Total	**716496**	**3298**	**2565**	**95.25***	**0.46**

* Calculation without laboratories 7 and 13 due to insufficient data regarding cards with poor sample quality

3 Quality parameters of the screening analysis

The quality of a test procedure is measured by the sensitivity, the specificity and the positive predictive value (PPV). In a screening procedure, the sensitivity (correct positive test results), but especially the specificity (proportion of healthy persons with negative test results), 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 lower the rate of control screening (recall rate) necessitated by suspicious first screening results, the higher the specificity. In 2014 the recall rate was 0.64%. If we consider only screening samples from full-term newborns collected more than 36 hours after birth, the recall rate is 0.46%, which means that out of 1000 tests only approximately 5 results require a control examination. With samples taken less than 36 hours after birth or before the 32nd WoG a secondary screening has to be done irrespective of the results.

The total specificity was 99.46%. The sensitivity cannot be stated because the number of the number of false negative neonates is not systematically recorded. Here, nationwide registers would be helpful.

Table 3 : Recall rates and cases found for Germany 2014, n= 716,496*

	Recall ≥36h and ≥32WoG		Recall <36h		Recall <32WoG		Recall Total	Confirmed cases
Disease	n	(%)	n	(%)	n	(%)	(%)	n
Hypothyroidism	544	0.08	285	5.02	39	0.45	0.14	213
CAH	1546	0.22	264	5.43	467	5.35	0.32	45
Biotinidase-Deficiency	195	0.03	12	0.15	13	0.15	0.03	27
Classic galactosaemia	287	0.04	2	0.02	12	0.14	0.04	8
PKU/HPA	193	0.03	17	0.21	25	0.29	0.03	137
MSUD	36	0.01	2	0.02	11	0.13	0.01	0
MCAD	124	0.02	2	0.02	2	0.02	0.02	65
LCHAD	37	0.01	0	0.00	1	0.01	0.01	3
VLCAD	142	0.02	1	0.01	0		0.02	6
CPT I-Deficiency	6	0.0009	3	0.04	2	0.02	0.002	0
CPT II-Deficiency	10	0.001	0	0.00	0		0.001	0
CACT-Deficiency	0		0	0.00	0			0
GA I	95	0.01	0	0.00	2	0.02	0.01	6
IVA	27	0.004	2	.02	18	0.21	0.01	9
Total	**3242**	**0.46**	**590**	**10.94**	**592**	**6.78**	**0.64**	**519**

* Primary screening Total: n= 716,496; Primary screening ≥ 36h and ≥ 32WoG n=669,709; Primary screening <36h n=8058; Primary screening <32WoG n=8729

3.1 Stratified recall rate and confirmed cases

The following tables show recall rates ≥36h and confirmed cases stratified by laboratory. The ≥36 hours category also always includes ≥32 weeks gestational age. The confirmed cases are based on all screening tests, irrespective of age and gestational age. The status of the report is Dec. 1st, 2015. Cases from birth year 2014 that were found at a later date are not included in this report. The plausibility check of cases reported as confirmed for metabolic diseases were performed by Professor Andreas Schulze and Dr. Regina Ensenauer, and for endocrine diseases by Dr. Oliver Blankenstein and PD Dr. Heiko Krude.

Excluded, and therefore not reported, are cases with missing confirmation diagnostics data (n=21) (table 3.1) and cases where the confirmation diagnostics could not be verified (n=3). As a result, the true prevalence of some diseases may be higher than reported here. Cases reported twice were counted only once. Feedback from the attending physicians regarding the confirmation diagnostics is sought for quality assurance of laboratory analysis and evaluation of the quality of the results. The DGNS provides appropriate forms and consent.

Table 3.1 : Cases that could not be confirmed due to missing information

Disease	Data missing n
Hypothyroidism	8
CAH	1
Biotinidase	2
PKU	4
MCAD	3
VLCAD	2
IVA	1
Total	**21**

In the following tables, recall rates <0.01% and very small n are not calculated, because for smaller numbers, random fluctuations would have a disproportionately large effect.

3.1.1 Hypothyroidism

Lab	Primary screening total	Primary screening ≥36h	Recall ≥36h	Recall rate(%)*	Confirmed cases
1	56065	54619	27	0.05	12
3	15911	15591	10	0.06	5
5	54074	52728	66	0.13	15
6	12898	12449	9	0.07	4
7	49772	48360	43	0.09	3
8	165561	162180	165	0.10	57
9	119373	116482	92	0.08	41
10	35957	35302	31	0.09	14
11	16858	16335	5		3
12	88611	86628	36	0.04	27
13	67020	65418	28	0.04	21
14	25830	25335	25	0.10	9
15	8566	8282	7	0.08	2
Total	**716496**	**699709**	**544**	**0.08**	**213**

* Recall rate recorded only if rate ≥ 0.01% and n > 5.

In addition, n=30 hyperthyrotropinaemia were reported and confirmed. These are not included in the calculation of prevalence.

3.1.2 Congenital adrenal hyperplasia (CAH)

Lab	Primary screening total	Primary screening ≥36h	Recall ≥36h	Recall rate(%)	Confirmed cases
1	56065	54619	5	0.01[a]	5
3	15911	15591	3	0.02	1
5	54074	52728	207	0.39	3
6	12898	12449	42	0.34	0
7	49772	48360	317	0.66	0
8	165561	162180	55	0.03[b]	12
9	119373	116482	374	0.32	9
10	35957	35302	121	0.34	4
11	16858	16335	27	0.17	2
12	88611	86628	241	0.28	4
13	67020	65418	92	0.14	4
14	25830	25335	37	0.15	0
15	8566	8282	25	0.30	1
Total	**716496**	**699709**	**1546**	**0.22**	**45**

[a] Lab used 2nd tier method [b] Lab used 2nd tier method for screening >36h and <32 WoG

Additionally, n=3 cases of congenital adrenal hyperplasia syndrome were reported that did not require treatment and were not included in the prevalence figures.

3.1.3 Biotinidase deficiency

Lab	Primary screening total	Primary screening ≥36h	Recall ≥36h	Recall rate(%)*	Confirmed cases	of which complete defect / no differentiation
1	56065	54619	11	0.02	2	2
3	15911	15591	1		1	1
5	54074	52728	7	0.01	1	1
6	12898	12449	13	0.10	0	
7	49772	48360	16	0.03	5	0
8	165561	162180	91	0.06	10	4
9	119373	116482	4		3	3
10	35957	35302	2		1	0
11	16858	16335	2		0	
12	88611	86628	21	0.02	1	1
13	67020	65418	15	0.02	1	1
14	25830	25335	2		1	1
15	8566	8282	10	0.12	1	0
Total	**716496**	**699709**	**195**	**0.03**	**27**	**14**

* Recall rate recorded only if rate ≥ 0.01% and n > 5.

3.1.4 Galactosaemia

Lab	Primary screening total	Primary screening ≥36h	Recall ≥36h	Recall rate(%)*	Confirmed cases**	Of which classic
1	56065	54619	11	0.02	2	1
3	15911	15591	2		0	
5	54074	52728	23	0.04	4	
6	12898	12449	4		1	
7	49772	48360	13	0.03	3	1
8	165561	162180	35	0.02	17	4
9	119373	116482	45	0.04	1	1
10	35957	35302	7	0.02	3	
11	16858	16335	2		0	
12	88611	86628	72	0.08	4	
13	67020	65418	61	0.09	1	1
14	25830	25335	4		1	
15	8566	8282	8	0.10	1	
Total	**716496**	**699709**	**287**	**0.04**	**38**	**8**

* Recall rate recorded only if ≥ 0.01% and n > 5

** Variations are not comprehensively recorded

3.1.5 Phenylketonuria (PKU) / Hyperphenylalaninemia (HPA)

Lab	Primary screening total	Primary screening ≥36h	Recall ≥36h	Recall rate(%)*	Confirmed cases	Including PKU
1	56065	54619	17	0.03	7	3
3	15911	15591	4		2	2
5	54074	52728	11	0.02	10	6
6	12898	12449	4		2	0
7	49772	48360	36	0.07	15	7
8	165561	162180	33	0.02	29	17
9	119373	116482	21	0.02	16	11
10	35957	35302	8	0.02	8	4
11	16858	16335	4		4	1
12	88611	86628	26	0.03	24	8
13	67020	65418	17	0.03	11	7
14	25830	25335	8	0.03	8	4
15	8566	8282	4		1	0
Total	**716496**	**699709**	**193**	**0.03**	**137**	70

* Recall rate recorded only if ≥ 0.01% and n > 5.

3.1.6 Maple Syrup Urine Disease (MSUD) – no cases reported

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

Lab	Primary screening total	Primary screening ≥36h	Recall ≥36h	Recall rate(%)*	Confirmed cases
1	56065	54619	8	0.01	5
3	15911	15591	4		3
5	54074	52728	5		5
6	12898	12449	7	0.06	1
7	49772	48360	6	0.01	3
8	165561	162180	15	0.01	13
9	119373	116482	24	0.02	14
10	35957	35302	38	0.11	8
11	16858	16335	4		3
12	88611	86628	3		3
13	67020	65418	6	0.01	6
14	25830	25335	4		1
15	8566	8282	0		0
Total	**716496**	**699709**	**124**	**0.02**	**65**

* Recall rate recorded only if rate ≥ 0.01% and n > 5.

3.1.8 Long-Chain-3-OH-Acyl-CoA-Dehydrogenase (LCHAD)-Deficiency

Lab	Primary screening total	Primary screening ≥36h	Recall ≥36h	Recall rate(%)*	Confirmed cases
1	56065	54619	17	0.03	0
3	15911	15591	0		0
5	54074	52728	3		1
6	12898	12449	0		0
7	49772	48360	0		0
8	165561	162180	2		1
9	119373	116482	8	0.01	1
10	35957	35302	1		0
11	16858	16335	1		0
12	88611	86628	2		0
13	67020	65418	3		0
14	25830	25335	0		0
15	8566	8282	0		0
Total	**716496**	**699709**	**37**	**0.01**	**3**

* Recall rate recorded only if rate ≥ 0.01% and n > 5.

3.1.9 (Very-)Long-Chain-Acyl-CoA-Dehydrogenase (VLCAD)-Deficiency

Lab	Primary screening total	Primary screening ≥36h	Recall ≥36h	Recall rate(%)*	Confirmed cases
1	56065	54619	20	0.04	0
3	15911	15591	3		1
5	54074	52728	2		0
6	12898	12449	8	0.06	0
7	49772	48360	29	0.06	1
8	165561	162180	4		2
9	119373	116482	50	0.04	1
10	35957	35302	20	0.06	0
11	16858	16335	0		0
12	88611	86628	0		0
13	67020	65418	3		1
14	25830	25335	1		0
15	8566	8282	2		0
Total	**716496**	**699709**	**142**	**0.02**	**6**

* Recall rate recorded only if rate ≥ 0.01% and n > 5.

3.1.10 CPT I-Deficiency, CPT II-Deficiency and CACT-Deficiency - no confirmed cases reported.

3.1.11 Glutaric aciduria Type I

Lab	Primary screening total	Primary screening ≥36h	Recall ≥36h	Recall rate(%)*	Confirmed cases
1	56065	54619	8	0.01	0
3	15911	15591	0		0
5	54074	52728	2		1
6	12898	12449	2		0
7	49772	48360	22	0.05	0
8	165561	162180	2		2
9	119373	116482	54	0.05	2
10	35957	35302	1		0
11	16858	16335	0		0
12	88611	86628	2		1
13	67020	65418	2		0
14	25830	25335	0		0
15	8566	8282	0		0
Total	**716496**	**699709**	**95**	**0.01**	**6**

* Recall rate recorded only if ≥ 0.01% and n > 5.

3.1.12 Isovalerianacidaemia (IVA)

Lab	Primary screening total	Primary screening ≥36h	Recall ≥36h	Recall rate(%)*	Confirmed cases
1	56065	54619	4		0
3	15911	15591	0		0
5	54074	52728	0		0
6	12898	12449	2		0
7	49772	48360	8	0.01	0
8	165561	162180	1		2
9	119373	116482	4		5[b]
10	35957	35302	2		0
11	16858	16335	2		0
12	88611	86628	3		1
13	67020	65418	1		1
14	25830	25335	0		0
15	8566	8282	0		0
Total	**716496**	**699709**	**27**		**9**

* Recall rate recorded only if ≥ 0.01% and n > 5.7

[b] n=1 Blood sample <36h, therefore not included in recall

3.2 Recall rate stratified according to time of primary screening

The number of positives, especially false positive screening results and therefore the recall rate is dependent on age and gestational age. Testing less than the 36 hours after birth and at gestational age of <32 weeks increases the risk of false negative and false positive results. This differs for the various targeted diseases. Therefore, in the following tables we stratify the recall rates by gestational age and timing of the sampling. Recall rate is recorded only if it exceeds 0.01% and n > 5 because for smaller numbers, random fluctuations would have a disproportionately large effect.

3.2.1 Hypothyroidism

	Primary Screening ≥ 36h			Primary Screening < 36h			Primary Screening < 32SSW		
Lab	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	27	0.05	783	12	1.53	663	2	0.30
3	15591	10	0.06	142	n.s.[a]		178	n.s.[c]	
5	52728	66	0.13	646	n.s.[a]		700	n.s.[c]	
6	12449	9	0.07	292	n.s.[a]		157	n.s.[c]	
7	48360	43	0.09	752	34	4.52	660	0	
8	162180	165	0.10	1436	116	8.08	1945	9	0.46
9	116482	92	0.08	1303	n.s. [a]		1588	7	0.44
10	35302	31	0.09	360	38	10.56	295	2	0.68
11	16335	5	0.03	318	39	12.26	205	0	
12	86628	36	0.04	843	30	3.56	1140	17	1.49
13	65418	28	0.04	814	3	0.37	788	1	0.13
14	25335	25	0.10	277	11	3.97	218	1	0.46
15	8282	7	0.08	92	2	2.17	192	0	
Total	**699709**	**544**	**0.08**	**8058**	**285**	**5.02**[b]	**8729**	**39**	**0.51**[d]

[a] For labs 3, 5, 6 und 9 no data available about the number of cases with a TSH level above the set cutoff. Elevated TSH levels are monitored here during the second screening after 36 hours.

[b] Calculations exclude Labs 3, 5, 6, 9.

[c] For labs 3, 5, 6 und 9 no data available about the number of cases with a TSH level above the set cutoff. Elevated TSH levels are monitored during the second screening after the end of the 32nd WoG.

[d] Calculations exclude Labs 3, 5, 6.

3.2.2 Congenital adrenal hyperplasia (CAH)

	Primary screening ≥ 36h			Primary screening < 36h			Primary screening < 32WoG		
Lab	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1[a]	54619	5		783	4	0.51	663	6	.90
3	15591	3	0.02	142	n.s.[c]		178	n.s.[d]	
5	52728	207	0.39	646	n.s.[c]		700	23	3.29
6	12449	42	0.34	292	n.s.[c]		157	3	1.91
7	48360	317	0.66	752	48	6.38	660	280	42.42
8[b]	162180	55	0.03	1436	93	6.48	1945	18	.93
9	116482	374	0.32	1303	n.s.[c]		1588	22	1.39
10	35302	121	0.34	360	16	4.44	295	31	10.51
11	16335	27	0.17	318	18	5.66	205	4	1.95
12	86628	241	0.28	843	77	9.13	1140	43	3.77
13	65418	92	0.14	814	n.s.[c]		788	12	1.52
14	25335	37	0.15	277	3	1.08	218	12	5.50
15	8282	25	0.30	92	5	5.43	192	13	6.77
Total	**699709**	**1546**	**0.22**	**8058**	**264**	**5.43**[e]	**8729**	**467**	**5.46**[f]

[a] Laboratory used second-tier process [b] Laboratory used second-tier process at screening >36h and <32 WoG

[c] For labs 3, 5, 6, 9 and 13 no data available about the number of cases with 17OHP levels above the set cutoff. Elevated 17OHP levels are monitored here during the second screening after 36 hours.

[d] For lab 3 no data available about the number of cases with 17OHP levels above the set cutoff. Elevated 17OHP levels are monitored here during the second screening after 32 WoG

[e] Calculations exclude Labs 3, 5, 6, 9, 13

[f] Calculations exclude Lab 3

3.2.3 Biotinidase deficiency (incl. partial defects)

	Primary screening ≥ 36h			Primary screening < 36h			Primary screening < 32WoG		
Lab	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	11	0.02	783	1		663	0	
3	15591	1		142	0		178	0	
5	52728	7	0.01	646	0		700	1	
6	12449	13	0.10	292	1		157	0	
7	48360	16	0.03	752	2		660	2	
8	162180	91	0.06	1436	5		1945	3	
9	116482	4		1303	0		1588	0	
10	35302	2		360	0		295	1	
11	16335	2		318	0		205	1	
12	86628	21	0.02	843	3		1140	2	
13	65418	15	0.02	814	0		788	3	
14	25.335	2		277	0		218	0	
15	8282	10	0.12	92	0		192	0	
Total	**699709**	**195**	**0.03**	**8058**	**12**	**0.15**	**8729**	**13**	**0.15**

3.2.4 Galactosaemia

	Primary screening ≥ 36h			Primary screening < 36h			Primary screening < 32WoG		
Lab	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	11	0.02	783	0		663	0	
3	15591	2		142	0		178	0	
5	52728	23	0.04	646	0		700	0	
6	12449	4		292	0		157	0	
7	48360	13	0.03	752	0		660	1	
8	162180	35	0.02	1436	0		1945	1	
9	116482	45	0.04	1303	0		1588	0	
10	35302	7	0.02	360	0		295	1	
11	16335	2		318	0		205	1	
12	86628	72	0.08	843	1		1140	5	0.44
13	65418	61	0.09	814	1		788	2	
14	25.335	4		277	0		218	1	
15	8282	8	0.10	92	0		192	0	
Total	**699709**	**287**	**0.04**	**8058**	**2**		**8729**	**12**	**0.14**

3.2.5 Phenylketonuria (PKU) / Hyperphenylalaninemia (HPA)

	Primary screening ≥ 36h			Primary screening < 36h			Primary screening < 32WoG		
Lab	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	17	0.03	783	2		663	1	
3	15591	4		142	0		178	0	
5	52728	11	0.02	646	0		700	0	
6	12449	4		292	0		157	0	
7	48360	36	0.07	752	7	0.93	660	19	2.88
8	162180	33	0.02	1436	0		1,945	0	
9	116482	21	0.02	1303	0		1588	1	
10	35302	8	0.02	360	3		295	0	
11	16335	4		318	0		205	0	
12	86628	26	0.03	843	3		1,140	1	
13	65418	17	0.03	814	1		788	1	
14	25.335	8	0.03	277	1		218	2	
15	8282	4		92	0		192	0	
Total	**699709**	**193**	**0.03**	**8058**	**17**	**0.21**	**8729**	**25**	**0.29**

3.2.6 Maple Syrup Urine Disease (MSUD)

	Primary screening ≥ 36h			Primary screening < 36h			Primary screening < 32WoG		
Lab	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	12	0.02	783	0		663	2	
3	15591	0		142	0		178	0	
5	52728	0		646	0		700	0	
6	12449	2		292	0		157	0	
7	48360	7	0.01	752	1		660	9	1.36
8	162180	0		1436	0		1945	0	
9	116482	5		1303	0		1588	0	
10	35302	7	0.02	360	1		295	0	
11	16335	2		318	0		205	0	
12	86628	1		843	0		1140	0	
13	65418	0		814	0		788	0	
14	25.335	0		277	0		218	0	
15	8282	0		92	0		192	0	
Total	**699709**	**36**	**0.01**	**8058**	**2**		**8729**	**11**	**0.13**

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

	Primary screening ≥ 36h			Primary screening < 36h			Primary screening < 32WoG		
Lab	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	8	0.01	783	0		663	0	
3	15591	4		142	0		178	0	
5	52728	5		646	0		700	0	
6	12449	7	0.06	292	0		157	0	
7	48360	6	0.01	752	0		660	1	
8	162180	15	0.01	1436	0		1945	0	
9	116482	24	0.02	1303	0		1588	0	
10	35302	38	0.11	360	2		295	1	
11	16335	4		318	0		205	0	
12	86628	3		843	0		1140	0	
13	65418	6	0.01	814	0		788	0	
14	25.335	4		277	0		218	0	
15	8282	0		92	0		192	0	
Total	**699709**	**124**	**0.02**	**8058**	**2**		**8729**	**2**	

3.2.8 Long-Chain-3-OH-Acyl-CoA-Dehydrogenase (LCHAD)-Deficiency

	Primary screening ≥ 36h			Primary screening < 36h			Primary screening < 32WoG		
Lab	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	17	0.03	783	0		663	0	
3	15591	0		142	0		178	0	
5	52728	3		646	0		700	0	
6	12449	0		292	0		157	0	
7	48360	0		752	0		660	0	
8	162180	2		1436	0		1945	0	
9	116482	8	0.01	1303	0		1588	1	
10	35302	1		360	0		295	0	
11	16335	1		318	0		205	0	
12	86628	2		843	0		1140	0	
13	65418	3		814	0		788	0	
14	25.335	0		277	0		218	0	
15	8282	0		92	0		192	0	
Total	**699709**	**37**	**0.01**	**8058**	**0**		**8729**	**1**	

3.2.9 (Very-)Long-Chain-Acyl-CoA-Dehydrogenase (VLCAD)-Deficiency

	Primary screening≥ 36h			Primary screening< 36h			Primary screening< 32SSW		
Lab	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	20	0.04	783	0		663	0	
3	15591	3		142	0		178	0	
5	52728	2		646	0		700	0	
6	12449	8	0.06	292	0		157	0	
7	48360	29	0.06	752	0		660	0	
8	162180	4		1436	0		1945	0	
9	116482	50	0.04	1303	1		1588	0	
10	35302	20	0.06	360	0		295	0	
11	16335	0		318	0		205	0	
12	86628	0		843	0		1140	0	
13	65418	3		814	0		788	0	
14	25.335	1		277	0		218	0	
15	8282	2		92	0		192	0	
Total	**699709**	**142**	**0.02**	**8058**	**1**	**0.01**	**8729**	**0**	

3.2.10 Carnitine-Palmitoyl-CoA-Transferase I (CPTI)-Deficiency

	Primary screening ≥ 36h			Primary screening < 36h			Primary screening < 32SSW		
Lab	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	5		783	0		663	1	
3	15591	0		142	0		178	0	
5	52728	0		646	0		700	0	
6	12449	1		292	0		157	0	
7	48360	0		752	0		660	0	
8	162180	0		1436	1		1945	1	
9	116482	0		1303	0		1588	0	
10	35302	0		360	2		295	0	
11	16335	0		318	0		205	0	
12	86628	0		843	0		1140	0	
13	65418	0		814	0		788	0	
14	25335	0		277	0		218	0	
15	8282	0		92	0		192	0	
Total	**699709**	**6**		**8058**	**3**		**8729**	**2**	

3.2.11 Carnitine-Palmitoyl-CoA-Transferase I (CPTI)-Deficiency or Carnitine-Acylcarnitine-Translocase (CACT)-Deficiency

	Primary screening ≥ 36h			Primary screening < 36h			Primary screening < 32WoG		
Lab	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	1		783	0		663	0	
3	15591	0		142	0		178	0	
5	52728	1		646	0		700	0	
6	12449	0		292	0		157	0	
7	48360	0		752	0		660	0	
8	162180	2		1436	0		1945	0	
9	116482	0		1303	0		1588	0	
10	35302	6	0.02	360	0		295	0	
11	16335	0		318	0		205	0	
12	86628	0		843	0		1140	0	
13	65418	0		814	0		788	0	
14	25335	0		277	0		218	0	
15	8282	0		92	0		192	0	
Total	**699709**	**10**		**8058**	**0**		**8729**	**0**	

3.2.12 Glutaric aciduria Type I (GA I)

	Primary screening ≥ 36h			Primary screening < 36h			Primary screening < 32WoG		
Lab	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	8	0.01	783	0		663	0	
3	15591	0		142	0		178	0	
5	52728	2		646	0		700	0	
6	12449	2		292	0		157	0	
7	48360	22	0.05	752	0		660	1	
8	162180	2		1436	0		1945	0	
9	116482	54	0.05	1303	0		1588	1	
10	35302	1		360	0		295	0	
11	16335	0		318	0		205	0	
12	86628	2		843	0		1140	0	
13	65418	2		814	0		788	0	
14	25335	0		277	0		218	0	
15	8282	0		92	0		192	0	
Total	**699709**	**95**	**0.01**	**8058**	**0**		**8729**	**2**	

3.2.13 Isovalerianacidaemia (IVA)

Lab	Primary screening ≥ 36h			Primary screening < 36h			Primary screening < 32WoG		
	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	3		783	0		663	3	
3	15591	2		142	0		178	0	
5	52728	0		646	0		700	0	
6	12449	2		292	0		157	0	
7	48360	7	0.01	752	1		660	11	1.67
8	162180	4		1436	0		1945	0	
9	116482	4		1303	1		1588	0	
10	35302	1		360	0		295	3	
11	16335	0		318	0		205	0	
12	86628	2		843	0		1140	1	
13	65418	2		814	0		788	0	
14	25335	0		277	0		218	0	
15	8282	0		92	0		192	0	
Total	**699709**	**27**		**8058**	**2**		**8729**	**18**	**0,21**

3.2.14 Abnormal MS/MS results that could not conclusively be attributed to one of the target diseases

Labor	Primary screening ≥ 36h			Primary screening < 36h			Primary screening < 32SSW		
	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate	Primary screening	Recall	Recall rate
1	54619	2		783	3	0.38	663	0	
3	15591	0		142	0		178	0	
5	52728	0		646	0		700	0	
6	12449	0		292	0		157	0	
7	48360	0		752	0		660	0	
8	162180	323	0.20	1436	7	0.49	1945	112	5.76
9	116482	0		1303	0		1588	0	
10	35302	90	0.25	360	3		295	33	11.19
11	16335	0		318	0		205	0	
12	86628	74	0.09	843	3		1140	16	1.40
13	65418	19	0.03	814	1		788	3	
14	25335	4		277	0		218	5	2.29
15	8282	1		92	0		192	0	
Total	**699709**	**513**	**0.07**	**8058**	**17**	**0.21**	**8729**	**169**	**1.94**

4 Process Time

4.1 Age at blood sample collection

According to the “Children’s Guidelines” (section 8 subsection 1) blood samples should be collected between 36 and 72 hours after birth, ideally between 36 and 48 hours. In 92% of cases in which the time of blood sampling was provided, collection took place in the designated time frame, in 6.8% not on until after 72 hours and in 1.2% before 36 hours (table 4.1). The proportion of samples which were collected after 72 hours - i.e. outside the designated time frame - could be lowered from 22.3 % in 2005 to 6.8% in 2014 (Figure 2).

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

Table 4.1: Age at blood sample collection - primary screening

	Total	<36h		36h-<48h		48h-<72h		≥72h	
Lab	n	n	%	n	%	n	%	n	%
1[a]	55994	894	1.6	14487	25.87	35769	63.88	4844	8.65
3[a]	15906	175	1.1	3945	24.8	11190	70.35	596	3.75
5	54074	704	1.3	40775	75.41	11374	21.03	1221	2.26
6	12898	307	2.38	5387	41.77	6763	52.43	441	3.42
7	49772	840	1.69	18034	36.23	24316	48.85	6582	13.22
8[a]	165128	1634	0.99	67151	40.67	85460	51.75	10883	6.59
9[a]	119314	1426	1.20	47664	39.95	61975	51.94	8249	6.91
10	35957	389	1.08	10593	29.46	22249	61.88	2726	7.58
11	16858	335	1.99	5071	30.08	10431	61.88	1021	6.06
12[a]	85956	903	1.05	36340	42.28	43961	51.14	4752	5.53
13	67020	872	1.30	32029	47.79	28787	42.95	5332	7.96
14[a]	24750	287	1.16	10903	44.05	11971	48.37	1589	6.42
15	8566	94	1.10	4082	47.65	4114	48.03	276	3.22
Total	**712193**	**8860**	**1.24**	**296461**	**41,63**	**358360**	**50,32**	**48512**	**6.81**

Due to missing data, the number of samples for which times are known is in some cases below the total number of primary screening samples (indicated with [a]).

4.2 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 (section 6 subsection 3). In 24.5% of cases in which the delivery times were stated, the sample did not reach the lab until more than 72 hours after the blood sample was taken. In 23.2% of the cases, the time period was between 48 and 72 hours. Efforts should be made to shorten transport times, particularly on weekends (table 4.2).

Table 4.2: Period between sample collection and receipt by the lab

	Total	≤24h		>24h-48h		>48h-72h		>72h	
Lab	n	n	%	n	%	n	%	n	%
1[a]	55887	15110	27.04	19561	35	11133	19.92	10083	18.04
3[a]	15790	4504	28.52	7556	47.85	2816	17.83	914	5.79
5	54074	14110	26.09	11838	21.89	15513	28.69	12613	23.33
6	12898	1360	10.54	5475	42.45	3467	26.88	2596	20.13
7	49772	12179	24.47	12509	25.13	7554	15.18	17530	35.22
8[a]	165128	16452	9.96	55157	33.40	44304	26.83	49215	29.80
9	119373	1060	8.89	29943	25.08	30596	25.63	48226	40.40
10	35957	4966	13.81	13488	37.51	9934	27.63	7569	21.05
11	16858	2623	15.56	6969	41.34	4802	28.48	2464	14.62
12[a]	86821	28852	33.23	31068	35.78	16681	19.21	10220	11.77
13	67020	19794	29.53	23669	35.32	13918	20.77	9639	14.38
14[a]	25211	14051	55.73	6175	24.49	3148	12.49	1837	7.29
15	8566	1752	20.45	3280	38.29	1695	19.79	1839	21.47
Total	**713355**	**146361**	**20.52**	**226688**	**31.78**	**165561**	**23.21**	**174745**	**24.50**

Due to missing data, the number of samples for which times are known is in some cases below the total number of primary screening samples in the previous tables (indicated with [a]).

4.3 Period between receiving the sample and reporting the results

In 82.8% of cases, the results are reported within 24 hours. In cases with borderline results, the time in the lab can increase due to repeated testing (quality control). (Table 4.3, figure 4)

Table 4.3 Period between receiving the sample and reporting the results

	Total	≤24h		>24h-48h		>48h-72h		>72h	
Lab	n	n	%	n	%	n	%	n	%
1[a]	56021	31090	55.50	15665	27.96	5275	9.42	3991	7.12
3[a]	15907	14380	90.40	900	5.66	322	2.02	305	1.92
5	54074	40549	74.99	10669	19.73	2574	4.76	282	0.52
6	12898	12361	95.84	344	2.67	159	1.23	34	0.26
7	n.s.	n.s.		n.s.		n.s.		n.s.	
8	165561	159094	96.09	5509	3.33	818	0.49	140	0.08
9[a]	119297	112384	94.21	5909	4.95	679	0.57	325	0.27
10	35957	28498	79.26	6496	18.07	825	2.29	138	0.38
11	16858	11730	69.58	4430	26.28	600	3.56	98	0.58
12[a]	86900	63563	73.14	16795	19.33	6220	7.16	322	0.37
13	67020	49469	73.81	11643	17.37	5256	7.84	652	0.97
14[a]	25216	22712	90.07	2108	8.36	230	0.91	166	0.66
15	8566	3957	46.19	4511	52.66	88	1.03	10	0.12
Total	**664275**	**549787**	**82.76**	**84979**	**12.79**	**23046**	**3.47**	**6463**	**0.97**

In some cases, the number of samples is smaller than the total number of primary screening samples in the previous (marked with [a]).

Figure 2: Age at the time the blood sample was collected 2005 to 2014

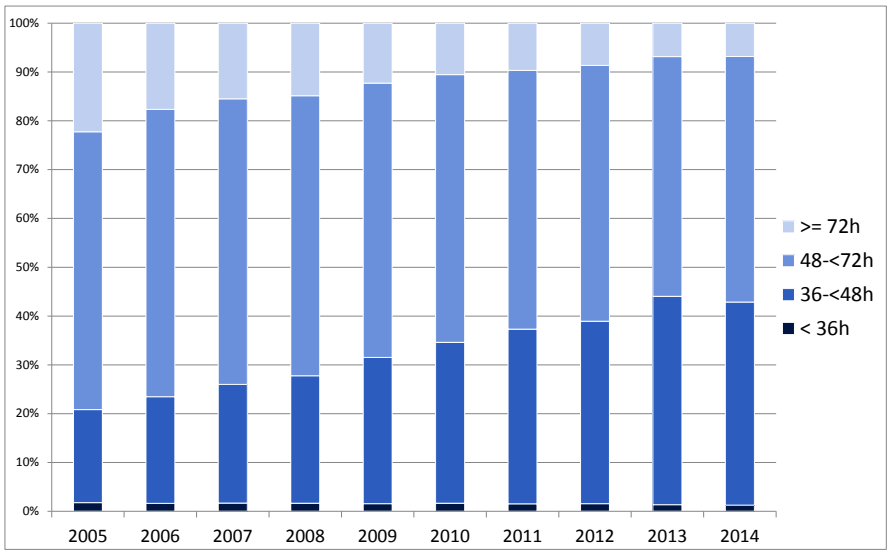


Figure 3: Time between sample collection and receipt by the lab 2005 to 2014

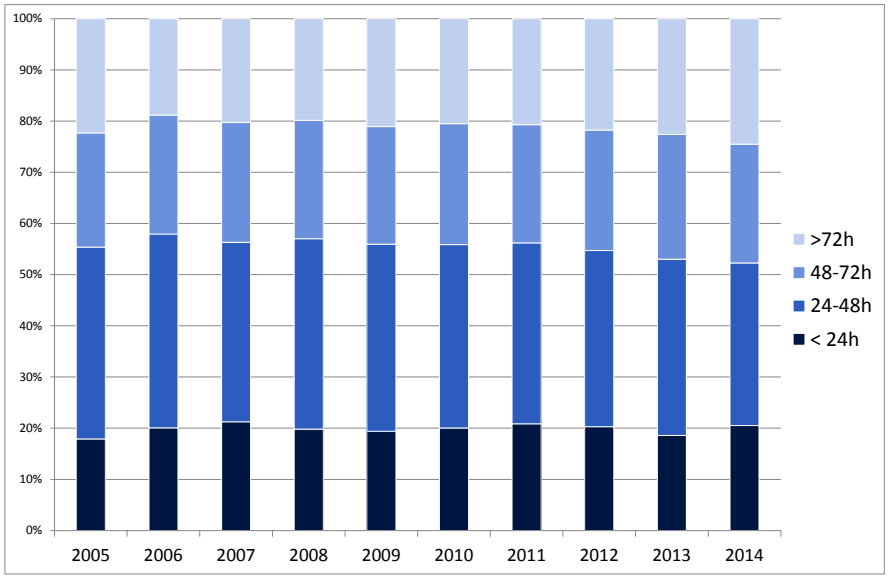
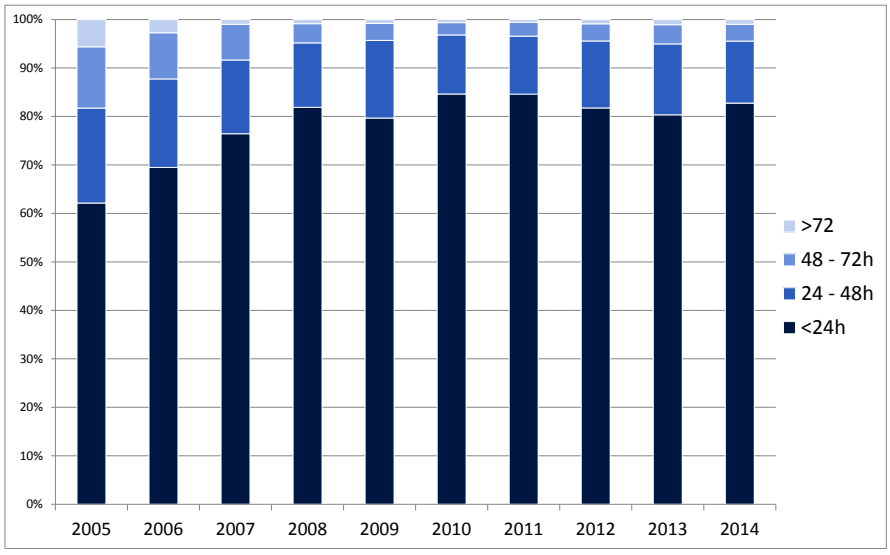


Figure 4: Time between receipt by the lab and report of the results 2005 to 2014



5 Time of screening in the confirmed cases

5.1 Primary screening

Decisive for the success of the screening are the reliability of the findings and the speed with which confirmation diagnostics are carried out and therapeutic measures are initiated in suspected cases. In accordance with the guidelines, the blood sample should not be collected less than 36 hours after birth or more than 72 hours after birth except in the case of early release. Any delay means a potential risk for affected children.

Table 5.1 shows the age at the time of first screening for children affected by one of the target diseases. For the sake of clarity, ages above 72 hours are reported in days; 5.2% of the affected children were already more than 72 hours old at the time the blood sample was taken.

Table 5.1 Time of primary screening in confirmed cases

Disease	36-72h	4-7d	>7d	<36h	<32WoG	≥36h, Time not specified [a]	Not specified [b]	Total
Hypothyroidism	168	11	1	2	29	1	1	**213**
CAH	32	4	0	6	1	1	1	**45**
Biotinidase	25	0	0	0	1	1	0	**27**
Galactosaemia	7	0	0	0	1	0	0	**8**
PKU/HPA	121	6	1	5	1	2	1	**137**
MCAD	61	2	1	1	0	0	0	**65**
LCHAD	3	0	0	0	0	0	0	**3**
VLCAD	6	0	0	0	0	0	0	**6**
GA I	5	0	0	0	0	1	0	**6**
IVA	7	1	0	1	0	0	0	**9**
Total	**435**	**24**	**3**	**15**	**33**	**6**	**3**	**519**

[a] ≥36h Not specified means no repeat testing due to premature blood sample collection or premature birth, but exact age at the time of blood sample collection was not specified.

[b] Not specified here means no information available about either gestational age or age at the time the blood sample was collected.

6 Confirmation of pathological final results

The following chapter describes the diagnostic measures taken to confirm the diagnosis, as far as they were known to the laboratories. This information is important for quality assurance in the laboratory but is not always communicated to the laboratories by the attending physicians. For 19 of the 519 confirmed cases in 2014, no detailed information is available on confirmatory diagnosis, but the information available makes it possible to assess the case as plausibly positive. There is so little information about another 21 children that the diagnosis cannot be considered confirmed. The latter have not been included in the evaluations.

6.1 Congenital hypothyroidism

Lab	Confirmed cases	TSH	T3	fT3	T4	fT4	Ultrasound	Thyroid antibodies
1	12	12	n.s.	1	n.s.	12	9	6
3	5	5	4	3	3	5	5	4
5	15	13	n.s.	11	1	13	10	13
6	4	4	n.s.	3	n.s.	4	2	4
7	3	2	n.s.	2	n.s.	2	1	2
8	57	56	1	44	2	55	46	39
9	41	38	8	29	8	38	11	18
10	14	14	n.s.	10	n.s.	13	10	8
11	3	3	n.s.	3	n.s.	2	2	n.s.
12	27	27	1	19	n.s.	26	18	15
13	21	21	n.s.	2	n.s.	21	1	n.s.
14	9	9	n.s.	3	n.s.	9	6	5
15	2	2	n.s.	2	n.s.	2	2	n.s.
Total	**213***	**206**	**14**	**132**	**14**	**202**	**123**	**114**

* incl n=2 cases without detailed information about confirmation diagnostics

6.2 Congenital adrenal hyperplasia (CAH)

Lab	Confirmed cases	17-OHP (Serum)	Steroid (Serum/TB)	Urinary steroids	Molecular genetic testing
1	5	4	4	n.s.	4
3	1	1	1	n.s.	n.s.
5	3	1	1	3	n.s.
8	12	5	11	2	11
9	9	8	3	1	5
10	4	4	3	n.s.	2
11	2	2	2	n.s.	1
12	4	4	3	2	2
13	4	4	2	2	1
15	1	n.s.	n.s.	n.s.	1
Total	**45**	**33**	**30**	**10**	**27**

6.3 Biotinidase deficiency

Lab	Confirmed cases	Biotinidase (Serum/TB)	Molecular genetic testing
1	2	2	n.s.
3	1	n.s.	n.s.
5	1	1	n.s.
7	5	5	n.s.
8	10	10	1
9	3	3	n.s.
10	1	1	n.s.
12	1	1	1
13	1	1	n.s.
14	1	1	1
15	1	n.s.	n.s.
Total	**27***	**25**	**3**

* incl n=2 cases without detailed information about confirmation diagnostics

6.4 Classic Galactosaemia

Lab	Confirmed cases	Enzyme assay	Galactose. Gal1P	Molecular genetic testing
1	1	1	1	n.s.
7	1	n.s.	n.s.	n.s.
8	4	2	2	2
9	1	1	1	n.s.
13	1	1	n.s.	n.s.
Total	**8**	**5**	**4**	**2**

6.5 Phenylketonuria (PKU) / Hyperphenylalaninaemia (HPA)

Lab	Confirmed cases	Phe (Serum/TB)	Phe/Tyr	BH4-Test	Molecular genetic testing	Pterine im Urine/TB	DHPR in dried blood
1	7	7	3	3	1	n.s.	7
3	2	2	2	2	n.s.	n.s.	2
5	10	8	8	2	2	n.s.	7
6	2	2	2	n.s.	n.s.	n.s.	2
7	15	n.s.	n.s.	n.s.	n.s.	10	10
8	29	24	10	10	3	5	17
9	16	13	13	n.s.	n.s.	5	16
10	8	8	6	1	1	5	8
11	4	4	n.s.	1	n.s.	n.s.	n.s.
12	24	22	14	6	6	12	22
13	11	11	6	1	n.s.	n.s.	7
14	8	7	6	4	1	5	8
15	1	n.s.	n.s.	1	n.s.	n.s.	n.s.
Total	**137***	**108**	**70**	**31**	**14**	**42**	**106**

* incl n=9 cases without detailed information about confirmation diagnostics

6.6 Maple syrup urine disease (MSUD)

No cases of maple syrup urine disease (MSUD) were reported.

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

Lab	Confirmed cases	Confirmation Serum/TB	Urinary organic acids	Enzyme activity	Molecular genetic testing
1	5	5	5	5	4
3	3	1	1	n.s.	n.s.
5	5	n.s.	1	n.s.	5
6	1	n.s.	n.s.	n.s.	1
7	3	1	1	1	2
8	13	10	10	2	10
9	14	12	11	5	5
10	8	8	8	6	7
11	3	3	3	n.s.	3
12	3	2	1	n.s.	3
13	6	2	n.s.	1	4
14	1	n.s.	n.s.	n.s.	1
Total	**65***	**44**	**41**	**20**	**45**

* incl n=5 cases without detailed information about confirmation diagnostics

6.8 Long-Chain-3-OH-Acyl-CoA-Dehydrogenase (LCHAD)-Deficiency

Lab	Confirmed cases	Confirmation Serum/TB	Urinary organic acids	Enzyme activity	Molecular genetic testing
5	1	n.s.	1	n.s.	1
8	1	1	n.s.	n.s.	1
9	1	1	1	n.s.	1
Total	**3**	**2**	**2**	**n.s.**	**3**

6.9 (Very-)Long-Chain-Acyl-CoA-Dehydrogenase (VLCAD)-Deficiency

Lab	Confirmed cases	Confirmation Serum/TB	Urinary organic acids	Enzyme activity	Molecular genetic testing
3	1	n.s.	1	1	n.s.
7	1	n.s.	n.s.	n.s.	1
8	2	2	1	1	1
9	1	n.s.	1	1	1
13	1	1	n.s.	1	n.s.
Total	**6**	**3**	**3**	**4**	**3**

6.10 CPT I-Deficiency. CPT II-Deficiency and CACT-Deficiency

No cases of CPT I-Deficiency. CPT II-Deficiency and CACT-Deficiency reported

6.11 Glutaric aciduria Type I (GA I)

Lab	Confirmed cases	Confirmation Serum/TB	Urinary organic acids	Enzyme activity	Molecular genetic testing
5	1	n.s.	1	n.s.	n.s.
8	2	n.s.	2	n.s.	2
9	2	2	1	n.s.	2
12	1	1	1	n.s.	n.s.
Total	**6**	**3**	**5**	**n.s.**	**4**

6.12 Isovalerianacidaemia (IVA)

Lab	Confirmed cases	Confirmation Serum	Urinary organic acids	Enzyme activity	Molecular genetic testing
8	2	2	2	k.A.	1
9	5	3	5	k.A.	1
12	1	k.A.	1	k.A.	k.A.
13	1	k.A.	k.A.	k.A.	k.A.
Total	**9***	**5**	**8**	**k.A.**	**2**

* incl n=1 cases without detailed information about confirmation diagnostics

7 Methods and Cutoffs used in Screening

7.1 Filter paper for sampling

Lab	Filter paper
1	ID Biological (Ahlstrom 226)
3	ID Biological (Ahlstrom 226)
5	TFN (Munktell)
6	PerkinElmer226
7	WS 903
8	TFN (Munktell)
9	WS 903
10	WS 903
11	ID Biological (Ahlstrom 226)
12	Munktell
13	Munktell
14	ID Biological (Ahlstrom 226)
15	ID Biological (Ahlstrom 226)

7.2 Hypothyrodism

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	AutoDELFIA
8	TSH	15 mU/l	DELFIA
9	TSH	15 mU/l	AutoDELFIA
10	TSH	15 mU/l	AutoDELFIA
11	TSH	15 mU/l	DELFIA
12	TSH	20 mU/l (<4 Days) 15 mU/l (<7 Days) <10 mU/l (≥7 Days)	AutoDELFIA
13	TSH	20 mU/l (<4 Days) 15 mU/l (<7 Days) <10 mU/l (≥7 Days)	AutoDELFIA
14	TSH	15 mU/l	AutoDELFIA
15	TSH	15 mU/l	AutoDELFIA

7.3 Congenital adrenal hyperplasia (CAH)

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

* Laboratory used 2nd tier process

7.4 Biotinidase deficiency

Lab	Parameter	Cutoff	Method
1	Biotinidase	30% panel mean	Colorimetrie qualitative
3	Biotinidase	30% daily median	Colorimetrie qualitative
5	Biotinidase	% of panel mean	Colorimetrie quantitative
6	Biotinidase	60 U	Flurometrie (PE)
7	Biotinidase	2.7 U/g Hb	Colorimetrie quantitative
8	Biotinidase	<30% Daily mean	Colorimetrie quantitative
9	Biotinidase	0.2	Colorimetrie qualitative
10	Biotinidase	< 30%	Colorimetrie qualitative
11	Biotinidase	n.s.	Colorimetrie qualitative
12	Biotinidase	< 30%	Fluorometrie quantitative
13	Biotinidase	< 30%	Fluorometrie quantitative
14	Biotinidase	< 30%	Colorimetrie quantitative
15	Biotinidase	< 30%	Colorimetrie quantitative

7.5 Galactosaemia

Lab	Parameter	Cutoff	Method
1	GALT	3.5 U/g Hb	Fluorometrie(PE)
	Galactose	20 mg/dl	BIORAD Quantase
3	GALT	2.3 U/g Hb	BIORAD Quantase
	Galactose	15 mg/dl	
5	GALT	3.5 U/g Hb	BIORAD Quantase
	Galactose	15 mg/dl	BIORAD Quantase
6	GALT	3.5 U/g Hb	Fluorometrie (PE)
7	GALT	3.5 U/g Hb	Fluorometrie quantitative
8	GALT	<20% daily mean	Fluorimetrie quantitative
	Galactose	30 mg/dl	Colorimetrie non kit
9	GALT	<2.3 U/g Hb	BIORAD Quantase
	Galactose	20 mg/dl	BIORAD Quantase
10	GALT	1 Jan to 22 Jul 2014: 2.3 U/g Hb	BIORAD Quantase
	GALT	23 Jul to 1 Oct 2014: 4.5 U/gHb	BIORAD Quantase
	GALT	from 2 Oct 2014: 3.5 U/gHb	BIORAD Quantase
	Galactose	1111 µmol/l	BIORAD Quantase
11	GALT	3.5 U/g Hb	Fluorometrie quantitative
12	GALT	<30%	Fluoro. quant.(non kit)
	Galactose	15 mg/dl	Colorimetrie non Kit
13	GALT	<30%	Fluoro. quant.(non kit)
	Galactose	15 mg/dl	Colorimetrie non Kit
14	GALT	<3.5 U/g Hb	BIORAD Quantase
	Galactose	>15 mg/dl	BIORAD Quantase
15	GALT	<3.5 U/g Hb	BIORAD Quantase
	Galactose	>15 mg/dl	BIORAD Quantase

7.6 MS/MS

Lab	Method
1	non derivatized non Kit
3	non derivatized Chromsystems
5	derivatized non Kit
6	non derivatized PE Kit
7	derivatized PE Kit
8	derivatized non Kit
9	derivatized non Kit
10	derivatized non Kit /from 7 May 2014 derivatized CS Kit
11	non derivatized non Kit
12	derivatized non Kit
13	derivatized non Kit
14	derivatized non Kit
15	derivatized non Kit

Literature

1) Decision on an amendment to the guidelines of the Federal Committee of Physicians and Health Insurance Companies on the early detection of diseases in children up to the age of 6 ("Children's Guidelines") for the introduction of the extended newborn screening from 16 Dec 2010; Https://www.g-ba.de/downloads/62-492-506/RL_Kinder_2010-12-16.pdf (Access on 1 May 2016)

2) Statistical Yearbook 2014. Publisher: Federal Statistical Office, Wiesbaden www.destatis.de