NEWBORN SCREENING **ONTARIO**
DÉPISTAGE NÉONATAL **ONTARIO**



Annual Report to the Newborn Screening Ontario Advisory Council Calendar Year 2025

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Executive Summary

Ontario continues to see increasing birth rates, with an estimated number of births surpassing 150,000 for the first time ever in 2025. Like all cases of inflation, these increases in birth rates do have an impact throughout the system. NSO has adapted over time to build in efficiencies and streamline processes to minimize the resource implications of higher birth rates.

Perhaps in small part due to the larger population, we have seen a jump in declined screens over previous years. While the total number of declines is still a very small percentage (0.27%) of the population, we have seen a 46% increase in declines in 2025 (408 in 2025 vs. 279 in 2024). This is a trend that needs attention as NSO continues to build a more prominent public image, and projects on genomic sequencing are beginning.

In spite of the larger population, NSO continued to reduce the number of screen positive referrals again this year, with a drastic decline in CAH referrals due to the implementation of a new algorithm. After a detailed validation, the addition of 21-deoxycortisol into the screening algorithm occurred on January 7, 2025, reducing the number of CAH referrals by 92%, with no false positives or reported false negatives.

Another significant work effort for the lab this year was the move to a non-butylated method of screening for many of the metabolic disorders, which went live in September 2025. The change was made to facilitate screening for X-linked adrenoleukodystrophy (XALD) and ASL deficiency but had a wide impact across many disorders that are screened via this multiplexed assay. Careful planning and monitoring have occurred to identify impacts and adjust algorithms as necessary.

The pilot for XALD began on March 5, 2025, using a 3-tier algorithm that has allowed NSO to trial a multi-site approach with Hamilton Health Sciences Centre. The initial pilot involved a random sample of the population and then moved to a full population pilot with the above-mentioned change in method on September 29, 2025. The disease will be officially added to the NSO panel in early 2026.

The quality improvement project evaluating the use of dried saliva samples for the screening of congenital Cytomegalovirus (cCMV) wrapped up in May of 2025. The goals of this project were to define the birth prevalence of cCMV in Ontario using saliva, determine the clinical sensitivity of cCMV PCR testing of blood versus saliva, and examine cost effectiveness. Challenges were evident with the feasibility of this initiative, and due to the high number of false positive screening results and the minimal additional diagnostic benefit, it was determined in September 2025 that dried saliva samples would not be adopted as a standard of care for infants in Ontario. Work continues on the evaluation of the project and future publications.

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The point of care and home-based screening programs for Critical Congenital Heart Disease (CCHD) and Biliary Atresia (BA), respectively, are both showing trends of reduced primary target identification. CCHD has seen fewer primary targets being picked up over time, but an increase in the identification of secondary targets. There is evidence that better prenatal diagnosis of CCHDs could be impacting the post-natal pickup, and that certain specific primary targets are more commonly missed by pulse oximetry screening. A new algorithm is being introduced in some international programs that is being evaluated to determine the impact on Ontario's population.

As Biliary Atresia screening is in its third year of operations, a large evaluation was done involving a survey of parents and health care providers (HCPs) to measure the reach and effectiveness of BA screening program. The results were very positive and showed good coverage, awareness and appreciation of the program. However, in opposition of these findings, the BA program has seen a decrease in calls and referrals, and no BA cases identified in 2025. Ten cases of BA diagnosed outside of NSO's program have been reported, and although the families did not contact NSO, there is evidence that most had and were using the card, and in some cases the education provided may have led to shorter timeframes to identification in community.

The survey findings also demonstrated strong public trust in NSO and widespread support for newborn screening as a cornerstone of early infant care. Parents value the screening process and the guidance provided by the NSO screening team. HCPs who interacted directly with NSO reported positive experiences marked by reliability, clarity, and responsiveness.

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1. SCREENING CHARACTERISTICS

In 2025, NSO estimated the birth rate to be 151,343, based on NBS data and data from BORN.

Table 1. The coverage of screening in Ontario for 2025.

Infants Screened	DBS Panel	CCHD	Hearing Panel
Fully	150,521	145,692	140,685
Partially	94		61
No screening	822	5,651	10,957
Total	**151,343**		

Fully screened infants had all aspects of the screen completed. In the case of the DBS and hearing panel this may have been through testing more than one sample or with the CCHD screen, having additional screens completed. Infants who were considered partially screened for a panel did not have all aspects of the screen completed including only partial panels completed in the case of laboratory unsatisfactory samples or test level unsats and transfused infants where a post transfusion sample was never received. Infants who had no screening included infants where an initial sample was received but was unsatisfactory for any testing and a subsequent sample was not received and infants where no sample was received.

For CCHD screening, there are circumstances where infants are not eligible for screening. While they are included in the count of no screening, they are appropriately not screened. There are no partial screens for CCHD as the screen was either completed to satisfaction or it was not.

The rate of non-screened infants increased for the hearing panel as this screen moved back to a consented model in early March 2024. More details regarding this can be found in Section 1.3.

1.1 DRIED BLOOD SPOT

The overall number of samples received by NSO in 2025 is higher than last year.

Table 2. Screening sample volumes between 2021-25.

Sample Type	2025	2024	2023	2022	2021
Satisfactory	152,002	149,013	141,186	139,779	145,785
Unsatisfactory*	2,677	2,475	1,909	1,639	1,560
Routine Screening – Total	**154,679**	**151,475**	**143,095**	**141,418**	**147,345**

*unsatisfactory in this table is defined as samples unable to be tested fully because of poor sample quality (i.e. laboratory unsats)

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1.1.1 INFANTS SCREENED

The total number of newborns screened and newborn screening samples received is shown in Figure 1.

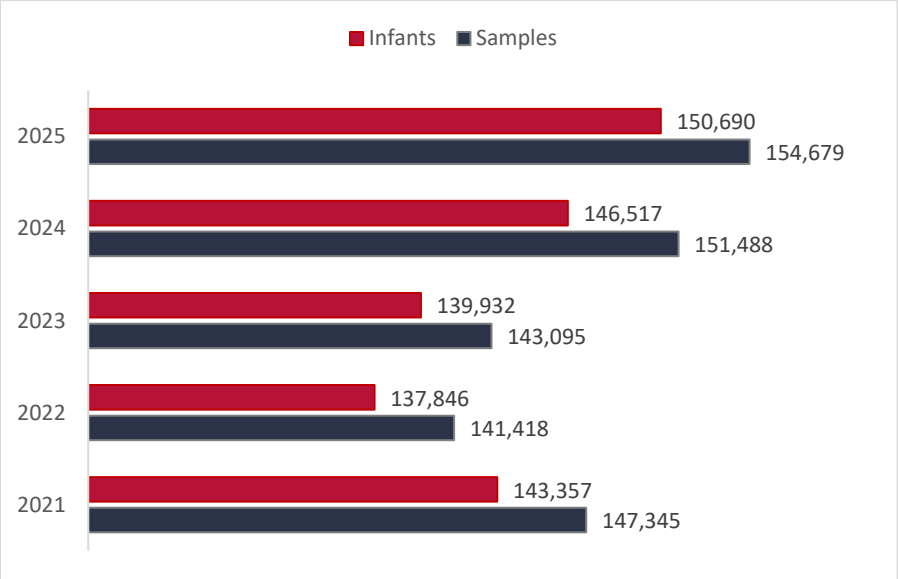


Figure 1: Total number of infants and samples screened between 2021-25.
The number of newborns is estimated by subtracting the number of samples determined to be from the same infant as another sample from the total number of received samples. This estimate be impacted by the efficiency of the linking algorithm as well as data quality. The number of infants tested is always lower than the number of samples received due to repeats required for transfusion, prematurity/low birth weight, and laboratory and data unsatisfactory samples.

1.1.2 DECLINED/DEFERRED SCREENING

If parents wish to decline or defer newborn screening, health care providers have the parents sign a decline/defer form included as part of the newborn screening card and submit the card with completed demographic information to NSO. This avoids unnecessary follow up in the case of a decline and allows formal documentation that screening was offered. Upon receipt of the decline form, NSO enters the information and generates a letter to the submitter documenting the receipt of the decline.

Similarly, in the case of a deferral, the information is entered and a letter is sent to the submitter. If a sample is not received by 14 days from the receipt of the deferral notice, NSO sends an additional reminder letter to the family directly.

In 2025, NSO received 1,091 completed decline/defer forms. The number of declines documented using this form has increased with 281 declines in 2025 compared with 248 in 2024. The remaining 810 forms received indicated a parent's desire to defer screening, and samples were eventually

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received for all but 18 of these deferred cases. In 16 cases the families declined (but 12 elected to have CCHD screening).

Table 3. Declined, deferred samples indicated on card between 2021-25.

Case Type	2025	2024	2023	2022	2021
Declined/deferred form received	1,091	983	861	758	819
Decline	281	248	168	151	96
Deferral	810	735	693	607	723

An additional 139 declined screens were also identified via missed screen alerts. There were 17 infants for which a decline form and a DBS missed screen decline case were received/created and 12 infants for whom a decline form was completed where a sample was received. In total there were 408 infants where newborn dried blood spot screening was declined (decline rate 0.27% of all infants screened), which is an in increase of >100 cases compared to the last two years. There were 268 families that declined the DBS screen but had the CCHD screen.

1.1.3 MISSED SCREENS

There were 570 potential missed screen alerts that required follow up in 2025. There were 428 potential missed cases logged that were not truly missed (top section of table above). There were 73 deceased/palliative cases logged and 139 declines. There were many cases where the sample was collected and received either the same day as the missed screen alert or after.

In 2025, there were 142 true missed newborn screen alerts that required follow up by NSO (bottom section of table above). Action on the part of NSO resulted in 92 of the 142 (65%) truly missed screens being completed.

1.1.4 AGE AT COLLECTION

The majority of newborn screening samples are collected between 24-48 hours of age. Greater than 99% of samples are collected by 48 hours of age. This statistic has remained at >99% for the last 4 years.

There is a grace period of 10 minutes for samples collected at <24 hours of age. There were 342 samples collected during this grace period – as such they did not require recollection but they are still counted in the <24 hour collection bracket.

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1.1.5 UNSATISFACTORY SAMPLES

The unsatisfactory rate increased in 2025, up to 1.73% compared to 1.31% in 2024. The majority of unsatisfactory samples (excluding <24 hour samples) are related to the quality of the blood sample collection directly, including too little or too much blood, or improper application of the blood on the card.

There were 49 samples that were deemed unsatisfactory for both a lab and a data reason. There were 139 unsatisfactory samples that did not require follow up as a repeat sample had already been received or testing of all analytes was able to be completed through two partially saturated samples. There were 2,489 unsatisfactory samples that required follow up.

Table 4. Unsatisfactory samples by reason between 2021-25.

			2025	2024	2023	2022	2021
SAMPLES	Satisfactory Samples		152,002	149,013	141,186	145,220	143,333
	Unsatisfactory Samples		2,677	2,475	1,909	2,215	2,332
	Unsatisfactory Rate		**1.73%**	**1.63%**	**1.33%**	**1.44%**	**1.63%**
	Samples Collected at <24hrs		464*	488	457	565	547
	Unsatisfactory Samples excluding <24hr samples		2,213	1,987	1,452	1,560	1,785
	Unsatisfactory Rate excluding <24hr samples		**1.43%**	**1.31%**	**1.01%**	**1.06%**	**1.25%**
REASONS	Lab Unsat Reasons	Quantity of blood insufficient	861	905	639	927	1,297
		Blood spots appear scratched or abraded	187	174	68	142	94
		Blood spots are supersaturated	94	47	21	35	42
		Blood spots appear clotted or layered	654	525	178	217	155
		Blood spots appear diluted	<5	<5	6	0	<5
		Blood spots exhibits serum rings	279	199	44	96	70
		Blood spots are wet and/or discolored	16	11	9	9	14
		Other	38	55	25	24	25
	Data Unsat Reasons	Blood dot collection paper is expired	11	11	49	54	38
		Insufficient data provided	0	6	<5	<5	11
		Damaged or delayed in transit	0	15	0	6	5
		Delivered to lab > 14 days after collection	29	22	81	38	33
		Sample collected at <24hrs	486	514	514	565	547
		Other/Mislabel	45	33	22	22	27

*Of the 486 samples collected at <24 hours, 22 had additional laboratory unsatisfactory reasons so were excluded from the <24 hours count. There were 26 samples that were unsatisfactory for both data and laboratory reasons.

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Of the 486 samples collected at <24 hours, the subsequent samples for these infants indicated a transfusion was given for 254 infants. Taking the pre-transfusion sample, even when collected at <24 hours, and a post-transfusion sample collected at ≥24 hours, often means that a subsequent 4-6 month sample is not required to complete screening for the infant as hemoglobin and galactosemia screening are not impacted by age at collection (but are impacted by packed red blood cell transfusions).

1.1.5.1 Repeat Rates for Unsatisfactory Specimens

The majority (~84%) of repeat samples are received within 2 weeks of the initial sample. By 6 weeks, ~94% of unsatisfactory samples have had screening completed via a repeat sample. This is comparable to the last 2 years. Of the 124 cases where a repeat sample was not received, partial testing was able to be completed on existing samples for 68 cases and another 19 were data unsats.

1.1.5.2 Priority Panels

Samples that are deemed unsatisfactory for the entire panel of testing are evaluated on whether there is sufficient blood for testing a smaller, priority panel of diseases. The priority panel is intended to expedite testing for the most aggressive, early onset diseases and include Metabolic diseases (AAAC platform), spinal muscular atrophy (SMN1), Galt deficiency, CH (TSH) and CAH (17OHP). SMA was added to the priority panel in June 2025. The priority panel also includes hemoglobinopathies to decrease the number of transfusion repeat requests that would be required 4 months post transfusion.

In 2025, NSO performed 1,301 priority panels for unsatisfactory samples that required follow up (~66.5% of laboratory unsatisfactory samples). These samples are still counted as unsatisfactory (Section 1.1.5), and a repeat is requested. The results of the priority diseases are also reported.

There were 23 cases where a 3rd repeat sample was not required as the first sample was an unsat priority panel and the second sample was an unsat balance panel where there was sufficient quantity of blood in the second sample to be able to complete the untested assays.

1.1.5.3 Test Level Unsats

Test level unsats (TLU) are samples that are initially satisfactory but are deemed unsatisfactory for reporting post testing due to poor quality results or insufficient sample to repeat or confirm testing. Samples that are unsatisfactory to complete initial testing require a routine repeat sample. These requests follow a similar workflow to regular unsatisfactory samples. Samples that are unsatisfactory to complete confirm testing require an urgent repeat sample. Urgent samples are requested to be sent to NSO within a week. If a repeat has not been received within a week (or a

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shorter timeframe if requested) the clinical team contacts the submitting hospital to obtain an update. If a family has not been reached or has declined coming back, the clinical team reviews the case with the appropriate Medical Scientist lead at NSO to determine next steps.

Regardless of urgency, results on these samples are reported only for those diseases where testing could be completed, and a repeat is requested when necessary. Repeats may not be required if a previous sample allowed for completion of the testing for a disease. There were 14 cases where a repeat sample was not required.

In 2025, there were 191 TLU cases. There were 7 TLU cases where a repeat was not received.

1.1.6 SCREENING TIMELINESS – RECEIPT AND AGE AT RESULTS

The purpose of the benchmarks was to establish days of age at which samples should be received, analyzed and resulted by the screening program, and screen positive infants should be referred, retrieved, have an initial and full diagnosis established. The goal would be to have 90% of the screened population meet the benchmarks.

Each cell contains the percentage of samples meeting benchmarks. Cells highlighted in green met the benchmarks. Cells highlighted in yellow had 70-89% of infants achieving benchmarks. Cells highlighted in red had benchmarks <70%.

Initial results refers to results from first tier screening. All samples undergo first tier testing. After interpretation the majority of samples will be screen negative and testing is complete. The second columns of numbers under each year are the age of their final results. There were 148,086 initial samples went through first tier screening in 2025. Of these, 138,643 (93.2%) were screen negative on all assays after first tier. Some samples require additional testing to determine if they are negative or positive. Age at final results is the subset of samples who required additional testing (through second and third tier screening) and the age that their results are final (either positive or negative).

There was an improvement in age at receipt this year (85% in 2025 compared to 84% in 2024). This resulted in increases in percentage meeting benchmarks for age at initial results and age at final results.

The SCID and SMA screening assays have a lower percentage reported by day 5 and 7 of life. The samples for these assays are punched a day after the biochemical assays. As well, the SCID and SMA assays include molecular testing as part of the first-tier testing (whereas cystic fibrosis and MPS1 are 2nd and 3rd tier) which takes 2 business days to complete. Unlike the biochemical laboratory, which is screening for the more aggressive disorders, the molecular laboratory does not operate on weekends. All of this leads to longer TAT for results of 3-5 days compared to the biochemical assays.

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Table 5. The percentage of infants meeting the defined benchmarks for each indicator for age at receipt and availability of initial and final results in 2024 and 2025.

	Screening (Initial Samples) 2025 Only			Screening (Initial Samples) 2024 Only		
Category	**Age at Receipt**	**Age at Initial Results**	**Age at Final Results**	**Age at Receipt**	**Age at Initial Results**	**Age at Final Results**
Benchmark (days)	**4**	**5**	**7**	**4**	**5**	**7**
CIT/ASA, CblA &B, CUD, FAOD, GA1, GAMT, HCY, IVA, LCHAD/TFP, MCAD, MSUD, PA/MMA, PKU, TYR1, VLCAD	**85%**	**85%**	**99%**	**84%**	**84%**	**98%**
Biotinidase Deficiency	**85%**	**85%**	**98%**	**84%**	**84%**	**98%**
Galactosemia	**85%**	**85%**	**99%**	**84%**	**84%**	**98%**
Mucopolysaccharidosis Type 1	**85%**	**84%**	**98%**	**84%**	**83%**	**98%**
Guanidinoacetate Methyltransferase Deficiency	**85%**	**85%**	**98%**	**84%**	**84%**	**98%**
Congenital Adrenal Hyperplasia	**85%**	**86%**	**99%**	**84%**	**84%**	**98%**
Congenital Hypothyroidism	**85%**	**85%**	**99%**	**84%**	**84%**	**98%**
Cystic Fibrosis	**85%**	**85%**	**95%**	**84%**	**84%**	**96%**
Hemoglobinopathies	**85%**	**63%**	**97%**	**84%**	**69%**	**97%**
Severe Combined Immune Deficiency	**85%**	**14%**	**66%**	**84%**	**13%**	**59%**
Spinal Muscular Atrophy	**85%**	**15%**	**67%**	**84%**	**14%**	**63%**

Note: The pilot phase of X-linked Adrenoleukodystrophy (XALD) began in 2025 but only moved to a full launch in early 2026. Data pertaining to XALD screening will be included in the 2026 annual report.

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Table 6. Median and 90th centile values for age of receipt of initial samples, and availability of initial and final results, 2024 and 2025. The 'n' column represents the total number of samples that went on for additional testing.

	Screening (Initial Samples) 2025 Only					Screening (Initial Samples) 2024 Only				
Category	Age at Initial Results		Age at Final Results			Age at Initial Results		Age at Final Results		
	Median	90th Centile	n	Median	90th Centile	Median	90th Centile	n	Median	90th Centile
CIT/ASA	4	6	50	6	8	4	6	31	6	8
CUD			459	5	7			239	5	7
FAOD			16	5	7			8	5	6
GA1			926	6	7			28	5	8
HCY			859	9	13			210	9	13
IVA			51	5	7			27	6	7
LCHAD/TFP			45	5	7			41	5	8
MCAD			30	5	8			14	5	9
MSUD			35	6	9			8	6	9
PA/MMA/CblA&B			318	6	8			145	6	8
PKU			156	5	7			156	5	7
TYR1			32	6	8			17	6	13
VLCAD			207	5	7			199	5	7
Biotinidase Deficiency			82	6	7			88	6	7
Galactosemia			117	6	8			123	6	8
GAMT			210	7	12			174	10	12
MPS1H			887	10	18			841	9	19
CAH			835	6	7			660	6	8
CH			904	5	7			874	5	7
Cystic Fibrosis			5,945	9	13			5,906	8	13
Hemoglobinopathies	5	7	134	6	8	5	7	135	6	9
SCID	7	9	833	9	13	7	10	723	11	15
SMA	7	9	22	8	11	7	10	11	9	13

The age at final results decreased for SCID and SMA between 2024 to 2025.

With the implementation of changes to the homocystinuria (HCY) screening algorithm to include total homocysteine as a second-tier test, the number of samples that went on to second-tier testing increased. This also resulted in a longer median time to report for HCY.

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Table 7. Median and 90th centile values for time from receipt to initial results, and time from receipt to final results, 2024 and 2025. The 'n' column represents the total number of samples that went on for additional testing.

Category	Screening (Initial Samples) 2025 Only					Screening (Initial Samples) 2024 Only				
	Receipt To Initial Results (hours)		Receipt To Final Results (hours)			Receipt To Initial Results (hours)		Receipt To Final Results (hours)		
	Median	90th Centile	n	Median	90th Centile	Median	90th Centile	n	Median	90th Centile
CIT/ASA	24	26	50	52	75	24	26	31	73	76
CUD			459	49	72			239	50	72
FAOD			16	52	100			8	63	82
GA1			926	54	98			28	49	72
HCY			859	146	196			210	146	196
IVA			51	50	74			27	49	73
LCHAD/TFP			45	50	73			41	50	73
MCAD			30	48	57			14	48	74
MSUD			35	49	73			8	49	50
PA/MMA/CblA&B			318	52	80			145	50	97
PKU			156	49	52			156	50	52
TYR1			32	52	74			17	73	75
VLCAD			207	50	73			199	50	72
Biotinidase Deficiency	25	26	82	50	73	25	26	88	50	73
Galactosemia	24	25	117	49	52	24	25	123	50	52
GAMT	24	26	210	77	172	24	26	174	146	195
MPS1H	25	26	887	172	341	25	26	841	148	386
CAH	24	25	835	51	98	24	25	733	51	98
CH	24	25	904	49	73	24	25	874	50	73
Cystic Fibrosis	24	26	5,945	145	220	24	26	5,906	124	222
Hemoglobinopathies	26	73	134	97	122	26	73	135	74	123
SCID	96	128	833	146	219	99	151	723	169	295
SMA	96	126	22	126	199	98	145	11	148	255

While the median age of initial results for hemoglobinopathies is reported as 5 days (Table 10b), when looking at the data in terms of hours (Table 11), it is only 1-2 hours from the other conditions reported which means initial results would be reported the same day as the metabolic, endocrine and cystic fibrosis conditions.

1.2 CRITICAL CONGENITAL HEART DISEASE

Submitters submit their Critical Congenital Heart Disease (CCHD) screen results to NSO via a tear off sheet on the standard NSO dried blood spot card. These may come with the dried blood spot, or separately, depending on hospital process. The total number of CCHD cards registered at NSO in 2025 was 154,140 representing 149,932 infants.

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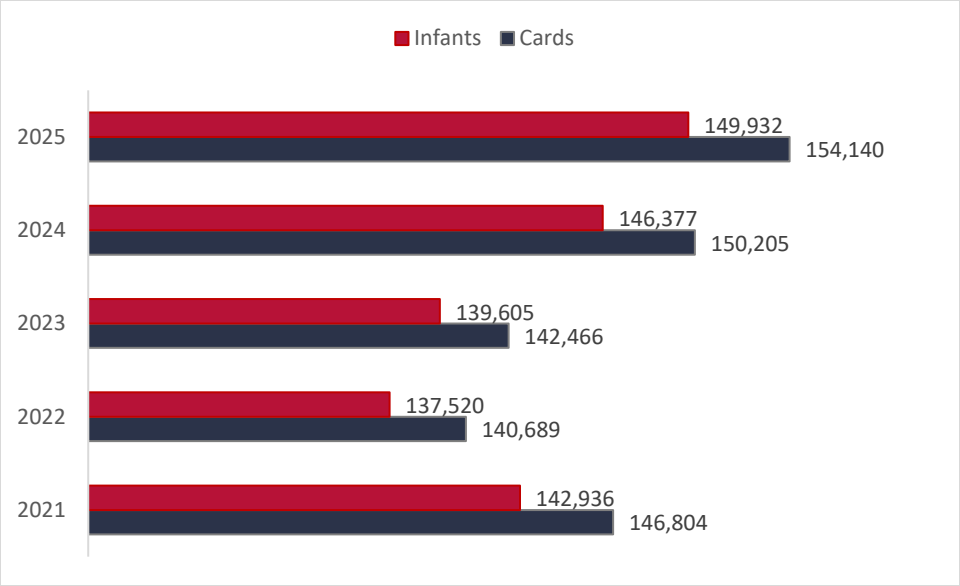


Figure 2. CCHD cards received at NSO and total number of infants between 2021-25.

There are also expected reasons why the CCHD screen would not be done, such as a long NICU stay or a prenatal diagnosis. These would also contribute to the lower estimate of infants screened, but efforts have been made to encourage submission of the form in these circumstances to document that the screen was not done. In 2025, 8,448 of the requisitions submitted did not include screening information.

Table 8. CCHD cards received from 2021-25.

CCHD Cards received	2025		2024		2023		2022		2021	
Screen Completed	145,692	94.52%	142,291	94.73%	135,004	94.76%	133,617	94.97%	139,264	94.86%
Screen Not Done	8,448	5.48%	7,914	5.27%	7,462	5.24%	7,072	5.03%	7,540	5.14%
	154,140		**150,205**		**142,466**		**140,689**		**146,804**	

1.2.1 Screens Completed

The NSO CCHD algorithm allows for up to 3 repeat tests done one hour apart prior to making a referral. In the cards where screening was done, 99.12% of the screens were resolved after just one test (most often this would be a pass, but this could also be an immediate referral), which is the same as last year. Only 0.75% required a second test and 0.14% required three tests to complete the screen. This has been consistent over the last 5 years.

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1.2.2 Screens Not Done

In 2025, CCHD screens were not done on 5.48% of the cards received. The most common reason that CCHD screening was not done is that the infant is expected to be in the NICU for > 7 days or was previously diagnosed with a heart defect.

Table 9. Reasons for CCHD Screen not done between 2021-25.

	2025		2024		2023		2022		2021	
'Screen Not Done' cards submitted	**8,448**		**7,928**		**7,462**		**7,072**		**7,540**	
Not eligible for screening (diagnosed pre- or postnatally with heart defect, NICU >7 days, on oxygen, etc)	4,962	58.74%	4,613	58.19%	4,599	61.63%	4,556	64.42%	4,993	66.22%
Already done	1,155	13.67%	893	11.26%	633	8.48%	503	7.11%	514	6.82%
Insufficient information provided/blank card	1,273	15.07%	1,430	18.04%	1,139	15.26%	1,062	15.02%	1,005	13.33%
Deferred/declined	810	9.59%	725	9.14%	747	10.01%	686	9.70%	786	10.42%
Other	248	2.94%	267	3.37%	344	4.61%	265	3.75%	242	3.21%

On September 30, 2024, NSO started to collect multiple reasons why a screen was not performed to try to better ascertain the number of cardiac defects in the province. There were 178 infants who had more than one reason why their screen was not performed (23 were diagnosed prenatally with a heart defect, 10 had a postnatal diagnosis of a heart defect, 52 additional infants were expected to be in the NICU for >7 days, and 97 had other reasons). These extra 182 reasons are not included in the table above.

Of the decline/deferred group (84) where the back of the form was not fully completed to know if the family was declining or deferring – 68 had a CCHD screen completed. There were 667 defer cards received. Of these 643 had a CCHD screen. Of the decline group (59) – 18 had a CCHD screen completed. In total, 77 families declined/did not complete CCHD screening (from the decline and defer forms received and the missed screen notifications). Of these 77 families, 48 declined both the CCHD screen and the DBS screen.

There were 1,155 cards that indicated that a previous screen had already been performed and 1,273 were just submitted completely blank. These blank cards often accompanied repeat dried blood spot specimens which may be reflected in the changes in numbers; the 'previous screen' reason increased in 2025 and the blank cards decreased.

1.2.3 CCHD Missed Screens

In 2025, 639 potential missed screens were identified. There has been a decrease in the number of missed screen notifications over the last few years. There was an increase in the category of 'infant not suitable for screening' and a decrease in the 'infant was screened – documentation not sent or sent late'. The majority of these alerts were due to improper documentation – either the infant was screened but documentation was not sent to NSO (166) or the infant was not suitable for screening

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and documentation was not sent to NSO (396). There were 71 CCHD screens that were missed for eligible infants. Infants are only eligible for CCHD screening up to 7 days of age. As these infants were >14 days of age, their health care providers were notified that the infant had not had CCHD screening in the newborn period.

1.2.4 Age at Time of CCHD Screen

The recommended age for CCHD screening is 24-48 hours of age, with an optimal window between 24 and 36 hours. The majority (~96%) of screening is completed by 48 hours of age which is similar to previous years.

1.2.5 Unsatisfactory CCHD Screens

Upon entry into the NSO database, unsatisfactory CCHD screens are identified when there has been a misinterpretation of the screening algorithm, the algorithm was not followed, or where the outcome is not adequately documented. This includes cases where the result should have been 'REFER' but a 'PASS' result was documented, and cases where the result should have been 'REPEAT' but a 'PASS' result was documented. NSO contacts the submitter who performed the screen to clarify the information provided and inform them of the unsatisfactory screen. If required the submitter will contact the family to bring the infant back to complete their CCHD screen.

The number of unsatisfactory screens in 2025 was 936, which was 0.61% of the cards received. The most frequent error was incomplete documentation – either of a repeat test done after 1 hour or missing screening values. With increased submitter education, the unsatisfactory rate decreased in 2020 and has remained below 0.75%.

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Table 10. Outcomes from unsatisfactory CCHD screen notifications 2021-25.

		2025	2024	2023	2022	2021
Unsatisfactory Screens		**936**	**953**	**953**	**829**	**1,057**
No screen/ rescreen recommended	Baby >7days	15 (1.6%)	26 (2.7%)	20 (2.4%)	42 (4.0%)	39 (3.3%)
	Baby in hospital	183 (19.6%	169 (17.7%)	131 (15.8%)	185 (17.5%)	203 (17.2%)
	Documentation inaccurate or incomplete	571 (61.0%)	596 (62.5%)	536 (64.7%)	653 (61.8%)	723 (61.3%)
	Missed - baby >7 days	<5 (<0.5%)	5 (0.5%)	6 (0.7%)	6 (0.7%)	6 (0.5%)
	No action needed	61 (6.5%)	52 (5.5%)	39 (4.7%)	50 (4.7%)	57 (4.8%)
Screen or physical exam recommended	Missed - baby ≤7 days	35 (3.7%)	31 (3.3%)	29 (3.5%)	40 (3.8%)	65 (5.5%)
	Physical exam recommended (screen positive)	<5 (<0.5%)	0	<5 (<0.5%)	<5 (<0.6%)	<5 (<0.5%)
	Rescreen recommended	68 (7.3%)	74 (7.8%)	64 (7.7%)	80 (7.6%)	83 (7.0%)
Total Screening Forms Submitted		**154,140**	**150,205**	**142,466**	**140,689**	**146,804**
Unsatisfactory Rate		**0.61%**	**0.63%**	**0.67%**	**0.59%**	**0.72%**

Note: No action needed includes infants that were later identified as a premature with no response from the submitter (information obtained from the dried blood spot card) or a satisfactory CCHD screen located that was previously unlinked to infant.

NSO performed follow up on 936 unsatisfactory screens, and in 88.9% of follow up cases the result was amended by the submitter due to incorrect completion of the form. In 7.3% of cases a rescreen was recommended. Through the follow up of unsatisfactory screens NSO was able to follow up with submitters for 104 infants that had not received a proper CCHD screen and needed to be screened (missed) or rescreened.

1.3 HEARING

The Infant Hearing Program (IHP) is Ontario's Early Hearing Detection and Intervention (EHDI) program, which is funded by the Ministry of Children, Community and Social Services (MCCSS). The IHP is a comprehensive provincial program that delivers universal newborn hearing screening (UNHS) and audiology assessment, and coordinates audiology interventions, language development and family support services for infants and young children with permanent hearing loss (PHL). Services are coordinated and delivered through 12 Lead Agencies across the province.

Since July 29, 2019, the IHP and NSO have offered screening for some risk factors for PHL using the newborn dried bloodspot (DBS). This screening analyzes DBS samples to look for congenital Cytomegalovirus (cCMV) infection and genetic risk factors associated with DFNB1 and DFNB4-

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associated PHL, including variants in the genes *GJB2/6* and *SLC26A4*. These represent some of the most common causes of childhood PHL and infants identified with these risk factors are at increased risk for congenital or early onset PHL.

In 2024, NSO and the National Centre for Audiology (NCA) at Western University became the IHP Oversight Agency, now operating as ***wave***. Through strategic guidance of the Ontario IHP, ***wave*** aims to achieve equitable access to person-centered, evidence-based and integrated services that lead to the best possible developmental outcomes. This oversight includes management of contracts and financial processes with IHP Lead Agencies, provision of operational support for service delivery, developmental and maintenance of clinical protocols, and training of IHP service providers. The second year of operation was marked by continued strengthening of relationships with Lead Agencies and system partners, province-wide procurement of new hearing screening equipment, the implementation of a revamped training model for IHP audiologists, and the establishment of ***wave*** as the provincial identity for IHP oversight.

1.3.1 Consent for Risk Factor Screening for PHL

When risk factor screening for PHL launched in 2019, parents/guardians were approached for consent as part of the UNHS process. With the onset of the COVID-19 pandemic in March of 2020, the requirement of additional consent for risk factor screening was waived due to disruptions to UNHS, which impacted the ability to obtain consent in a timely manner. As such, all DBS from babies born between March 26, 2020, and March 3, 2024, were screened for CMV and genetic risk factors for PHL without the requirement of additional consent through the IHP.

Consent for risk factor screening for PHL was re-introduced for babies born on or after March 4, 2024. Consent options were simplified for families (i.e. consent for UNHS includes consent for both the physiologic screening and the DBS risk factor screening), and an electronic interface was established between program information systems to transmit consent.

Risk factor screening for PHL continued with a consent-based approach throughout the beginning of 2025. A technical issue affecting the transmission of consent to NSO was identified, and the decision was made to waive consent and perform risk factor screening on all eligible newborn screening samples beginning on November 24, 2025. This approach remained in place through the end of 2025, while focused efforts were undertaken to monitor transmission through the interface. Future work will focus on reviewing and optimizing the consent approach for risk factor screening, alongside continued monitoring and strengthening of the interface to ensure timely, reliable data transmission.

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1.3.2 Screens Completed for PHL Risk Factor Screening

The table below shows the number of infants screened for CMV and genetic risk factors for PHL. NSO screened 150,201 infants in 2025. Risk factor screening for PHL (CMV and genetics) was completed for 140,685 infants (93.7%) in 2025.

Table 11. Number of babies screened for risk factors for PHL between 2022-25.

	2025		2024		2023	2022
	Jan 1 – Nov 23	Nov 24 – Dec 31	Jan 1 - Mar 3	Mar 4 - Dec 31		
Infants screened at NSO	135,295	14,906	24,448	122,069	139,931	137,849
IHP screening form received	126,145	N/A	N/A	113,470	N/A	N/A
Consent for risk factor screening	126,025	N/A	N/A	113,291	N/A	N/A
Babies screened for CMV and genetic risk factors	125,828	14,857	24,402	113,092	139,620	136,724
Babies screened for CMV	125,850	14,857	24,403	113,107	139,639	137,619
Babies screened for genetic risk factors	125,883	14, 863	24,434	113,222	139,837	136,885

*Note that risk factors for PHL data is pulled by date of birth instead of date of receipt.

1.4 BILIARY ATRESIA

The biliary atresia (BA) screening program was launched in January 2023. Healthcare providers distribute infant stool colour cards (ISCC) to parents shortly after the birth of their baby and instruct them to screen their newborn's stool colour at home for the first month of life (or for one month past their due date for babies born less than 37 weeks gestation). If parents detect pale stool, the ISCC provides guidance about how to contact the NSO BA clinical team so they can conduct a telephone assessment and facilitate referral to a Pediatric Academic Health Science Centre (PAHSC) hepatologist for evaluation.

Program Education and Awareness Strategies
Strategies to improve program education and awareness for our community partners continue through hospital rounds, webinars, and newsletters.

Family Advisors
Collaboration with family advisors is ongoing, including the review and provision of feedback on the parent BA program evaluation survey. We look forward to welcoming additional parent advisors to our team in 2026.

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APP work
NSO could not identify a suitable collaboration opportunity to develop a digital AI-assisted version of the ISCC. Despite requests from parents for a digital version of the ISCC, this will not be pursued at this time given the challenges standardizing colour interpretation across various platforms.

1.4.1 Survey

In 2025, NSO conducted a survey to assess parent and healthcare provider satisfaction, evaluate the reach and impact of screening services, and identify future opportunities, with a focus on awareness of and experiences with the recently introduced Biliary Atresia screening initiative.

The Evidence Network & Amplify Engagement conducted the survey of healthcare providers (HCPs) and parents from May to October 2025. A representative sample of 1,197 parents and 609 HCPs from across Ontario completed the survey. Parents were reached through community organizations, parent groups, and social media. HCPs were reached through newsletters, professional networks, and outreach across a wide range of healthcare settings. The respondent sample reflected the geographic and ethnic composition of Ontario's population.

Summary Results of Survey
The survey findings demonstrated strong public trust in NSO and widespread support for newborn screening as a cornerstone of early infant care. Parents value the screening process and the guidance provided by the NSO screening team. HCPs who interacted directly with NSO reported positive experiences marked by reliability, clarity, and responsiveness.

Parent and HCP responses about the BA screening program confirmed widespread ISCC distribution and strong acceptance and appreciation of the program. Parents who had contacted NSO about pale stool concerns unanimously reported outstanding experiences interacting with the NSO BA team.

Building on this strong foundation, there are clear opportunities to enhance consistency and coordination across the system to further optimize newborn screening across Ontario. Parent and HCP infographics outlining key findings of the survey are posted on the NSO website.

1.4.2 Infant Stool Colour Card (ISCC) Distribution

VWR continues to coordinate orders and supply stock to submitters for ISCCs through the well-established DBS pathway. To track orders, reports are received from VWR and NSO tracks ISCC ordering monthly. Along with this broad oversite, NSO sent fax reminders every three months to submitters to ensure they have a robust supply of ISCC and their ordering process was well established. In 2026, submitters with a six-month birth rate exceeding 1,000 births will receive fax reminders every three months, while all other submitters will receive reminders every six months.

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It is difficult to accurately confirm the distribution of ISCC to families. To bridge this gap, NSO added a checkbox to indicate ISCC distribution to parents on the CCHD screening card. While this is still not broadly utilized, full implementation of this change is expected in 2027 with plans to include completion of this checkbox on the submitter report card. From quarter 1 to quarter 4, indication of ISCC distribution to parents via CCHD screening card checkbox completion increased from 21% to 34% and the rate of non-completion decreased from 69% to 63%.

A short audit of submitter stock levels of ISCCs and ISCC ordering processes was conducted to identify submitters who required more assistance with developing and sustaining processes for maintaining adequate ISCC stock levels.

1.4.3 BA Screening Process

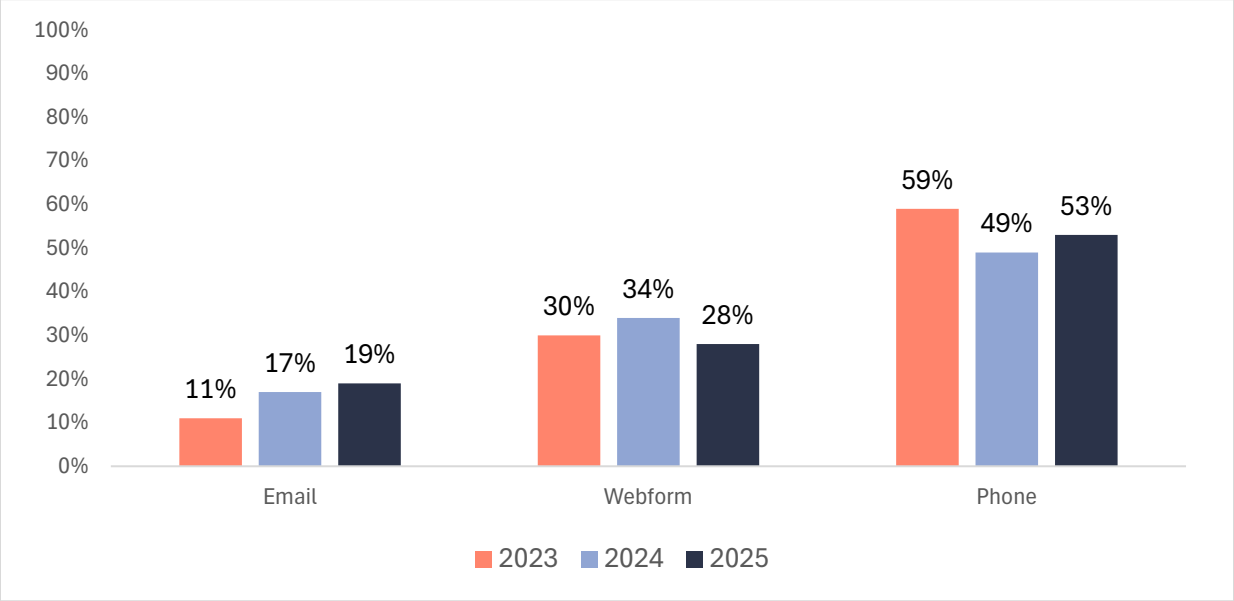


Figure 3. Method of initial parent contact to NSO.

The minimum age at first contact in days was 1 day, the maximum age was 647 days, and the mean was 63 days of age. These late calls occurred despite written instructions on the ISCC, and verbal instructions provided to families at the time of card distribution that recommends families screen for a total of four weeks post birth for infants born ≥37 weeks gestational and if ≤37 weeks gestation, four weeks past the baby's due date. Despite this, families continued to contact NSO beyond the recommended screening window. There were 11 calls after 1 year of age.

For infants older than 120 days of corrected age with a primary concern of pale stool, parents were instructed to present to their healthcare provider and request that a conjugated or direct bilirubin

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be measured to rule out cholestasis. For all other concerns, the family was redirected to the most appropriate healthcare provider.

Table 12. Summary table of BA screening characteristics and outcomes

	2025	2024	2023
Number of Contacts	267	349	271
Mean age at contact (Min-MAX)	63 (min 1 – max 647)	51 (min 1- max 521)	33 (min 2 – max 229)
Number of calls >120 days	33 (12%)	26 (7%)	6 (2%)
Number of calls about Pale Stool	126 (47%)	162 (46%)	117 (44%)
Number of screen positive referrals (%)	17 (13%)	43 (27%)	41 (35%)
Screen positive referrals with cholestasis	<5 (<29%)	6 (14%)	12 (29%)
Mean age at referral	35	43	49
Number of days to receive bloodwork	2.2 (0-11) *	1.3 (0-4 days)	1.5 (0 -7 days)
BA cases identified through screening	0	<5	<5
Median DOL for BA Dx	N/A	36	46
Median DOL for Kasai	N/A	38	50

* Includes an outlier where bloodwork was repeated three times due to specimen clotting in a community lab

1.4.4 Biliary Atresia Clinical Assessments

In 2025, there were 267 contacts to NSO by families or caregivers regarding infant stool concerns, of which 265 telephone clinical assessments of the cases were conducted. The number of contacts is down 23% from 2024.

Stool photos were received for 157 telephone triage assessments, and a total of 732 photos were submitted, de-identified, and uploaded to an NSO private drive with restricted access. 57% of the time the colour identified by the family on the ISCC aligned with the stool photo colour interpretation by the BA clinical team. Image #5 on the ISCC was the most frequent parent-identified stool colour overall and was the most frequent parent-identified stool colour that was not aligned with the clinical team's impression of the stool colour. There are plans to make changes to the layout and design of the ISCC to improve usability and accessibility.

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141/265 (53%) of the calls were unrelated to pale stool concerns. Common reasons for these unrelated calls remained similar to previous years; blood in the stool, other stool colour concerns (a colour not shown on the card), consistency/texture issues and constipation. In these cases, reassurance was given, or the callers were directed to seek an assessment from another HCP for their concern. A general resource about normal stools in infants was posted to the BA page of the NSO website.

Some cases resulted in an inability to contact the family, despite our team's best efforts, which included 3 attempts at re-contacting families who had reached out to us.

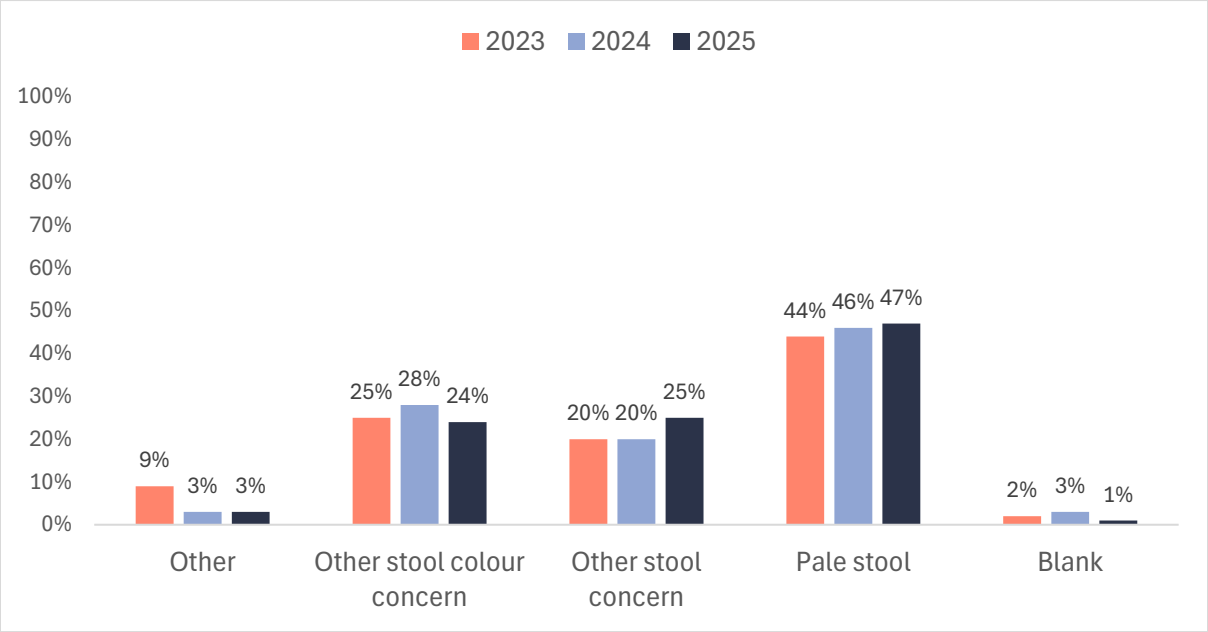


Figure 4. Reason for call to NSO.

Some cases require more than one contact by NSO with the family to make a screening determination. 94 calls required 2 contacts to make a screening determination, 44 required 3 contacts and 16 required 4 contacts to make a screening determination. These subsequent contacts allowed the clinical team to gather more stool photos and information about the infant over time. 45% of our contacts for term infants happen within the first 2 weeks of life.

In 50/172 (29%) cases, the parents reached out to HCPs (family doctor, walk-in clinic, emergency room visit) while waiting for the NSO team to reach them to conduct the telephone clinical assessment. Frequently this was due to a weekend or after hours contact to our program. 18/50 (36%) reached out to HCPs prior to making their first call to NSO. Midwives frequently redirected to call the NSO screening program. In other cases, the parents subsequently contacted NSO because they could not be seen by a doctor, or they were not satisfied with the response to their pale stool concerns. There are plans to include clearer instructions on the ISCC for parents to bring the ISCC

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with them to HCP appointments related to pale stool concerns. There will also be more efforts made in the coming year to increase primary care practitioner awareness about the BA screening program.

54% of contacts from parents for pale stool concerns indicated the presence of pale stool for <24 hours, and 46% had pale stool for >24 hours. 109/126 (87%) of calls about pale stools that had a clinical assessment were screen negative.

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2. SCREEN POSITIVES

2.1 DRIED BLOOD SPOT REFERRALS

2.1.1 Referrals by Treatment Centre

The number of referrals over the last 5 calendar years to the five Ontario treatment centres and the Winnipeg treatment centre are depicted in the graph above. 'Other' represents infants referred to treatment centres outside of Ontario/ Winnipeg, such as Quebec or the United States, or a centre in Ontario that is outside of the standard treatment centres. The proportion of referrals received by each of the five Ontario regional treatment centres has been relatively unchanged.

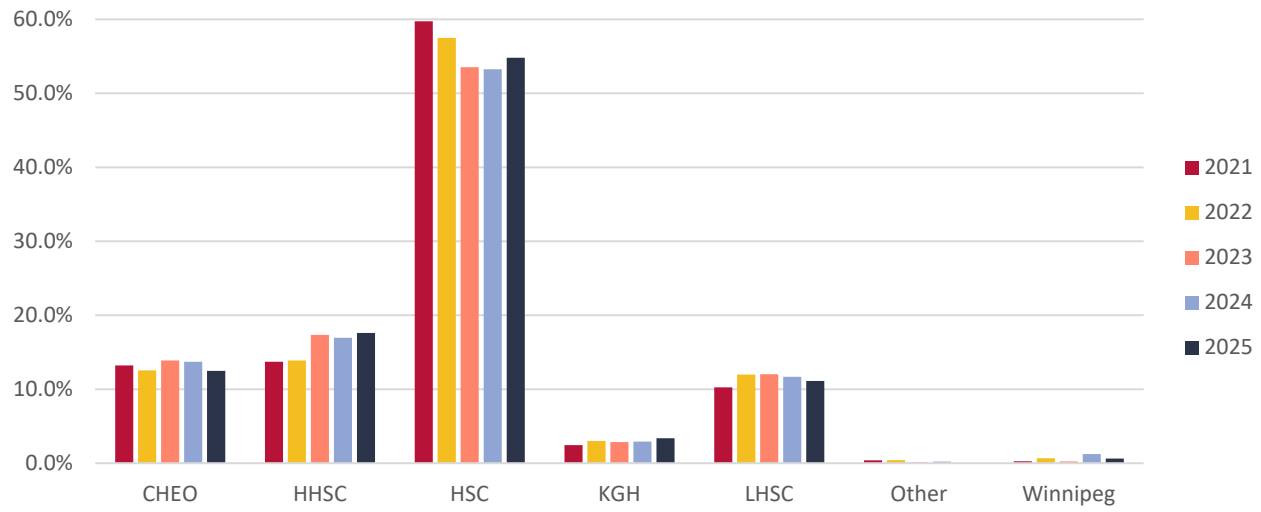


Figure 5. The percentage of referrals by treatment centre between 2021-25.

2.1.2 DBS REFERRALS BY YEAR

In 2025, there were 801 screen positive referrals. This represents ~0.5% of the total number of infants screened by NSO.

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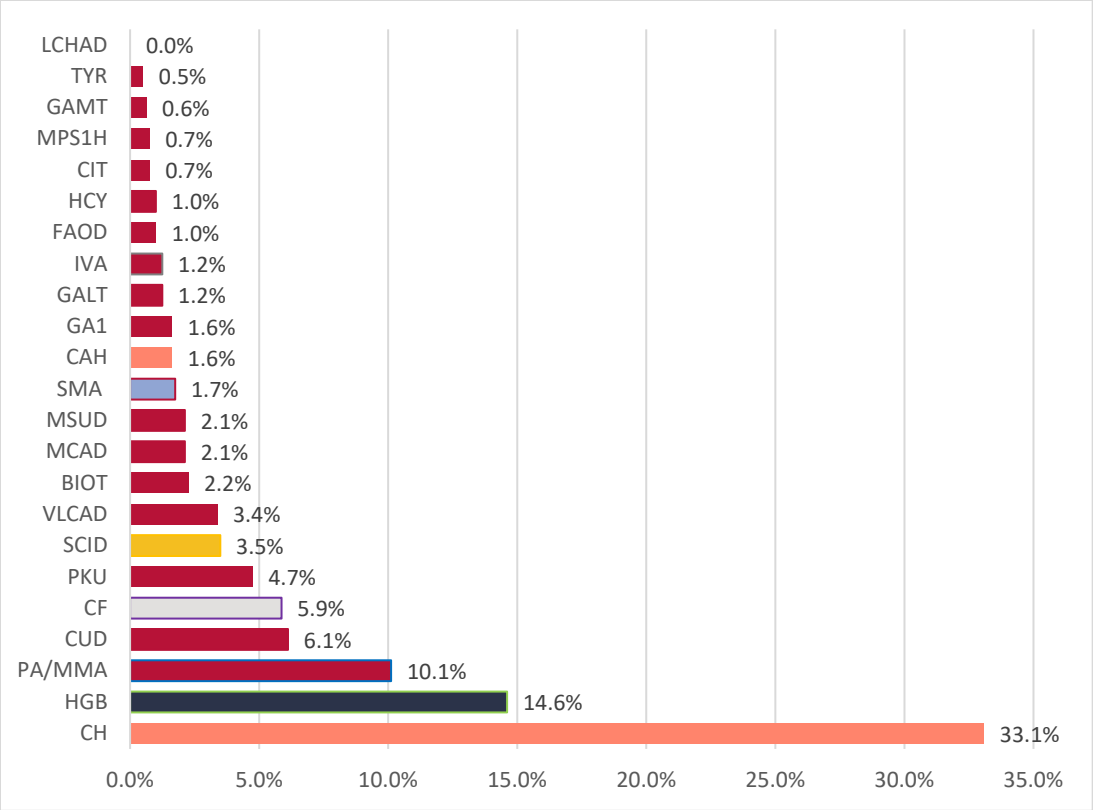


Figure 6. The percentage of screen positive referrals by disorder in 2025.

The number of screen positive infants referred in 2025 decreased from 2024 (801 vs. 890). The decline is principally explained by a disorder logic change for Congenital Adrenal Hyperplasia (CAH) in early 2025 (more details to follow below).

Endocrinopathies and Metabolics represent ~34.7% and ~39.6% of screen positives respectively. SCID screen positive referrals decreased in 2025 and now represent 3.5% of total screen positive referrals. The number of Cystic Fibrosis referrals decreased in 2025 and now represent 5.9% of total screen positive referrals. Hemoglobinopathies represent approximately 14.6% of screen positive referrals, which is unchanged from last year. SMA represents 1.7% of referrals.

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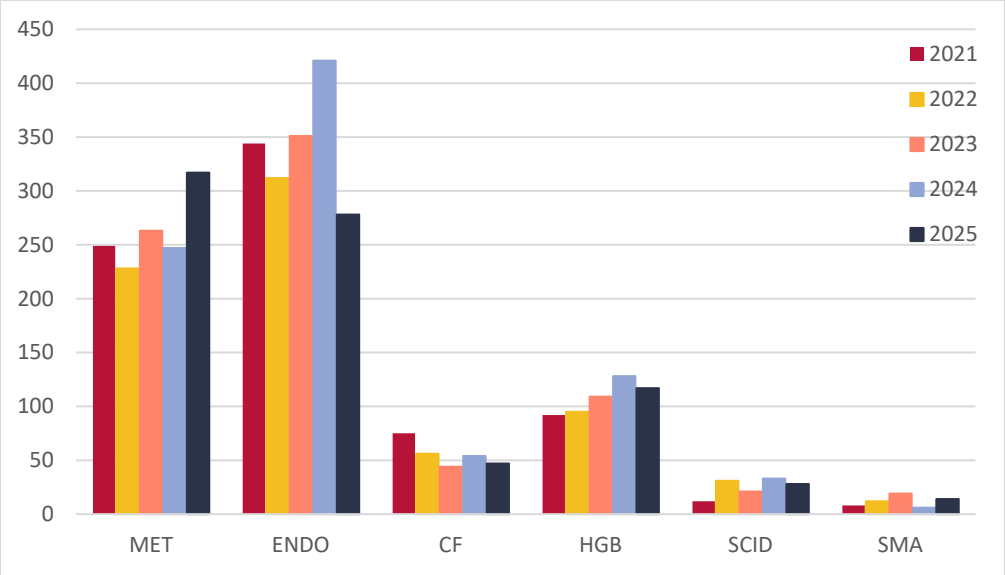


Figure 7. The total number of screen positives by disease grouping between 2021-25.

The number of screen positive referrals per disease grouping decreased for most referral types with the exception of metabolic and spinal muscular atrophy (SMA).

2.1.3 Diagnostic Feedback

Approximately 1.9% (15 cases) of diagnostic evaluation report forms (DERFs) remain pending for the referrals made in 2025 as of April 1, 2026. The percentage of pending DERFs is the lowest it has been for any annual report. With the use of preliminary data obtained during confirmation of retrieval and initial diagnosis, an outcome was obtained for 6 of these pending DERF cases.

Based on DERF data returned by the Treatment Centres, outcomes for each referral can be determined. A detailed explanation of the disease classifications can be found in Appendix C. NSO began to track initial diagnosis for all urgent and semi-urgent referrals in mid 2019. This was to ensure with a high PPV referral the correct infant was being referred (ruling out requisition errors) and if the correct infant was referred and found to be not affected, identifying a reason why the screen was positive (maternal factors, infant factors, or sample quality). This information is available earlier than DERF completion and is also a way to incorporate information into data analysis.

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Table 13. The outcome classifications for all referrals in 2025.

Disease	DERFs Pending	Outcomes Unknown	PRIMARY	VARIANT	INCIDENTAL	NOT AFFECTED	OTHER	Total No. Referred	Current PPV
Congenital Hypothyroidism	0	0	50	72	34	106	<5	265	20.2%
Congenital Adrenal Hyperplasia	0	0	10	0	0	<5	0	13	100.0%
Hemoglobinopathies	<5	0	64	<5	48	0	<5	117	56.7%
Cystic Fibrosis	0	0	21	19	6	<5	0	47	43.1%
Type 1	0	0	21	0	<5	0	0	22	95.7%
Type 2	0	0	0	<5	0	0	0	<5	1.3%
Type 3	0	0	0	18	5	<5	0	24	7.6%
SCID	<5	<5	<5	0	9	13	<5	28	13.9%
SMA	0	0	14	0	0	0	0	14	100.0%
Biotinidase Deficiency	0	0	<5	9	<5	6	0	18	7.4%
Citrullinemia	0	0	<5	<5	0	<5	0	6	16.2%
CUD	0	0	<5	0	18	26	<5	49	9.0%
FAO (CPT1, CPT2, and GA2)	0	0	<5	0	<5	6	0	8	9.6%
Galactosemia	0	0	<5	0	<5	5	0	10	12.5%
GAMT	0	0	0	0	<5	<5	<5	5	9.1%
Glutaric Aciduria Type 1	0	0	0	<5	0	12	0	13	9.3%
Homocystinuria	0	0	0	0	0	8	0	8	7.0%
Isovaleric Acidemia	0	0	<5	0	0	8	0	10	18.0%
LCHAD	0	0	0	0	0	0	0	0	82.4%
MCAD	0	0	11	<5	<5	0	0	17	53.9%
MPS1H	<5	<5	0	<5	<5	0	0	6	25.0%
MSUD	0	0	5	0	0	12	0	17	10.3%
PA/MMA	8	5	<5	0	27	40	6	81	4.2%
Phenylketonuria	0	0	<5	12	<5	17	<5	38	19.3%
Tyrosinemia	0	0	0	0	<5	<5	0	<5	9.2%
VLCAD	0	0	<5	0	11	15	0	27	7.4%
Total No. Positive	**15**	**9**	**197**	**120**	**165**	**288**	**22**	**801**	

DERF data is pulled April 1, 2026. The DERF Pending column is a total of all pending DERFs. The outcomes unknown column reflects cases without an initial or final diagnosis where the DERF is pending. The total number of infants referred is a tally of outcomes unknown, primary, variant, incidental, not affected and other.

2.2 HEMATOLOGY

The number of screen positives (117) in 2025 decreased slightly compared to 2024 (129).

Table 14. The PPV calculations for the current and past screening algorithms.

Additional information	PPV (Primary)	PPV (Primary + Variant)	PPV (Primary + Variant + Secondary)	% of Outcomes Unknown
Past (Nov 1, 2010 - Jul 31, 2015)	64.3%	65.1%	84.3%	0.8%
Current (Aug 1, 2015 - Dec 31, 2025)	**56.7%**	**57.3%**	**91.1%**	**0.7%**

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2.2.1 Hemoglobin Carriers

Table 15. Hemoglobin carrier requests between 2021-25.

	2025	2024	2023	2022	2021
Requests from high-risk population	69	54	41	23	unknown
Total Requests	**144**	**80**	**51**	**37**	**49**
Number of carriers	31	13	17	13	17

NOTE: The way hemoglobin carrier requests are logged was changed in 2021. Therefore, the number of requests from high-risk populations was unknown for that year.

Table 16. Carriers identified in 2025.

HGB Pattern	Carriers Identified
FAC	383
FAD	297
FAS	1,929
FAX	76
Grand Total	**2,685**

There were 144 hemoglobin carrier requests in 2025. Of the 31 carriers, all were from high-risk populations or where risk status was not indicated.

Fewer than 1% of carriers request their results with the number of hemoglobin carrier requests remaining low compared to the number of carriers. There was a task force of the NSO-AC that examined different carrier disclosure models that could be considered in Ontario due to the low uptake in carrier requests. NSO has responded to the report from that task force by submitting a proposal to the Ontario Ministry of Health with a plan to implement the recommended changes. These changes are anticipated to be completed towards the end of 2026.

2.3 Cystic Fibrosis

There were 47 referrals this year compared to 54 in 2024. The Type 1 referrals have genotypes consistent with a high risk of a diagnosis of CF, the Type 2 referrals have genotypes consistent with a high risk for a *CFTR* -related disorder NOT meeting CF diagnostic criteria and the Type 3 referrals have genotypes of uncertain clinical significance.

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Table 17. The PPV calculations for the current and past screening algorithms.

Additional information	PPV (Primary)	PPV (Primary + Variant)	PPV (Primary + Variant + Secondary)	% of Outcomes Unknown
Past (Jul 28, 2019 - Mar 18, 2020) Cat A	83.3%	100.0%	100.0%	0.0%
Past (Jul 28, 2019 - Mar 18, 2020) Cat B	2.1%	10.3%	10.3%	1.3%
Past (Jul 28, 2019 - Mar 18, 2020) Cat C	0.0%	1.7%	1.7%	1.4%
Past (until Mar 18, 2020) ALL	8.1%	15.3%	15.3%	1.2%
Current (Mar 19, 2020 - Dec 31, 2025) Type 1	95.7%	99.3%	99.3%	0.0%
Current (Mar 19, 2020 - Dec 31, 2025) Type 2	1.3%	98.7%	98.7%	1.3%
Current (Mar 19, 2020 - Dec 31, 2025) Type 3	7.6%	74.8%	74.8%	0.0%
Current (Mar 19, 2020 - Dec 31, 2025) ALL	**43.1%**	**90.4%**	**90.4%**	**0.3%**

2.4 ENDOCRINOLOGY

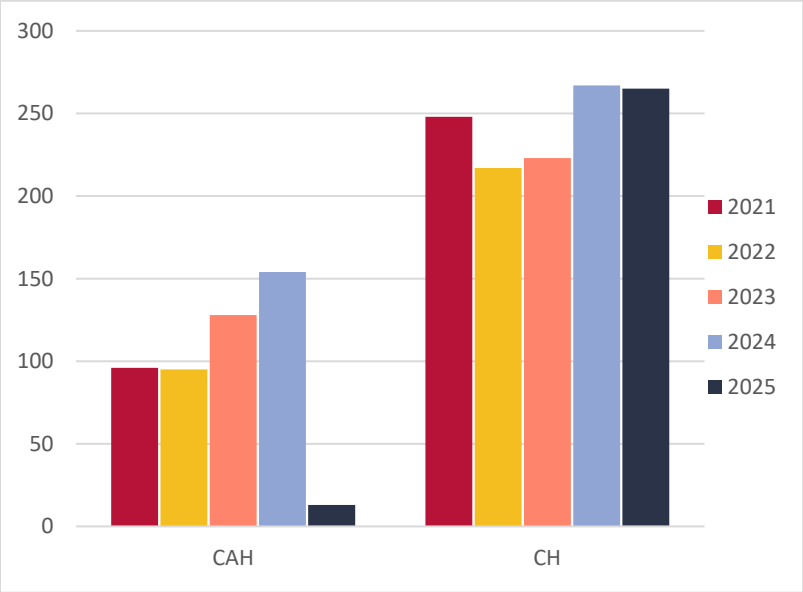


Figure 8. The total number of congenital adrenal hyperplasia and congenital hypothyroidism screen positives between 2021-25.

The number of screen positives for CAH decreased dramatically due to the addition of 21-deoxycortisol into the screening algorithm on January 7, 2025. Since the change took place there have been no false positive referrals and the number of referrals decreased from 154 in 2024 to 13 in 2025. There have been no reported false negatives during this time. The number of screen positives for CH was similar to 2024.

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Table 18. The PPV calculations for the current and past screening algorithms.

Disease	Additional information	PPV (Primary)	PPV (Primary + Variant)	PPV (Primary + Variant + Secondary)	% of Outcomes Unknown
Congenital Hypothyroidism	Past (Jun 12, 2018 - Jul 3, 2019)	16.0%	23.8%	23.8%	0.2%
	Current (Jul 4, 2019 - Dec 31, 2025)	**20.2%**	**38.9%**	**38.9%**	**0.1%**
Congenital Adrenal Hyperplasia	Past (Jun 12, 2018 - Jan 6, 2025)	4.0%	6.1%	6.5%	0.6%
	Current (Jan 7, 2025 - Dec 31, 2025)	**100.0%**	**100.0%**	**100.0%**	**0.0%**

2.5 METABOLIC

On September 29, 2025, NSO moved to a non-butylated method of screening for many of the metabolic disorders. The change allowed NSO to measure C26:0-LPC as a first-tier marker for X-linked adrenoleukodystrophy (XALD) and argininosuccinic acid as a first-tier marker for ASL deficiency. After the method change an increase in certain referrals was observed:

1. a slight negative bias was observed for C0 resulting in an increase in screen positive referrals for carnitine uptake disorders (CUD). To address the analytical shift, the C0 screening thresholds for CUD were decreased as of November 22, 2025.
2. an increased mean values for methylcitric acid (MCA) lead to an increase in the number of propionic acidemia and methylmalonic acidemia. On October 20, 2025, the MCA cutoff was increased.
3. glutaric acidemia type 1 (GA1) referrals increased prompting a first-tier disorder logic change on December 8, 2025.

A pilot for XALD began on March 5, 2025, using a 3-tier algorithm; very long-chain fatty acids are measured on tiers one and two and a count of the ABCD1 locus on tier three. During the initial pilot approximately 300 random samples were screened daily for XALD. On September 29, 2025, the pilot transitioned to a full population screen. XALD was officially added to the Ontario newborn screening panel in January 2026. As such, screen positive outcomes will be reported in the 2026 annual report.

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Table 19. The PPV calculations for the current and past (where applicable) screening algorithms.

Disease	Additional information	PPV (Primary)	PPV (Primary + Variant)	PPV (Primary + Variant + Secondary)	% of Outcomes Unknown
GAMT Deficiency		**9.1%**	**9.1%**	**9.1%**	**0.0%**
Glutaric Aciduria type 1	Past (until Dec 7, 2025)	**9.3%**	**9.7%**	**22.7%**	**0.0%**
	Current (Dec 8 - Dec 31, 2025)	-	-	-	
Isovaleric Acidemia	Past (until Feb 17, 2020)	3.0%	4.2%	4.2%	0.0%
	Current (Feb 18, 2020 - Dec 31, 2025)	**18.0%**	**18.0%**	**18.0%**	**0.0%**
PA/MMA/CblA/CblB	Past (Apr 22, 2013 - Sep 28, 2025)	4.2%	4.4%	8.8%	2.7%
	Current (Dec 1, 2025 - Dec 31, 2025)	**0.0%**	**0.0%**	**0.0%**	**13.3%**
Fatty Acid Oxidation Defects - Other		**9.6%**	**51.4%**	**52.4%**	**0.5%**
LCHAD/TFP		**82.4%**	**82.4%**	**94.1%**	**0.0%**
VLCAD	Past (until Dec 14, 2021)	7.1%	12.1%	13.9%	0.7%
	Current (Dec 15, 2021 - Dec 31, 2025)	**7.4%**	**15.6%**	**16.4%**	**0.0%**
CUD	Past (Mar 5, 2014 - Sep 28, 2025)	9.3%	9.7%	9.7%	0.7%
	Current (Nov 21, 2025 - Dec 31, 2025)	-	-	-	
MCAD	Past (Sep 1, 2016 - Jul 28, 2019)	18.8%	20.1%	21.5%	0.6%
	Current (Jul 29, 2019 - Dec 31, 2025)	**53.9%**	**68.7%**	**68.7%**	**0.0%**
Citrullinemia/ASA		**16.2%**	**21.1%**	**21.1%**	**0.4%**
Homocystinuria	Past (until Jul 29, 2019 - July 21, 2024)	3.0%	3.0%	27.0%	0.0%
	Current (Jul 22, 2024 - Dec 31, 2025)	**7.0%**	**7.0%**	**7.0%**	**0.0%**
Phenylketonuria	Past (until Jul 28, 2019)	14.3%	27.5%	27.5%	0.0%
	Current (Jul 29, 2019 - Dec 31, 2025)	**19.3%**	**47.2%**	**47.2%**	**0.0%**
MSUD	Past (until Nov 14, 2011)	2.6%	2.6%	2.6%	0.0%
	Current (Nov 15, 2011 - Dec 31, 2025)	**10.3%**	**11.6%**	**11.6%**	**0.6%**
Tyrosinemia	Past (Sep 14, 2009 - Sep 19, 2011)	1.4%	1.4%	1.4%	0.0%
	Current (Sep 20, 2011 - Dec 31, 2025)	**9.2%**	**9.2%**	**11.5%**	**1.1%**
Galactosemia	Past (Jan 13, 2014 - Feb 10, 2025)	19.5%	38.2%	38.2%	0.0%
	Current (Feb 11 - Dec 31, 2025)	**12.5%**	**12.5%**	**12.5%**	**0.0%**
Biotinidase Deficiency	Past (Jan 13, 2014 - Jul 2, 2014)	2.0%	36.7%	36.7%	0.0%
	Current (Jul 3, 2014 - Dec 31, 2025)	**7.4%**	**44.8%**	**44.8%**	**0.4%**
MPS1H		25.0%	62.5%	62.5%	10.7%

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2.6 IMMUNOLOGY

The number of screen positive referrals for SCID decreased from 33 in 2024 to 28 in 2025.

Table 20. The PPV calculations for the current and past screening algorithms.

Additional information	PPV (Primary)	PPV (Primary + Variant)	PPV (Primary + Variant + Secondary)	% of Outcomes Unknown
Past (Jan 6, 2020 - Feb 28, 2021)	19.0%	19.0%	19.0%	0.0%
Current (Mar 1, 2021 - Dec 31, 2025)	**13.9%**	**14.8%**	**15.7%**	**0.8%**

2.7 NEUROLOGY

Since screening began in 2020, the number of screen positive referrals for SMA has fluctuated by year, ranging from 6 referrals in 2024 and 2020, to 19 referrals in 2023. In 2025, there were 14 referrals.

Table 21. The PPV calculations for the current screening algorithm.

PPV (Primary)	PPV (Primary + Variant)	PPV (Primary + Variant + Secondary)	% of Outcomes Unknown
100.0%	**100.0%**	**100.0%**	**0.0%**

2.8 CARDIOLOGY

There were 168 CCHD screen positives in 2025, just up from 157 in 2024. Most were screened within 24-48 hours of life. The 6 true positives were screened between 24-36 hours of age.

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Table 22. Definitive diagnosis for CCHD Screen Positives (individual years and cumulative)

Definitive Diagnosis Categorization	2025	2024	2023	2022	2021
Primary target	6	<5	<5	6	9
Secondary target	98	85	89	61	69
Incidental Finding	18	30	27	31	38
Not affected	46	38	48	49	51
Lost to follow up		0	0	<5	0
Grand Total	168	157	168	148	167

* In December 2024 the decision was made to add critical pulmonary or aortic valve stenosis to the list of secondary conditions.

Of the 168 screen positives received in 2025, 6 were diagnosed with a critical congenital heart defect, 116 had a secondary CHD target or were diagnosed with an incidental finding such as pulmonary disease or infection, and 46 were found to be not affected.

2.8.1 CCHD Definitive Diagnosis Data and Positive Predictive Values

In 2025, the Positive Predictive Value (PPV) for CCHD screening was 3.57% for primary targets and 61.90% for primary and classical secondary target diseases. Cumulatively since the beginning of the program, the PPV is 4.65% for primary targets, and 42.72% for primary and classical secondary target diseases. Of the 1,444 screen positives since the initiation of CCHD screening (the lost to follow up DERFs have been excluded from analysis), 536 (37.17%) have been determined to be not affected after diagnostic follow up.

Data from a recent publication examining prenatal identification of CCHD was 69.8% in Alberta and Ontario (Eckersley et al, *Ultrasound Obstet Gynecol* 2025; **66**: 606–613). This is higher than what was predicted when CCHD screening was implemented which also corresponds with data from the paper that indicates that the prenatal detection of CCHD rose almost 10% from mid 2015 to 2022. This may account for some of the decreasing detection of CCHD by screening as outlined in the table below.

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Table 23. PPV calculations for CCHD Screen Positives 2019-25 and cumulative.

Data set	PPV (Primary)	PPV (Primary + Secondary)	Total No. Screen Positive
2019	9.00%	30.50%	167
2020	5.60%	30.10%	197
2021	5.40%	46.70%	167
2022	4.08%	45.58%	148
2023	2.38%	55.36%	168
2024	2.55%	56.69%	157
2025	3.57%	61.90%	168
Cumulative	4.65%	42.72%	1,444

2.9 HEARING

Table 24. Number of risk factor screen positive babies between 2021-25.

Risk Factor	2025 # screen positives (% rate)	2024 # screen positives (% rate)	2023 # screen positives (% rate)	2022 # screen positives (% rate)	2021 # screen positives (% rate)
CMV	178 (0.13%)	172 (0.13%)	184 (0.13%)	190 (0.13%)	140 (0.10%)
Genetics	35 (0.025%)	31 (0.023%)	43 (0.031%)	37 (0.027%)	32 (0.022%)

The table above shows the number of risk factor screen positive infants for the last six years. In 2025, there were 178 CMV screen positive infants. The CMV screen positive rate was 0.13%. There was an increase in 6 cases from the year previously.

There were 35 infants with genetic screen positive results in 2025. Since October 19, 2020, reflexive screening for the GJB2 p.(V37I) variant has been performed for infants who have a single GJB2 variant identified on the common variant panel use for genetic risk factor screening. The GJB2 p.(V37I) variant is therefore only reported in compound heterozygous state with another variant on the panel (homozygosity for this variant is not assessed). Improvements to the care pathway for infants who screen positive for genetic risk factors is ongoing, in preparation for reporting infants who are homozygous for the GJB2 p.(V37I) variant in the coming future. This work includes development of genetic counselling modules to support families with timely, evidence-based information.

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There are fewer SLC26A4-releated cases annually than GJB2. This is expected as SLC26A4-related PHL is less common than GJB2/6-related PHL. We continue to evaluate the frequency of variants screened in our population and they are as expected.

2.9.1 CMV DBS screen positive referrals and outcomes

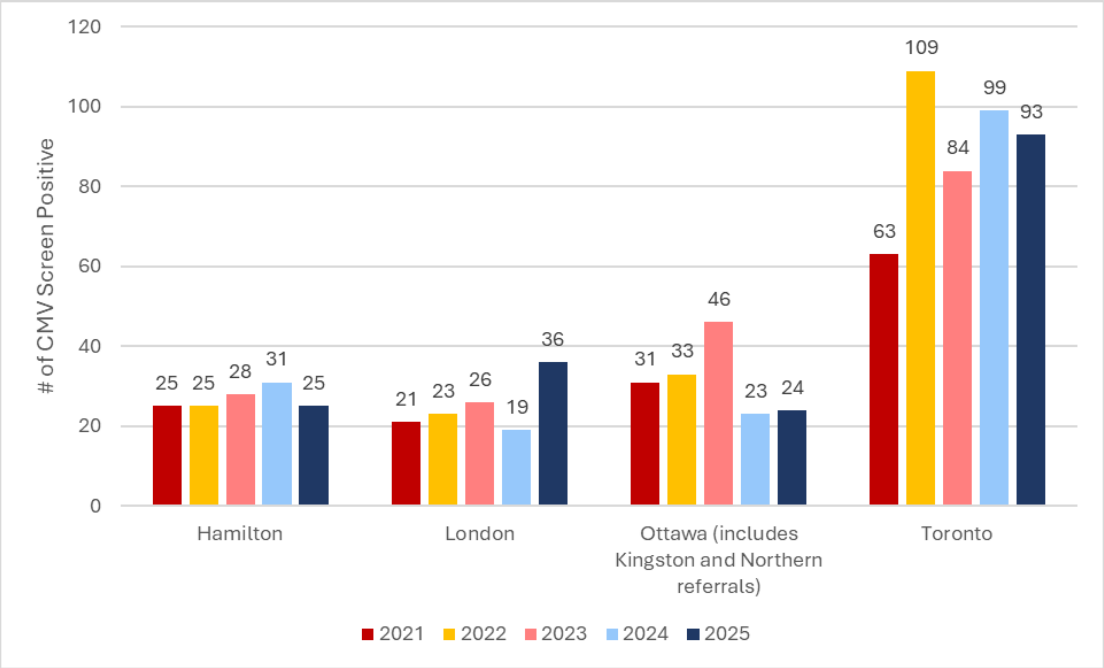


Figure 9. CMV screen positives by referral region

The figure above shows the breakdown of CMV screen positive referrals by region. Similarly to the previous year, Toronto received the largest number of CMV referrals (93/178, 52%), followed by London (36/178, 20%), Hamilton (25/178, 14%) and Ottawa (24/178, 14%). The Ottawa referral region includes the following areas: Ottawa, Kingston and Northern Ontario. This distribution is similar to that observed in 2024; however, London experienced an increase in referrals (17 more than the previous year), for which no clear contributing factors were identified.

The majority of CMV screen positive infants were referred to a community pediatrician for their initial assessment (146/178, 82%). Thirty infants were referred directly to ID for their initial assessment (30/178, 17%). This was an increase from the previous year where only 8% were seen by ID for their initial assessment. Reasons for a direct referral to ID were geographical/travel related, coverage for pediatricians, the infant was hospitalized at time of referral, or the infant had symptoms of cCMV at the time of retrieval. It is unclear from the data what led to this increase in 2025.

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Table 25. CMV screen positive infants

DBS Screening Result	GRAND TOTAL 2025	GRAND TOTAL 2024	GRAND TOTAL 2023
Robust	165	166	173
Borderline	13	6	11
TOTAL	178	172	184

Urine CMV PCR results are available for 174 (98%) of the screen positive infants. Of these, 158 (91%) had positive/detected results. There were 16 cases (9%) where the DBS was positive, but the confirmatory urine CMV PCR results were negative/not detected. On June 26th, 2023, NSO started a new initiative with Public Health Ontario (PHO) where all urine samples were sent to the PHO lab for initial testing and if the result is negative further testing is completed (viral culture and CMV PCR at NSO). Since implementing this initiative there were 62 negative urine results, and all reflexive testing was negative, therefore on February 10th, 2025, this initiative was completed. After the end of the initiative, clinical guidelines and algorithms were updated to assist with establishing a definitive diagnosis in CMV screen positive infants with negative CMV PCR results. All infants that screen positive and have a negative urine result, are now discharged as a false positive result with no further testing completed. This updated guideline allows us to discharge families in a timely manner when their urine results are negative and allows families to have a definitive false positive diagnosis.

NSO introduced a result category of "borderline positive" in 2021 to help parse out the screen positives with weaker viral amplification that may be more likely to have negative urine CMV PCR results. Data from 2025 showed that 33% of borderline screen positives had negative urine CMV PCR results as compared to 7% of robust screen positives.

Table 26. Definitive diagnoses for CMV screen positive infants between 2022-25.

Definitive Diagnosis	2025 Total	2024 Total	2023 Total	2022 Total
Asymptomatic cCMV	**98**	**92**	**123**	**145**
Symptomatic cCMV	**60**	**53**	**36**	**22**
Indeterminate/ Inconclusive	**0**	**<5**	**7**	**6**
cCMV excluded (false positive)	**14**	**18**	**8**	**6**
Non-congenital CMV	**<5**	**<5**	**<5**	**-**
LTFU	**5**	**<5**	**7**	**<5**
Pending	**0**	**<5**	**<5**	**8**
TOTAL	**178**	**172**	**184**	**190**

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Of the CMV screen positive infants with positive confirmatory urine CMV PCR results, 62% (98/157) were deemed to have asymptomatic cCMV infection and 38% (59/157) were classified as symptomatic, with the remainder being lost to follow-up. Based on the literature, we would expect that approximately 10-15% of babies with cCMV would be symptomatic however this data is similar to the year previous when we had 36% of infants classified as symptomatic.

Fourteen (9%) infants with symptomatic cCMV infection were ascertained clinically prior to newborn screening results being available. This underscores the importance of screening, as symptoms of cCMV infection can be subtle and non-specific, making clinical diagnosis a challenge. In the symptomatic group, 9 (15%) infants had PHL identified at the initial diagnostic audiology assessment. The importance of ongoing hearing surveillance must be underscored for all CMV screen positive infants as there is risk of developing PHL for both asymptomatic (~10%) and symptomatic (~30%) cases.

2.9.1.1 CMV DSS screen positive referrals and outcomes

On February 11, 2024, NSO started a pilot to receive dried saliva samples for CMV testing from the following ten birth hospitals; Mount Sinai, St. Michael's, Sunnybrook Health Sciences, Ottawa General Hospital, Ottawa Civic Hospital, Hopital Montfort, Kingston Health Science Centre, Hamilton Health Sciences Centre, St. Joseph's Healthcare, and London Health Sciences Center. The following were possible results; positive/borderline DBS and positive/borderline DSS, positive/borderline DBS and negative DSS, and negative DBS and positive/borderline DSS. The goals of this project are to define the birth prevalence of cCMV in Ontario using DSS, determine the clinical sensitivity of CMV PCR testing of DBS versus DSS, and examine cost effectiveness. This project ended on May 31, 2025, once the supply of cards and swabs were depleted. Overall, 39,112 DSS samples were tested during the pilot, and we are currently preparing a report outlining the sensitivity, specificity, and feasibility of this initiative. Due to the high number of false positive screening results - which placed a considerable burden on families - and the minimal additional diagnostic benefit, it was determined in September 2025 that dried saliva samples would not be adopted as a standard of care for infants in Ontario.

In 2025 there were 13,830 DSS samples received and 42 referrals with screen positive results. A large proportion of infants (96% (25/26)) that screened positive on both the DBS and DSS had confirmed cCMV, however a much smaller proportion (38% (6/16)) of infants that had a positive or borderline DSS had confirmed cCMV.

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2.9.2 Genetic screen positive outcomes

In 2025, there were no genetic screen positive infants from the following IHP regions: Essex Kent, North Eastern, Thunder Bay, or Kenora Rainy River. Referral numbers were generally lower in 2024 than in previous years.

Table 27. Genetic screen positive results and outcomes.

	Genotype Class					
Outcome	Panel/Panel Genotype	Panel/V37I Genotype	TOTAL 2025	TOTAL 2024	TOTAL 2023	TOTAL 2022
Hearing loss	21	<5	25	24	27	26
Surveillance*	0	9	9	7	13	11
Pending	<5	0	<5	0	0	0
LTFU	0	0	0	0	<5	0
TOTAL	22	13	35	31	43	37

* Infants with normal hearing were offered audiologic surveillance in accordance with IHP protocols

Overall, there were 35 genetic risk factor screen positive infants in 2025, which was a slight increase form 2024. There were 22 infants with panel/panel genotypes, and as expected, the majority of these were confirmed to have permanent hearing loss.

There were 13 infants with panel/V37I genotypes. The majority (9/13, 69%) had normal hearing at their initial diagnostic audiology assessment and were enrolled in audiologic surveillance. The proportion of infants with normal hearing at their initial assessment was closer to the 2023 data (in 2023 72% had normal hearing at their first assessment). As more infants are screened, we will learn more about the true risks associated with the V37I variant and will continue to monitor this to ensure we are providing accurate risk estimations to families. Evaluation of audiologic surveillance outcomes of genetic risk factor screen positive infants is underway, with the goal of informing implementation of evidence-based improvements to the IHP surveillance protocol.

2.10 BILIARY ATRESIA

In 2025, NSO made the determination that 17/126 pale stool calls (13%) were screen positive for BA, which is defined as a stool colour assessed to be between #1-6 on the ISCC. These cases were then referred to one of the 5 PAHSC hepatologists for measurement and interpretation of a fractionated bilirubin level. Toronto received the largest percentage (55%) of referrals.

The average age in days at time of referral to a PAHSC was 35 and 70% of referrals happened within 1 day (range 0-4 days) of the family's initial contact with the NSO BA clinical team. After

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referral to a PAHSC, it took an average of 2.2 days (range of 0-11 days) to complete the initial bloodwork.

Of the 7 referred cases that underwent confirmatory testing, 88% were discharged from the PAHSC due to fractionated bilirubin measurements below the threshold for cholestasis. No cases of biliary atresia were identified though the screening program for 2025.

Table 28. The definitive diagnosis classification of the screen positive BA referral cases in 2023-25.

Definitive Diagnosis Categorization	2025	2024	2023
Primary Target – Biliary Atresia	0	<5	<5
Secondary Target- Classic	<5	<5	-
Infection-Other	-	-	<5
Incidental – Idiopathic Cholestasis	-	<5	6
Not Affected	14	34	28
Lost to Follow up	<5	<5	<5
Grand Total	17	43	41

Table 29. The positive predictive value of BA screening.

Year	Positive Predictive Value		Total No. of Screen Positives
	PPV (Primary Target)	PPV (Primary Target + Classic Secondary)	
2023	7.6%	12.8%	41
2024	2.5%	12.5%	43
2025	0%	6.2%	17

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3. SCREENING SYSTEM SUPPORT

3.1 BIOCHEMICAL

NSO receives samples for biochemical testing – both for diagnostic testing and monitoring of affected patients. In 2025, NSO received 6,564 samples from 2,640 patients. This was a decrease of over 1,000 samples compared to last year (7,778). Monitoring samples accounted for 1,385 of the samples received. Screen positive follow up accounted for 816 of the 6,564 samples received.

3.2 MOLECULAR

NSO performs molecular diagnostic testing for targets of newborn screening, nuclear mitochondrial conditions, and primary immune deficiencies. The number of requests have increased annually, with 484 samples received in 2020 to 1,269 in 2024. In 2025, 1,353 samples were received of which 151 were requests for targets of screening following a positive NBS referral to one of the regional treatment centres.

3.3 SURVEILLANCE FOR FALSE NEGATIVES

Discrepant result cases are situations in which an infant either screen positives for a condition with a high PPV and diagnostic investigations are normal without a clinical explanation for the screening results or an infant has a negative NBS but is picked up clinically (either symptomatic or through familial cascade testing) to have a condition for which NSO screens. NSO tracks discrepant results for all the screen program – dried blood spot, CCHD and BA.

3.4 RESEARCH

NSO's research program focuses on program development and evaluation, developing novel laboratory methods and clinical biomarkers, most recently using metabolomics and genomics, studying policy, and clinical research in newborn screening. NSO is involved in approximately 12 research projects, 8 of which are led by NSO. NSO's involvement varies depending on the project, such as secondary use of NSO data and data analysis, secondary use of NSO samples, testing of external samples, review and analysis of screening data, and pilot newborn screening studies. Where needed, NSO research studies are reviewed and approved by the CHEO Research Ethics Board, and studies using residual newborn screening samples are performed in accordance with NSO's policy on Storage and Secondary Use of Newborn Screening Samples.

Information about NSO's research, recent publications, and opportunities to participate in NSO-led studies are now posted on NSO's website under About us > Current Research (Current Research |

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Newborn Screening Ontario). NSO scientists and research team contributed to 7 publications in 2025.

Below are some of NSO's study activities and publications from 2025, as well as upcoming activities:

SMA screening cost-utility model and analysis
A cost-utility analysis of newborn screening for SMA in Canada was published this year. The study showed that screening and early treatment of SMA in Canada is cost-saving compared to not screening. This provides critical Canadian data on the cost-effectiveness of SMA screening and treatment, aiding policy and decision-makers.

Congenital CMV ANd HEARing in Ontario: Optimizing Screening to Improve Child Health Outcomes (CAN HEAR Ontario)
Dried saliva specimens (DSS) were collected alongside the standard of care dried blood specimen (DBS) from more than 40,000 babies born in selected hospitals in Ontario from February 2024 to May 2025 to compare newborn screening methods for CMV. Babies who had a DSS sample and DBS sample collected and screened positive on either sample type had the opportunity to participate in a long-term follow-up research study where clinical information is collected about the infants over time. Recruitment for the study began in 2024 and concluded in 2025. Study activities which include data collection through study visits, surveys and review of investigation results that are stored at NSO are currently underway. Evaluation of the results of the quality improvement project are underway, with publications anticipated in 2026. DSS will not be added to NSO's CMV screening protocol at this time.

Canadian Population Screening for Risk of Type 1 Diabetes Research Consortium (CanScreenT1D)
CanScreenT1D (Home - CanScreen T1D Research Consortium) is a research consortium led by SickKids Hospital focused on studying how to create Canada's first screening program for Type 1 Diabetes (T1D). NSO is co-leading the pilot of the T1D screening system, which is based upon either genetic risk score or autoantibody testing and surveillance. This pilot study is being done to understand the acceptability and feasibility of T1D screening and to generate evidence to support policy decisions around its implementation. CanScreenT1D projects informing the development of the pilot study progressed in 2025, and a pilot protocol was drafted. Recruitment for the pilot is expected to begin in 2026.

NBS Target Chart Review/MPS I Case Series
Research Ethics Board (REB) approval has been obtained to conduct retrospective chart reviews of patients who screened positive for and/or were diagnosed with a newborn screening (NBS) targeted condition. This protocol aims to characterize the overall health status and clinical features of these individuals, comparing those who experienced morbidity and/or mortality with those who

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did not. The protocol can be leveraged for program evaluations and publications, with the correct permissions.

A retrospective chart review is currently underway, focusing on the first five years of MPS I screening in Ontario. This analysis will help evaluate how effectively NBS meets its long-term objectives in this population—specifically, improvements in health, growth, development, and functional outcomes.

First years of GAMT newborn screening - publication

A manuscript on the first years of GAMT newborn screening is underway.

Identifying at risk Newborns From the Analysis of NGS Testing (INFANT)

Kristin Kernohan, Director of the Molecular Diagnostic laboratory at NSO, is leading a project entitled Identifying at risk Newborns From the Analysis of NGS Testing (INFANT). This study, funded by a $12M grant from Genome Canada is part of the Canadian Precision Health Initiative (CPHI). INFANT is evaluating the use of next generation sequencing in newborn screening (also called genetic Newborn Screening or gNBS). INFANT has four study activities: 1. developing a gNBS panel of diseases and variants to be used for targeted genome sequencing analysis; 2. genome sequencing of 9,000 unselected infants and associated data collection (standard of care newborn screening results, health records, metabolomics); 3. genome sequencing of 1,000 infants who have screened positive on standard of care newborn screening, to assess sensitivity of gNBS for those conditions; and 4. contributing data to the Pan Canadian Genome Library (PCGL). In 2025, a gNBS panel of diseases and variants was finalized (Activity 1), and protocols and processes were developed for Activities 2 and 3. Recruitment and enrolment began in 2026. The study will conclude in 2029 and will provide valuable information to NSO on introduction of gNBS.

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Appendix A: Classifications of True/False Positives

NSO has developed a classification system for true positives to take into account the variability of definitive diagnoses and the impact of variant conditions and incidental findings. The definitions are as follows:

Table 1A. The definitions of the classification of true positive.

True Positive?	Definition	Example
Primary	confirmed diagnosis of a targeted condition	Classical PKU
Not Affected	confirmed to be NOT affected by a target or related disease	Not Affected
Other	lost to follow up; family refused follow up; infant deceased prior to completion of diagnostic evaluation	Deceased
Variant	confirmed diagnosis of a variant of the targeted condition	CF indeterminate or gray zone
Incidental	not affected by target or variant disease but not unaffected; affected with secondary target or other condition; carriers; reason intrinsic to baby or mother that caused the baby to screen positive	Vitamin B12 deficient (PA/MMA screen positive), maternal Grave's disease (CH screen positive)

The category of incidental is a large group – consisting of reasons due to mom and baby. Now that the DERF information is captured in BORN, we have added additional classifications to allow for more useful data extractions in the future.

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Table 2A. The true positive categories.

True Positive Categories	
Generic	Detailed
Not Affected	Not Affected
Primary	Primary Target – Classic
Variant	Primary Target – Variant or Indeterminate
Incidental	Secondary Target – Classic
	Secondary Target – Variant or Indeterminate
	Untargeted Disease
	Persistent Laboratory Abnormalities
	Carrier
	Maternal Disease
	Maternal Persistent Laboratory Abnormalities
Other	Lost to Follow Up
	Deceased
	Other
Twin	Twin (Screen Negative)

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Appendix B: Disease Prevalence and PPV for High PPV Referrals

High PPV referrals are referrals where the screening values are suggestive of disease. These referrals are classified as semi-urgent or urgent and are sometimes also alert referrals with same day confirmation testing.

Table 1B. The disease prevalence rates (including both screen positive and missed cases) for each primary target screened by NSO via dried blood spot screening and positive predictive value calculations for high PPV referrals.

Diseases	Date Screening Initiated	% of Outcomes Unknown	Disease Prevalence of Primary Targets	Positive Predictive Value (PPV) for High PPV Referrals
Congenital Hypothyroidism (CH)	04-Apr-06	0.4%	1 in 2,243	83%
Congenital Adrenal Hyperplasia (CAH)	14-May-07	0.6%	1 in 22,411	35%
Sickle Cell Disease	24-Nov-06	0.6%	1 in 2,704	95%
Cystic Fibrosis (CF)	09-Apr-08	0.4%	1 in 4,749	77%
Severe Combined Immune Deficiency (SCID)	12-Aug-13	0.1%	1 in 51,008	45%
Glutaric Aciduria type 1 (GA1)	09-Aug-06	0.0%	1 in 139,839	100%
Guanidinoacetate Methyltransferase Deficiency (GAMT)	17-Oct-22	0.0%	1 in 465,341	14%
Isovaleric Acidemia (IVA)	09-Aug-06	0.0%	1 in 147,199	61%
Propionic Acidemia (PA)	09-Aug-06	1.7%	1 in 215,136	38%
Methylmalonic Acidemia (MMA)			1 in 155,376	
Cobalamin A Deficiency			1 in 932,258	
Cobalamin B Deficiency			1 in 1,398,387	
Long-chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD)	09-Aug-06	0.0%	1 in 215,136	89%
Trifunctional Protein Deficiency (TFP)			1 in 2,796,774	
Very-long-chain Acyl-CoA Dehydrogenase Deficiency (VLCAD)	09-Aug-06	0.4%	1 in 75,588	61%
Carnitine Uptake Defect (CUD)	09-Aug-06	0.3%	1 in 66,590	21%
Medium-chain Acyl-CoA Dehydrogenase Deficiency (MCADD)	04-Apr-06	0.2%	1 in 15,551	88%
Citrullinemia (CIT)	09-Aug-06	0.4%	1 in 116,532	40%
Argininosuccinic Acid Lyase Deficiency (ASA)			1 in 215,136	
Homocystinuria due to Cystathionine beta-synthase (CBS) deficiency	09-Aug-06	0.3%	1 in 699,194	3%
Phenylketonuria (PKU)	04-Apr-06	0.0%	1 in 17,143	67%
Maple Syrup Urine Disease (MSUD)	09-Aug-06	0.9%	1 in 116,532	30%
Tyrosinemia type 1	09-Aug-06	0.5%	1 in 279,677	71%
Galactosemia (GALT)	19-Feb-07	0.5%	1 in 51,353	22%
Biotinidase Deficiency (BIOT)	19-Feb-07	0.3%	1 in 56,702	21%
Mucopolysaccharidosis type 1 Hurler (MPS1H)	27-Jul-20	10.7%	1 in 129,814	25%
Spinal Muscular Atrophy (SMA)	13-Jan-20	0.0%	1 in 12,980	100%

*Diseases with >10% of DERFs pending are highlighted in red.

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Appendix C: Glossary

17OHP = 17-hydroxyprogesterone
AAAC = amino acid and acylcarnitine
ASA = argininosuccinic acid lyase deficiency
BA = biliary atresia
BIOT = biotinidase deficiency
CAH = congenital adrenal hyperplasia
CblA = cobalamin A defects
CblB = cobalamin B defects
CBS = Cystathionine beta-synthase deficiency
CCHD = critical congenital heart disease
cCMV = congenital cytomegalovirus
CF = cystic fibrosis
CH = congenital hypothyroidism
CHD = congenital heart defect
CHEO = Children's Hospital of Eastern Ontario
Cit = citrullinemia
CMV = cytomegalovirus
CPT1 = carnitine palmitoyltransferase type 1
CPT2 = carnitine palmitoyltransferase type 2
CUD = carnitine uptake disorder
DBS = dried blood spot
DERF = diagnostic evaluation report form
ENDO = endocrinology
FAOD = fatty acid oxidation defects/disorders
GA1 = glutaric acidemia type 1
GA2 = glutaric acidemia type 2
GALT = galactosemia
GAMT = guanidinoacetate methyltransferase
GI = gastroenterologist
GUAC = guanidinoacetate
HbS/β Th = sickle cell beta-thalassemia
HbSC = hemoglobin sickle C Disease
HbS/HPFH = sickle hemoglobin and hereditary persistence of fetal hemoglobin
HbSS = Sickle Cell Disease
HCP = health care provider
HCY - homocystinuria
HGB = hemoglobinopathy
HHSC = Hamilton Health Sciences Centre
HSC = The Hospital for Sick Children
ID = infectious disease
IHP = Infant Hearing Program
ISCC = infant stool colour card
IV = intravenous
IVA = isovaleric acidemia
KGH = Kingston General Hospital
KP = Kasai procedure
LCHAD = long chain 3-hydroxyacyl-CoA dehydrogenase deficiency
LC-MS/MS = liquid chromatography with tandem mass spectrometry
LHSC = London Health Sciences Centre
LTFU = lost to follow up
MCA = methylcitric acid
MCAD = medium chain acyl-CoA dehydrogenase deficiency
MCCSS = The Ministry of Children, Community and Social Services
MET = metabolic
MMA = methylmalonic acidemia
MPS1 = mucopolysaccharidosis type 1
MPS1H = mucopolysaccharidosis type 1 Hurler
MSUD = maple syrup urine disease
NBS = newborn screen
NICU = neonatal intensive care unit
NSO = Newborn Screening Ontario
NSO-AC = Newborn Screening Ontario Advisory Council
PA = propionic acidemia
PAHSC = Pediatric Academic Health Science Centre
PCR = polymerase chain reaction
PHL = permanent hearing loss
PHO = Public Health Ontario
PKU = phenylketonuria
PPHN = persistent pulmonary hypertension
PPV = positive predictive value
RTCs = Regional Treatment Centres
RN = registered nurse
SCID = severe combined immune deficiency

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SMA = spina muscular atrophy
TAT = turn around time
TFP = trifunctional protein deficiency
TLU = test level unsatisfactory
TPN = total parenteral nutrition
TSH = thyroid stimulating hormone
TYR = tyrosinemia
TYR1 = tyrosinemia type 1
Unsat = unsatisfactory
VLCAD = very long chain acyl-CoA dehydrogenase deficiency
XALD = X-linked adrenoleukodystrophy

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