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

Annual Report to the Newborn Screening Ontario Advisory Council Calendar Year 2021

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Executive Summary

The second year of COVID-19 brought the promise of vaccines and a hopefulness for the end of the pandemic. Amid tentative reopening of public activities, between repeated waves and new strains of the virus emerging, the stress on health care human resources has become a common theme. In addition to fatigue, the isolation and testing requirements for symptomatic staff and contacts presented challenges for staffing in the NSO laboratory during the peak times, but ultimately did not impact patient care. However, NSO indirectly experienced the impacts of staff shortages, with delays in shipping, supply chain interruptions, virtual equipment service, and incomplete diagnostic feedback. The day-to-day operations of the program were complicated by the prolonged impacts of COVID-19, requiring much more frequent troubleshooting and intervention to ensure maintenance of a quality service.

Supporting this is evidence that no major changes to quality indicators were observed in 2021; indeed changes were primarily improvements which are documented in this report. The number of babies screened in Ontario has returned to pre-COVID-19 levels, and yet the rates of unsatisfactory samples or missed screens have dropped. There are fewer screen positives from blood spot screening, owing to the first full year with the new Cystic Fibrosis screening algorithm, and other algorithm changes aimed at improving positive predictive value of screening. It is also the first full year of SMA and MPS1 screening. The timeliness of screening results continues to improve, but some challenges remain. Molecular testing was much more severely impacted by supply chain and quality issues during the year, causing increased delays for those diseases with molecular assays.

In the CCHD screening program, missed screens have decreased substantially, and more babies are being screened in the most appropriate timeframe. The rate of false positives has decreased as all sites have discontinued the use of third-party interpretation software, as per NSO recommendation. While the number of primary target true positives remains constant, there was an increase this year in secondary targets identified, which are babies in need of care that might have otherwise been discharged home.

The Infant Hearing Risk Factor Screening program continues to operate under a waiver of consent due to the challenges of ensuring full coverage for the Infant Hearing Program during the pandemic. The number of screen positives for CMV remains lower than expected in the population and has not changed significantly during the pandemic. Of the 140 cases referred for CMV, 91% were confirmed by urine CMV testing and of those 14% were symptomatic. Of the symptomatic cases, less than 40% were identified prior to screening, highlighting the importance of the risk factor screen for identifying both symptomatic and asymptomatic babies. The genetic risk factor screen produced a higher number of screen positives, as it was the first full year of reflexive testing for the *GJB2* p.(V37I) variant.

The lack of significant impacts, and rather the slow and steady improvements to NSO's quality indicators throughout this report, are a testament to the hardworking staff in the NSO laboratory and program, as well as the referral centres, who persevered through the challenges of the second pandemic year to provide high quality care to the infants of Ontario.

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1. Screening Samples in 2021

Table 1. Screening sample volumes between 2017-2021.

Sample Type	2021	2020	2019	2018	2017
Satisfactory	145,785	141,548	146,099	145,724	145,405
Unsatisfactory*	1,560	1,785	1,356	1,365	2,248
Routine Screening – Total	**147,345**	**143,333**	**147,455**	**147,089**	**147,653**

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

1.1 Screening Samples

The overall number of samples received by NSO in 2021 is comparable to pre-COVID years, as is the unsatisfactory rate.

1.1.1 Infants Screened

The total number of samples received for newborn screening purposes only is depicted in Figure 1, along with the number of infants screened. The number of infants tested is an estimate which may 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.

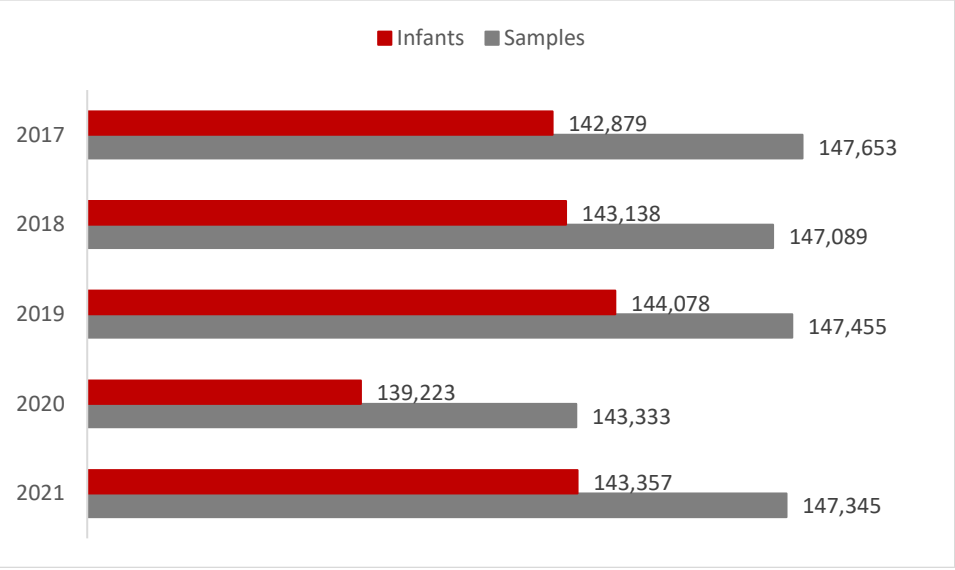

Figure 1: Total number of infants and samples screened between 2017-2021.

The overall number of infants tested has returned values observed in the years prior to COVID. Based on defers/ declines (Section 1.1.2), missed screen alerts and deceased infants from BORN (Section 1.1.3), and newborn screening sample counts (Table 1), NSO estimates the total number of infants in Ontario as 143,888

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and the rate of screening uptake in 2021 as 99.6% (compared to 139,750 infants and the same screening uptake of 99.6% in 2020).

1.1.2 Declined/Deferred Testing

If parents wished 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 2021, NSO received 819 completed decline/defer forms (Table 2), a continued increase from previous years. The number of declines documented using this form has increased with 96 declines in 2021 compared with 76 in 2020. The remaining 723 forms received indicated a parent's desire to defer screening, and samples were eventually received for all but 10 of these deferred cases. The COVID-19 pandemic may have contributed to the increase in newborn screening deferrals, as some families were opting for shorter stays in hospital. When these families were discharged home <24h, some may have chosen to defer their screen, avoiding a <24h collection.

Table 2. Declined, deferred samples indicated on card between 2017-2021.

Case Type	2021	2020	2019	2018	2017
Declined/deferred form received	819	713	607	603	499
Decline	96	76	68	62	50
Deferral	723	637	539	541	449

Table 3. Overall declined screens between 2017-2021.

Infants with declined newborn screening test				
2021	2020	2019	2018	2017
137	136	131	120	127

An additional 80 declined screens were also identified via missed screen alerts. There were 33 infants who were identified as declining through the missed screen process and then a decline form was also received and 6 infants where a sample was received. In total there were 137 infants with declined newborn screening tests (Table 3). There were 92 families that declined the DBS screen but had the CCHD screen.

1.1.3 Missed Screens

There were 415 potential missed cases logged that were not truly missed. There were 73 deceased/palliative cases logged (double from last year) and 80 declines (higher than last year). There were many more cases where the sample was collected and received either the same day as the missed screen alert or after. Of these

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cases, 98 of the samples were batched by the submitter, 58 experienced shipping delays by Purolator, and 7 were both batched and also had Purolator shipping delays.

In 2021, there were 148 true missed newborn screen alerts that required follow up by NSO. Hospitals were the responsible facility in 84% of the missed screen alerts and midwives were involved in roughly 16% of the cases. Of the 148 cases counted as true misses, 26 were due to expired cards from one institution. Instead of submitting samples on expired cards, the institution discharged patients with no documentation sent to NSO. NSO has since conducted submitter education regarding card stock management. Action on the part of NSO resulted in 97 of the 148 (66%) truly missed screens being completed. While slightly lower than previous years, is comparable to the rates in the previous two years.

1.1.4 Hemoglobin Carriers

Table 4. Hemoglobin carrier requests between 2017-2021.

	2021	2020	2019	2018	2017
Requests from high risk population	unknown	23	35	46	61
Total Requests	**49**	**32**	**40**	**55**	**69**
Number of carriers	17	12	16	18	18

Table 5. Carriers identified in 2021.

HGB Pattern	Carriers Identified
FAC	368
FAD	237
FAE	242
FAS	1373
FAX	117
Grand Total	**2,337**

In 2021, approximately 0.7% of carriers requested their results. The number of hemoglobin carrier requests has increased over the last year, however, is still low compared to the number of carriers. The way hemoglobin carrier requests are logged was changed in 2021. Therefore, the number of requests from high risk populations was unknown. This will be captured in 2022.

The NSO-AC struck a task force in 2020 to examine different carrier disclosure models that could be considered in Ontario due to the low update in carrier requests. While the task force is looking at Sickle Cell Disease in particular, the modeling could be applied to other conditions screened by NSO, such as Cystic Fibrosis and MPS1H. The task force work should be complete in 2022.

1.1.5 Age at Collection

Table 6. Age at collection for 2019-2021, initial samples only.

Age at Collection	Number of Initial Samples (2021)	% of Initial Samples (2021)	% of Initial Samples (2020)	% of Initial Samples (2019)
Less than 24 hours	852	0.59%	0.66%	0.69%
24-47 hours (1-2 days)	140,588	98.09%	97.48%	96.36%
48-71 hours (2-3 days)	1,279	0.89%	1.34%	1.99%
72-168 hours (3-7 days)	456	0.32%	0.38%	0.50%
Greater than 168 hours (7 days)	156	0.11%	0.14%	0.46%

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The majority of newborn screening samples are collected between 24-48 hours of age. Greater than 98% of samples are collected by 48 hours of age (Table 6). There has been a positive shift towards samples being collected between 24-48 hours of age following the official change to NSO's recommended age of collection in January 2017.

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2. Unsatisfactory Samples

Table 7. Unsatisfactory samples by reason between 2017-2021.

			2021	2020	2019	2018	2017
SAMPLES	Satisfactory Samples		145,220	143,333	146,099	145,045	144,717
	Unsatisfactory Samples		2,125	2,332	2,044	2,044	2,936
	Unsatisfactory Rate		**1.44%**	**1.63%**	**1.40%**	**1.41%**	**1.99%**
	Samples Collected at <24hrs		565	547	697	575	577
	Unsatisfactory Samples excluding <24hr samples		1,560	1,785	1,347	1,469	2,359
	Unsatisfactory Rate excluding <24hr samples		**1.06%**	**1.25%**	**0.90%**	**1.01%**	**1.60%**
REASONS	Lab Unsat Reasons	Quantity of blood insufficient	927	1,297	919	710	1,471
		Blood spots appear scratched or abraded	142	94	118	292	531
		Blood spots are supersaturated	35	42	97	176	185
		Blood spots appear clotted or layered	217	155	202	403	639
		Blood spots appear diluted	<5	<5	<5	<5	5
		Blood spots exhibits serum rings	96	70	82	168	200
		Blood spots are wet and/or discolored	9	14	10	38	<5
		Other	24	25	50	88	62
	Data Unsat Reasons	Blood dot collection paper is expired	54	38	14	12	77
		Insufficient data provided	<5	11	9	11	29
		Damaged or delayed in transit	6	5	5	45	8
		Delivered to lab > 14 days after collection	38	33	19	8	23
		Sample collected at <24hrs	565	547	697	575	577
		Other/Mislabel	22	27	6	90	47

There were 12 samples that were deemed unsatisfactory for both a lab and a data unsat reason. There were 174 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 1,951 unsatisfactory samples that required follow up.

Of the 565 samples collected at <24 hours, the subsequent samples for these infants indicated a transfusion was given for 132 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).

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2.1 Sample Quality – Laboratory Unsats

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.

The unsatisfactory rate increased in 2020, due in large part to an increase in laboratory unsats due to insufficient quantity of blood (Table 8). In July 2020, NSO started screening for Mucopolysaccharidosis Type 1H (MPS1H). The addition of MPS1H to the panel led to a minimum of 2 additional sample punches required to complete a full newborn screen. In October 2020, the first tier assay of the MPS1H screen was updated to be run without replication, reducing the minimum number of punches required to complete a full screen. The insufficient quantity of blood unsat numbers for 2021 are similar to previous years

2.2 Repeat Rates for Unsatisfactory Specimens

The majority (80%) of repeat samples are received within 2 weeks of the initial sample (Table 8). By 6 weeks, 90.4% of unsatisfactory samples have had screening completed via a repeat sample.

Table 8. Repeats received on unsatisfactory samples from 2019-2021.

Time to receipt of unsatisfactory repeat sample	2021		2020		2019	
Total unsatisfactory samples	**1,951**		**2,332**		**2,044**	
< 1 week	1,255	64.3%	1,314	56.3%	1,654*	80.9%
1 - <2 weeks	310	15.9%	410	17.7%		
2 - <3 weeks	95	4.9%	155	6.6%		
3 - <6 weeks	103	5.3%	128	5.5%	109	5.3%
≥ 6 weeks	31	1.6%	33	1.4%	34	1.7%
Not received	157	8.0%	292	12.5%	247	12.1%

*Prior to 2020, the unsatisfactory repeat workflow took place in 3 week increments.

2.3 Priority Panels

Priority Panels are a testing panel that became available with the launch of the new laboratory information system (OMNI) in July 2019. 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), Galt deficiency, CH (TSH) and CAH (17OHP).

In 2021, NSO performed 1,030 priority panels (71% of laboratory unsatisfactory samples) (Table 9). These samples are still counted as unsatisfactory (in Table 7), and a repeat is requested. The results of the priority diseases are also reported.

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Table 9. Repeat samples for priority panel unsats 2020-2021.

Time to receipt of priority panel repeat sample	2021		2020	
Total priority panels	**1,030**		**1255**	
< 1 week	617	58.3%	682	54.3%
1 - <2 weeks	209	19.8%	278	22.2%
2 - <3 weeks	57	5.5%	87	6.9%
3 - <6 weeks	54	5.1%	68	5.4%
≥ 6 weeks	20	1.9%	15	1.2%
Not received	73	7.5%	125	10.0%

There were 25 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.

2.4 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 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 out 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.

In 2021 there were 8 TLU where a repeat was not received due to declined repeat testing, families not returning to the birth hospital (despite contact from the submitter and NSO) and infant death. Table 10 shows the time to receipt of repeat samples after a TLU.

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Table 10. Repeat samples for TLU 2020-2021.

Time to receipt of TLU repeat sample	2021		2020	
Total Test Level Unsats – Routine	**81**		**74**	
< 1 week	43	53.10%	28	37.8%
1 - <2 weeks	20	24.70%	19	25.7%
2 - <3 weeks	6	7.40%	7	9.5%
3 - <6 weeks	5	6.20%	9	12.2%
≥ 6 weeks	<5	1.20%	<5	4.1%
Not received	6	7.40%	8	10.8%
Total Test Level Unsats - Urgent	**69**		**50**	
< 1 week	28	40.60%	29	58.0%
1 - <2 weeks	23	33.30%	12	24.0%
≥2 weeks	16	23.20%	8	16.0%
Not received	<5	2.90%	<5	2.0%

2.5 Data Quality and Process Related Unsats

The number of samples ultimately deemed unsatisfactory related to insufficient information (Table 7, data unsat reasons) remains consistently low, due to the efforts made by NSO to contact submitting providers for missing data fields.

There were 54 unsatisfactory samples due to expired filter paper, up from 38 in 2020 and 14 in 2019 (Table 7). Expired cards can fluctuate year to year, depending on when the lots of cards expire. There were two lots of cards that expired in 2021, at the end of January and June. NSO sends out bulletin reminders to submitters when an expiry date is approaching, asking them to check and circulate their stock. In addition, Track-Kit, NSO's shipping tracking system, alerts submitters if a card they are preparing to ship is expired or near expiry. When a submitter is alerted, Track-Kit recommends that they still ship the expired card, and to also recollect a sample on a valid card. The pop-up message also reminds them to verify their inventory, and discard and re-order cards as needed.

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3. Screen Positives

In 2021, there were 780 screen positive referrals (Figure 2). This represents ~0.54% of the total number of infants screened by NSO.

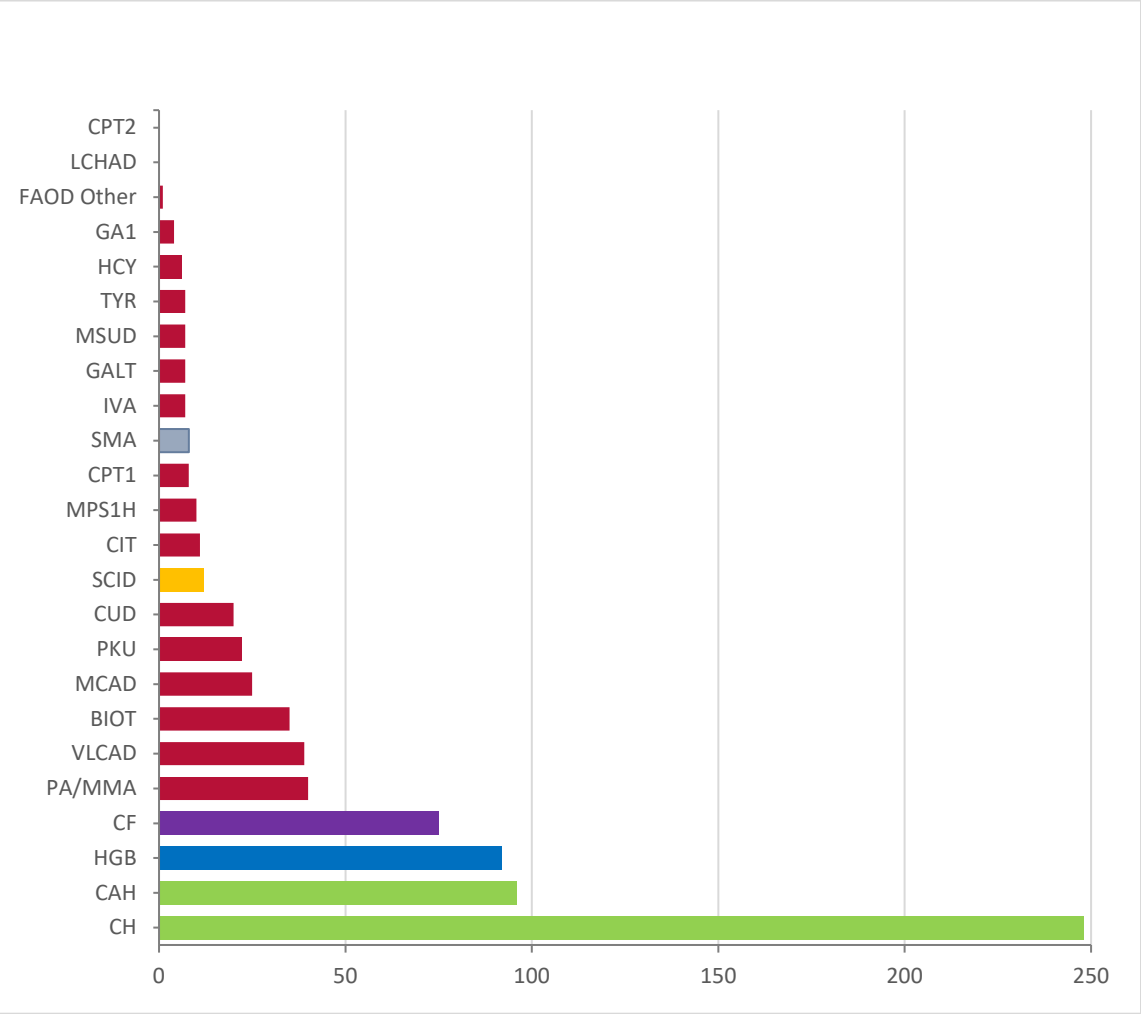

Figure 2. Total number of screen positive referrals by disease in 2021

The number of screen positive infants referred in 2021 decreased slightly from 2020 (881 vs. 780). This is discussed further in Section 3.4.

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3.1 Referrals by Treatment Centre

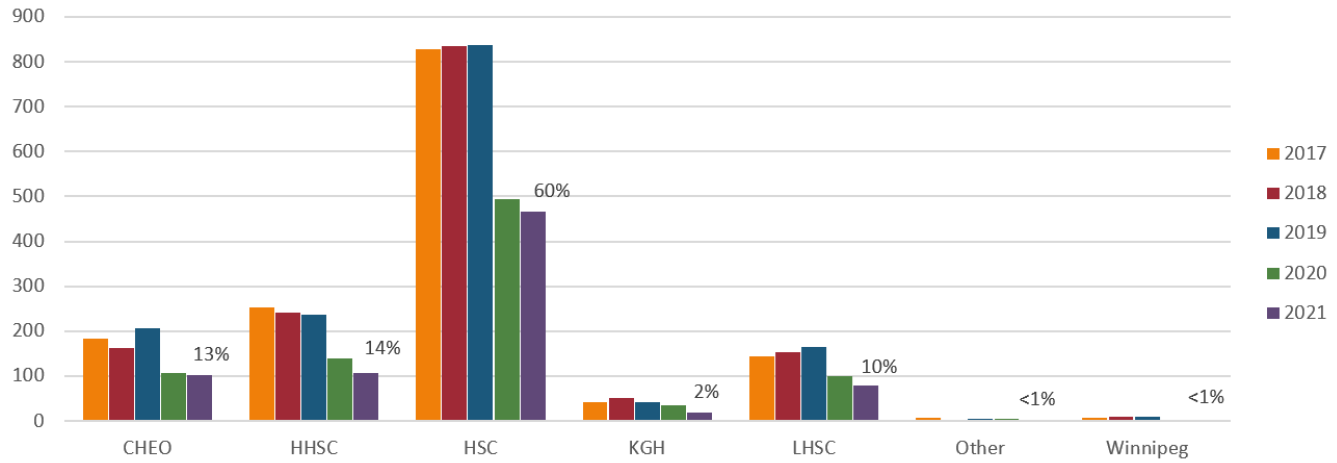

Figure 3a. The total number of referrals by treatment centre between 2017-2021.

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 (Figure 3a). 'Other' represents infants referred to treatment centres outside of Ontario/ Winnipeg, such as Quebec or the USA, 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 changed slightly over the last year with CHEO and HHSC receiving a similar proportion of referrals and HSC receiving approximately 60% of referrals (Figure 3b).

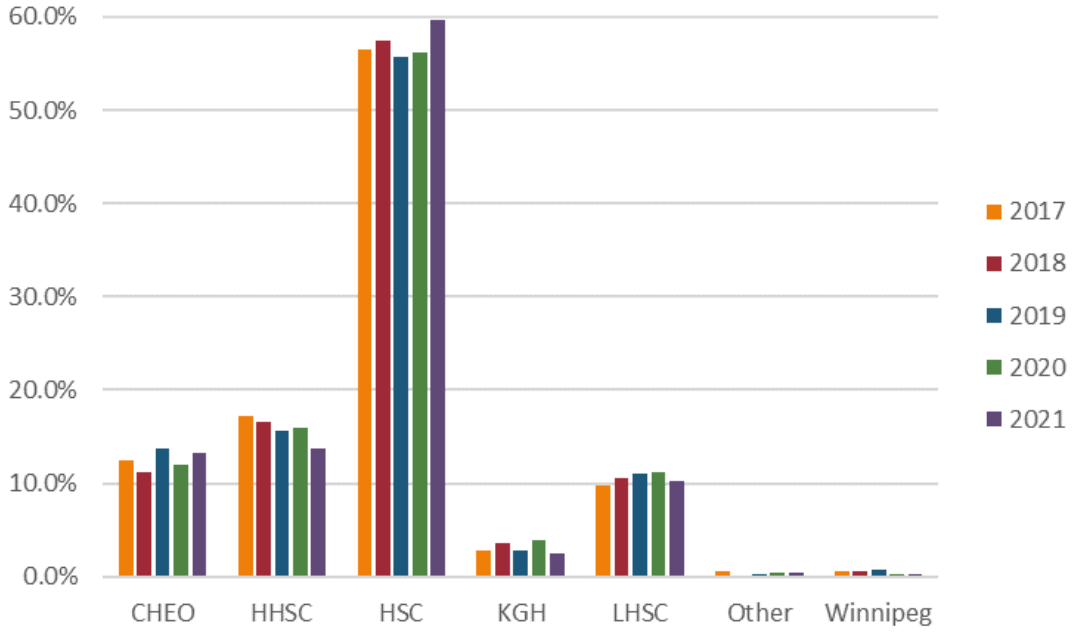

Figure 3b. The percentage of referrals by treatment centre between 2017-2021.

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3.2 Screen Positive Referrals by Disorder Group

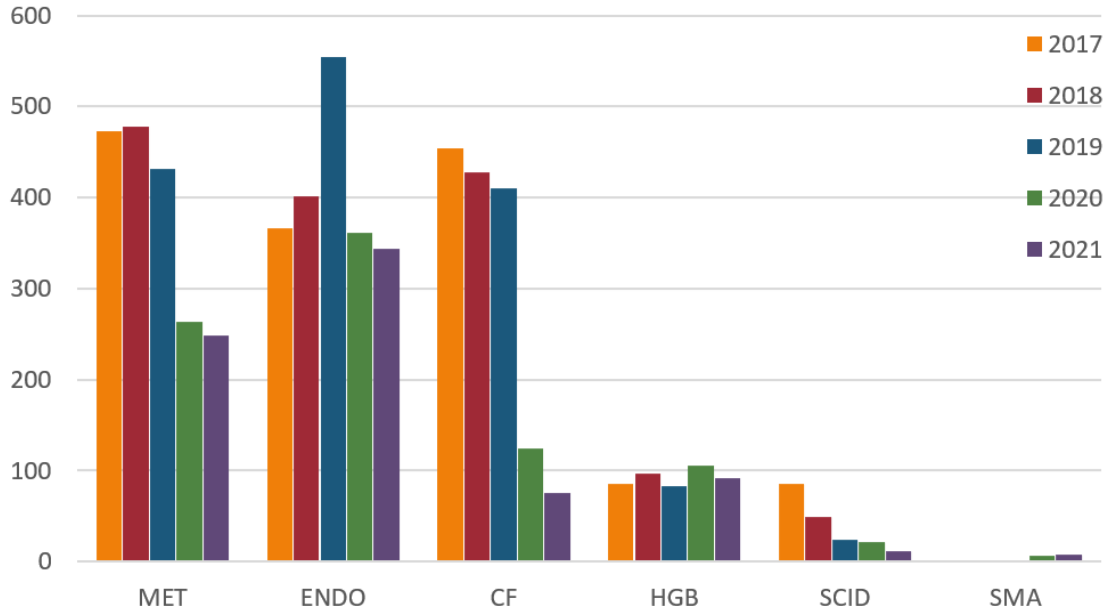

Figure 4. The total number of screen positives by disease grouping between 2017-2021.

The number of screen positive referrals per disease grouping decreased for all referral types except SMA (Figure 4). The CF algorithm changed in March 2020 with the addition of 3rd tier sequencing of the *CFTR* gene and only infants with 2 or more CFTR variants being referred as positive. This accounts for the decrease in CF referrals (discussed more in section 3.4.2). Each disease group is discussed further in section 3.4.

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3.2.1 Percentage of Screen Positive Referrals by Disorder in 2021

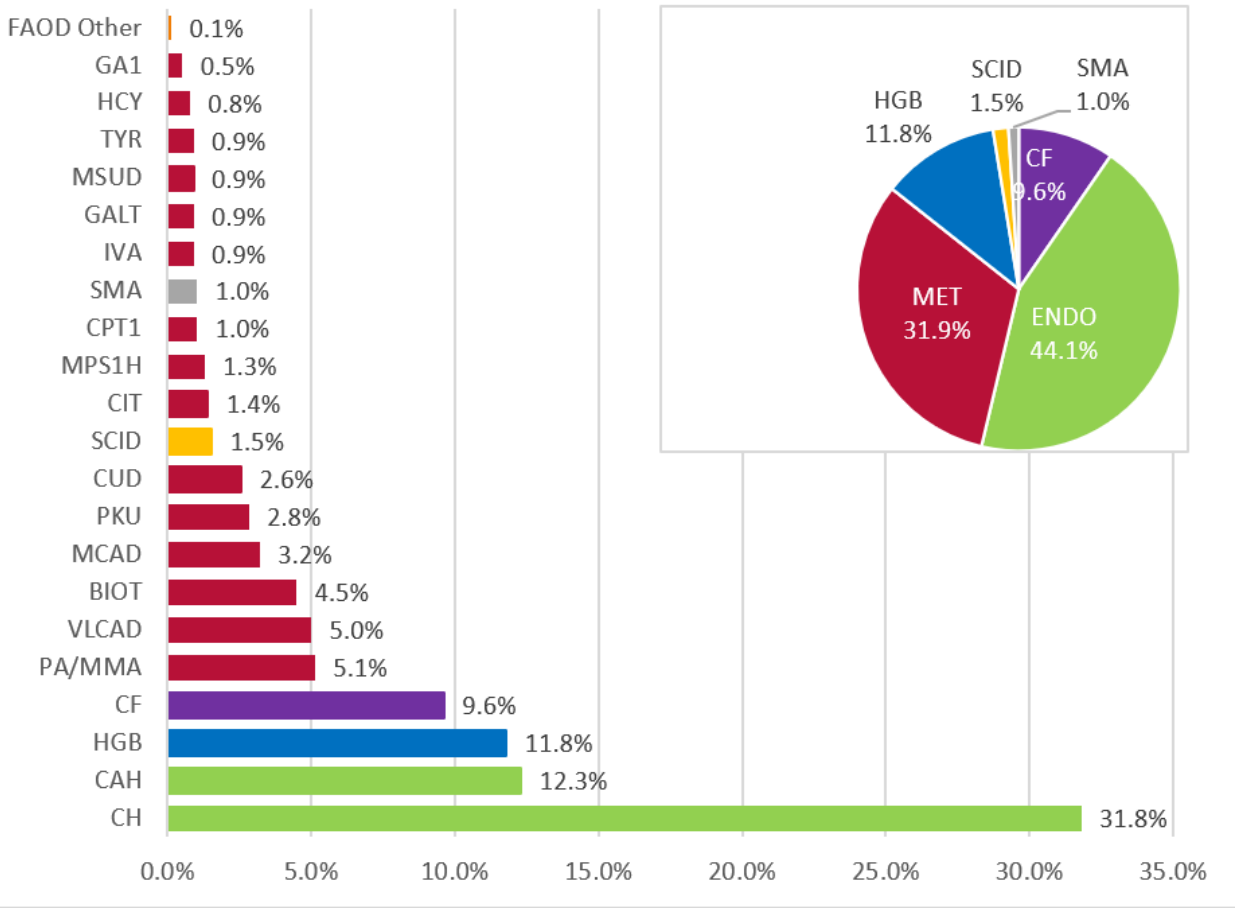

Figure 5. The percentage of screen positive referrals by disorder in 2021.

Endocrinopathies and Metabolics represent ~44% and ~32% of screen positives respectively (Figure 5). SCID screen positive referrals decreased in 2021 and now represent 1.5% of total screen positive referrals. The number of Cystic Fibrosis referrals continued to decrease in 2021 and now represent 9.6% of total screen positive referrals (see Section 3.4.2 for discussion). Hemoglobinopathies represent approximately 11.8% of screen positive referrals, which is unchanged from last year. SMA represents 1.0% of referrals.

3.3 Diagnostic Feedback

Approximately 31.9% (249 cases) of diagnostic evaluation report forms (DERFs) remain pending for the referrals made in 2021 as of April 1, 2022. The percentage of pending DERFs is higher than previous years, however, with the use of preliminary data obtained during confirmation of retrieval and initial diagnosis, an outcome was obtained for 153 of these pending DERF cases.

3.4 Definitive Diagnosis Data and Positive Predictive Values

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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 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.

Table 12. The outcome classifications for all referrals in 2021 (DERF data pulled April 1, 2022). 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.

Disease	DERFs Pending	Outcomes Unknown	PRIMARY	VARIANT	INCIDENTAL	NOT AFFECTED	OTHER	Total No. Referred
Congenital Hypothyroidism	43	33	55	24	17	119		248
Congenital Adrenal Hyperplasia	15	13	5	<5		77		96
Hemoglobinopathies	64	27	51		11	<5	<5	92
Cystic Fibrosis	39	7	27	33	7		<5	75
Type 1	18	<5	26					27
Type 2	<5	<5		11				15
Type 3	17	<5	<5	22	<5		<5	33
SCID	8	8			<5	<5		12
SMA	<5		8					8
Biotinidase Deficiency	14	10	6	<5	<5	14		35
Citrullinemia	<5	<5	<5			9		11
CUD	10	10	<5		<5	5		20
FAO (CPT1, CPT2, and GA2)	<5	<5	<5	6		<5		9
Galactosemia	<5	<5	<5	<5	<5	<5		7
Glutaric Aciduria Type 1						<5		<5
Homocystinuria	<5	<5				<5		6
Isovaleric Acidemia	<5		<5			6		7
LCHAD								0
MCAD	10	7	10	<5		6		25
MPS1H	7	7			<5			10
MSUD	<5	<5				5		7
PA/MMA	17	14	<5		10	15		40
Phenylketonuria	6	<5	5	<5		11		22
Tyrosinemia	<5	<5			<5	5		7
VLCAD	5	5		<5	17	15		39
Total No. Positive	**249**	**153**	**175**	**76**	**73**	**301**	<5	**780**

3.4.1 Hemoglobinopathies

The number of screen positives in 2021 decreased by 13 referrals from 2020.

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Table 13. The PPV calculations for the current and past screening algorithms.

Disease	Additional information	PPV (Primary)	PPV (Primary + Variant)	PPV (Primary + Variant + Secondary)	% of DERFs Pending
Hemoglobinopathies	Past (Nov 1, 2010 - July 31, 2015)	64.5%	65.3%	84.3%	1.1%
	Current (Aug 1, 2015 - Dec 31, 2021)	**62.5%**	**63.1%**	**90.8%**	**10.7%**

3.4.2 Cystic Fibrosis

The number of screen positives in 2021 decreased from 2020 as it was the first full year of third tier sequencing of the *CFTR* gene (introduced in March 2020). There were 75 referrals this year compared to 410 in 2019 and 124 in 2020. This is an 82% reduction in referrals compared to 2019. There were 27 Type 1 referrals (genotypes consistent with a high risk of a diagnosis of CF), 15 Type 2 referrals (genotypes consistent with a high risk for a *CFTR* -related disorder NOT meeting CF diagnostic criteria) and 33 Type 3 referrals (genotypes of uncertain clinical significance).

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

Disease	Additional information	PPV (Primary)	PPV (Primary + Variant)	PPV (Primary + Variant + Secondary)	% of DERFs Pending
Cystic Fibrosis	Past (Jul 28, 2019 - Mar 18, 2020) Cat A	81.3%	100.0%	100.0%	5.9%
	Past (Jul 28, 2019 - Mar 18, 2020) Cat B	2.2%	8.1%	8.1%	8.6%
	Past (Jul 28, 2019 - Mar 18, 2020) Cat C	0.0%	1.8%	1.8%	2.9%
	Past (until Mar 18, 2020) ALL	7.7%	13.5%	13.5%	6.7%
	Current (Mar 19, 2020 - Dec 31, 2021) Type 1	100.0%	100.0%	100.0%	2.1%
	Current (Mar 19, 2020 - Dec 31, 2021) Type 2	0.0%	100.0%	100.0%	38.6%
	Current (Mar 19, 2020 - Dec 31, 2021) Type 3	7.5%	82.5%	82.5%	8.9%
	Current (Mar 19, 2020 - Dec 31, 2021) ALL	**43.4%**	**93.8%**	**93.8%**	**16.2%**

*Cells are highlighted in red when >10% of DERFs are outstanding for a particular disorder or group of disorders.

3.4.3 Endocrinopathies

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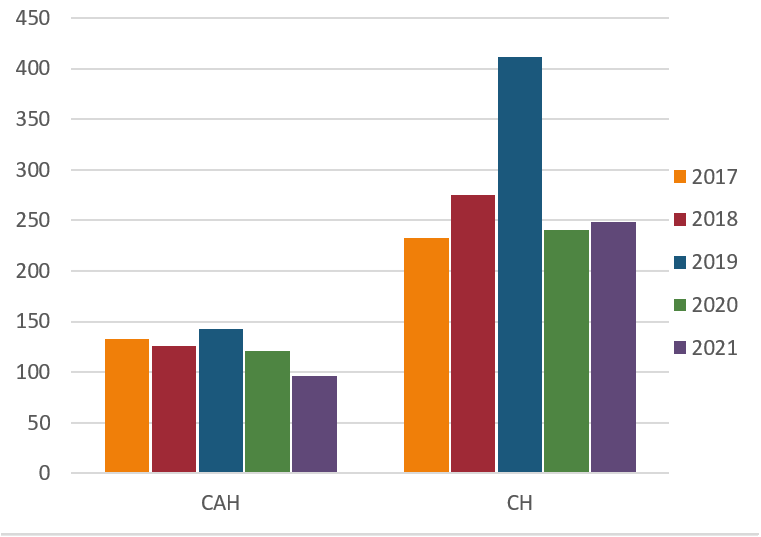

Figure 6. The total number of congenital adrenal hyperplasia and congenital hypothyroidism screen positives between 2017-2021.

The number of screen positives for CAH has decreased over the last few years. NSO has maintained the disorder logic that includes both birth weight and gestational age and does not refer extremely premature infants on their repeat sample if their initial sample was screen negative.

The number of screen positives for CH was similar to 2020.

Table 15. The PPV calculations for the current and past screening algorithms.

Disease	Additional information	PPV (Primary)	PPV (Primary + Variant)	PPV (Primary + Variant + Secondary)	% of DERFs Pending
Congenital Hypothyroidism	Past (Jun 12, 2018 - Jul 3, 2019)	16.6%	22.2%	22.2%	1.3%
	Current (Jul 4, 2019 - Dec 31, 2021)	**23.4%**	**36.2%**	**36.2%**	**10.1%**
Congenital Adrenal Hyperplasia	Past (Sept 2, 2016 - Jun 11, 2018)	7.0%	7.0%	7.5%	3.0%
	Current (Jun 12, 2018 - Dec 31, 2021)	**4.9%**	**5.4%**	**5.8%**	**7.8%**

*Cells are highlighted in red when >10% of DERFs are outstanding for a particular disorder or group of disorders.

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3.4.4 Metabolics

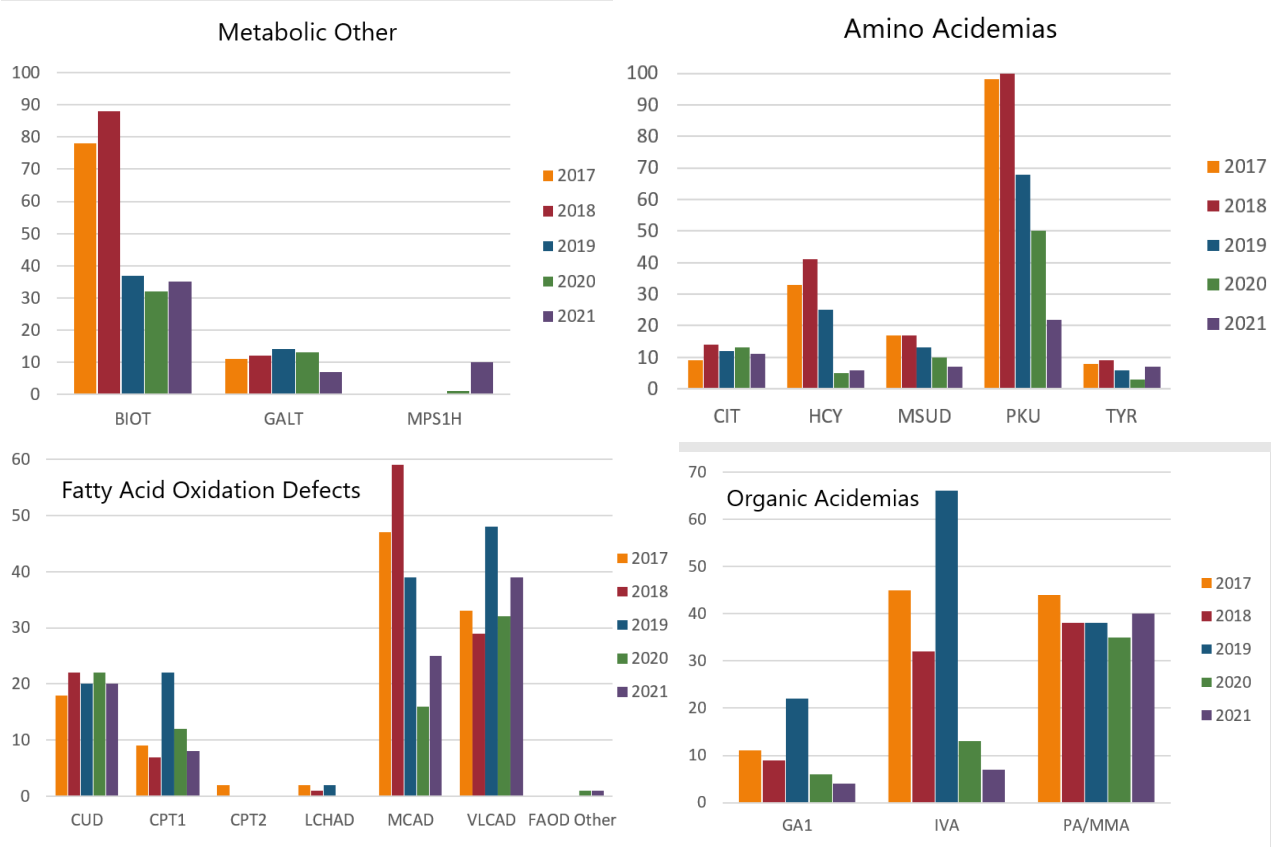

Figure 7. The number of metabolic screen positives between 2017-2021 by disease

NSO began screening for MPS1H on July 27, 2020. Eleven infants were referred as screen positive for MPS1H between 2020 and 2021, which is in keeping with what NSO had projected (~1 referral per month across the province) prior to implementing MPS1H screening. The majority of DERFs are still pending.

There was a general reduction in the number of referrals for amino acidopathies. This is likely in part due to the disorder logic changes implemented mid 2019 but could also be due to the TPN hold initiative underway across some of the NICUs in the province. By holding TPN for 3 hours prior to obtaining the newborn screening sample it is predicted that this would lead to a reduction in the amino acidopathies false positive referrals. In 2020, 6 hospitals were participating and in 2021, 20 hospitals were participating. 969 requisitions were received indicating TPN had been held. Internal reviews are still ongoing to determine if holding TPN prior to NBS collection has had an impact. There is also a research study underway comparing a TPN hold of 1 vs. 3 hours.

VLCAD had disorder logic change in mid December 2021 with the C14:1 cutoff increasing from 0.65 to 0.75uM. The effects of that change will be reported in the 2022 annual report. The anticipated change is that the referral rate will be about half of what it has been (~30 annually to 15).

The C5 cutoff for IVA was changed from 0.67 to 1.00 on Feb 18, 2020. This resulted in a significant decrease in the number of IVA referrals in 2020 which continued in 2021.

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Table 16. 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 DERFs Pending
Glutaric Aciduria type 1		**9.0%**	**9.0%**	**24.9%**	**1.0%**
Isovaleric Acidemia	Past (until Feb 17, 2020)	3.0%	4.2%	4.2%	0.5%
	Current (Feb 18, 2020 - Dec 31, 2021)	**20.0%**	**20.0%**	**20.0%**	**28.6%**
PA/MMA/CblA/CblB	Past (Feb 16, 2010 - Apr 21, 2013)	3.7%	3.7%	8.3%	0.9%
	Current (Apr 22, 2013 - Dec 31, 2021)	**4.9%**	**5.6%**	**10.5%**	**10.9%**
CPTI		**5.5%**	**60.3%**	**60.3%**	**2.0%**
CPTII		**12.1%**	**12.1%**	**12.1%**	**0.0%**
LCHAD/TFP		**81.3%**	**81.3%**	**93.8%**	**0.0%**
VLCAD	Past (until Dec 14, 2021)	7.3%	12.6%	14.4%	4.4%
	Current (Dec 15 - Dec 31, 2021)	**0.0%**	**0.0%**	**0.0%**	**33.3%**
CUD	Past (until Mar 4, 2014)	5.5%	5.5%	5.5%	0.0%
	Current (Mar 5, 2014 - Dec 31, 2021)	**5.6%**	**5.6%**	**5.6%**	**12.8%**
MCAD	Past (Sep 1, 2016 - Jul 28, 2019))	18.9%	20.3%	21.6%	1.3%
	Current (Jul 29, 2019 - Dec 31, 2021)	**64.1%**	**76.9%**	**76.9%**	**18.8%**
Citrullinemia/ASA		**18.8%**	**21.0%**	**21.0%**	**3.2%**
Homocystinuria	Past (until Jul 28, 2019)	**0.4%**	**0.4%**	**4.0%**	**3.1%**
	Current (Jul 29, 2019 - Dec 31, 2021)	**0.0%**	**0.0%**	**0.0%**	**42.9%**
Phenylketonuria	Past (until Jul 28, 2019)	**14.2%**	**27.4%**	**27.4%**	**1.3%**
	Current (Jul 29, 2019 - Dec 31, 2021)	**21.9%**	**34.2%**	**34.2%**	**15.2%**
MSUD	Past (until Nov 14, 2011)	3.8%	3.8%	3.8%	0.0%
	Current (Nov 15, 2011 - Dec 31, 2021)	**7.5%**	**8.5%**	**8.5%**	**5.2%**
Tyrosinemia	Past (Sep 14, 2009 - Sep 19, 2011)	1.4%	1.4%	1.4%	0.0%
	Current (Sep 20, 2011 - Dec 31, 2021)	**11.1%**	**11.1%**	**14.3%**	**8.3%**
Galactosemia	Past (until Jan 12, 2014)	35.7%	41.4%	41.4%	1.4%
	Current (Jan 13, 2014 - Dec 31, 2021)	**18.0%**	**31.0%**	**31.0%**	**5.5%**
Biotinidase Deficiency	Past (Jan 13, 2014 - Jul 2, 2014)	2.1%	37.5%	37.5%	0.0%
	Current (Jul 3, 2014 - Dec 31, 2021)	**6.3%**	**38.0%**	**38.0%**	**6.6%**
MPS1H		25.0%	25.0%	25.0%	63.6%

*Cells are highlighted in red when >10% of DERFs are outstanding for a particular disorder or group of disorders.

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3.4.5 Severe Combined Immune Deficiency

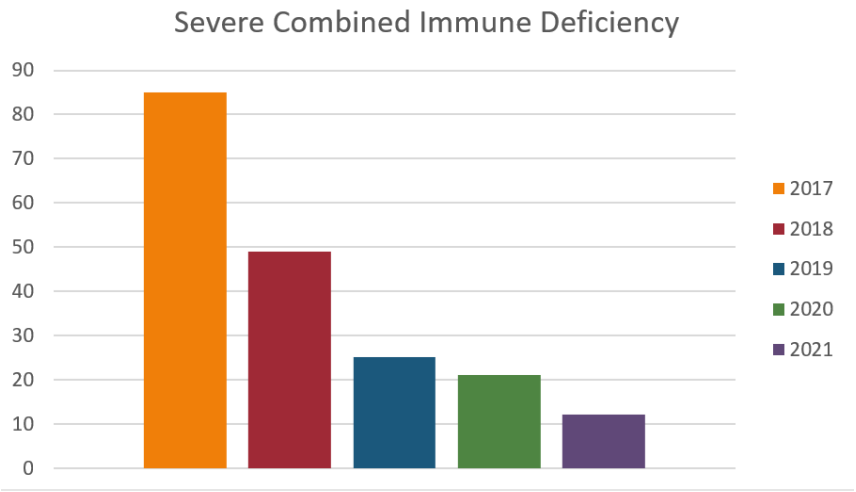

Figure 8. The number of SCID screen positives between 2017-2021.

The overall number of screen positive results for SCID decreased in 2021. In March 2021, purines were moved to a 3rd tier assay instead of being run in parallel as a 2nd tier assay.

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

Disease	Additional information	PPV (Primary)	PPV (Primary + Variant)	PPV (Primary + Variant + Secondary)	% of DERFs Pending
Severe Combined Immune Deficiency	Past (Jan 6, 2020 - Feb 28, 2021)	30.0%	30.0%	30.0%	43.5%
	Current (Mar 1 - Dec 31, 2021)	**0.0%**	**0.0%**	**0.0%**	**80.0%**

*Cells are highlighted in red when >10% of DERFs are outstanding for a particular disorder or group of disorders.

3.4.6 Spinal Muscular Atrophy

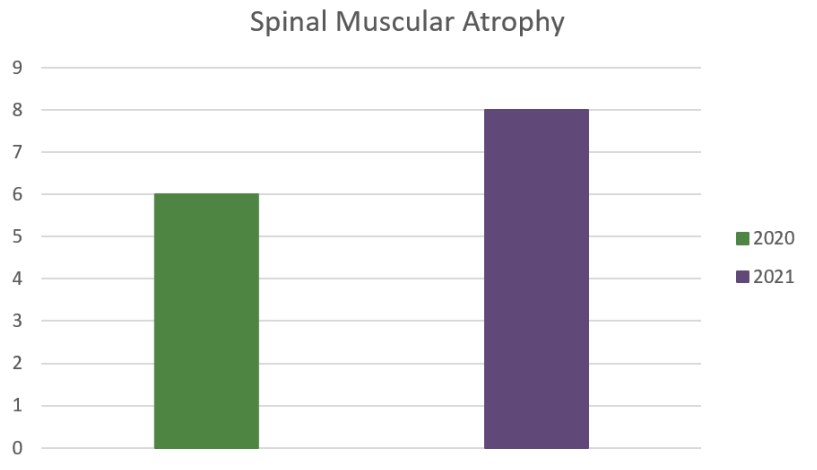

Figure 9. The number of SMA screen positives from 2020-2021.

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Spinal Muscular Atrophy (SMA) was added as a pilot to the newborn screening panel on Jan 13, 2020 and officially to the panel on July 27, 2020. SMA screening is performed by screening for homozygous deletions or conversions of the *SMN1* gene and copy number identified of 4 or less of the *SMN2* gene (*SMN2* copy number >4 are screen negative). Carriers are not identified through this screening methodology. Since screening began 6 infants were screen positive for SMA in 2020 and 8 in 2021.

Table 18. The PPV calculations for the current screening algorithm.

Disease	PPV (Primary)	PPV (Primary + Variant)	PPV (Primary + Variant + Secondary)	% of DERFs Pending
Spinal Muscular Atrophy	**100.0%**	**100.0%**	**100.0%**	**0.0%**

3.5 Disease Prevalence

Disease prevalence varies considerably between conditions on the NBS panel. The most common conditions screened by NSO include Congenital Hypothyroidism, Sickle Cell Disease, and Cystic Fibrosis. Diagnostic feedback has not yet been received for >50% of MPS1H referrals so an incidence was not calculated.

Of note, the prevalence was calculated based on NSO screen positive data and does not take into account reported potential false negative cases.

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Table 19. The disease prevalence rates 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 DERFs Pending	Disease Prevalence of Primary Targets	Positive Predictive Value (PPV) for High PPV Referrals
Congenital Hypothyroidism (CH)	4-Apr-06	2.5%	1 in 2,083	87%
Congenital Adrenal Hyperplasia (CAH)	14-May-07	1.8%	1 in 22,256	28%
Sickle Cell Disease	24-Nov-06	5.0%	1 in 2,847	94%
Cystic Fibrosis (CF)	9-Apr-08	1.4%	1 in 4,748	88%
Severe Combined Immune Deficiency (SCID)	12-Aug-13	8.7%	1 in 71,194	33%
Glutaric Aciduria type 1 (GA1)	9-Aug-06	1.0%	1 in 138,862	100%
Isovaleric Acidemia (IVA)	9-Aug-06	1.4%	1 in 158,699	55%
Propionic Acidemia (PA)/ Methylmalonic Acidemia (MMA)/ Cobalamin A & B Defects	9-Aug-06	4.9%	1 in 79,350	36%
Long-chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD)/ Trifunctional Protein Deficiency (TFP)	9-Aug-06	0.0%	1 in 170,907	88%
Very-long-chain Acyl-CoA Dehydrogenase Deficiency (VLCAD)	9-Aug-06	4.6%	1 in 79,350	60%
Carnitine Uptake Defect (CUD)	9-Aug-06	4.7%	1 in 92,575	20%
Medium-chain Acyl-CoA Dehydrogenase Deficiency (MCADD)	4-Apr-06	2.1%	1 in 15,553	91%
Citrullinemia (CIT)/Argininosuccinic Acid Lyase Deficiency (ASA)	9-Aug-06	3.2%	1 in 67,327	48%
Homocystinuria (HCY)	9-Aug-06	5.0%	1 in 2,221,789	Unknown
Phenylketonuria (PKU)	4-Apr-06	2.5%	1 in 16,455	66%
Maple Syrup Urine Disease (MSUD)	9-Aug-06	2.9%	1 in 201,981	26%
Tyrosinemia type 1	9-Aug-06	3.6%	1 in 246,865	71%
Galactosemia (GALT)	19-Feb-07	3.9%	1 in 49,924	19%
Biotinidase Deficiency (BIOT)	19-Feb-07	3.8%	1 in 61,335	14%
Mucopolysaccharidosis type 1 Hurler (MPS1H)	27-Jul-20	63.6%	Unknown	Unknown
Spinal Muscular Atrophy (SMA)	13-Jan-20	0.0%	1 in 20,123	100%

*Cells are highlighted in red when >10% of DERFs are outstanding for a particular disorder or group of disorders.

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4. Screening Timeliness

4.1 Initial Samples

Initial results refers to results from first tier screening. All infants undergo first tier testing. After interpretation the majority of infants will be screen negative and testing is complete. The first two columns of numbers are the age of their final results. There were 142,005 initial samples went through first tier screening in 2021. Of these, 129,440 were screen negative on all assays after first tier. Some infants require additional testing to determine if they are negative or positive. Age at final results is the subset of infants who required additional testing (through second and third screening) and the age that their results are final (either positive or negative).

Table 20. Median and 90th centile values for age of receipt of initial samples, and availability of initial and final results, 2020 and 2021.

	Screening (Initial Samples) 2020 Only					Screening (Initial Samples) 2021 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	52	5	8	4	6	49	6	8
CUD			178	5	8			189	5	7
FAOD			19	6	19			12	5	8
GA1			30	5	7			39	5	7
HCY			31	6	9			42	5	8
IVA			62	5	8			44	5	7
LCHAD/TFP			13	6	7			11	6	9
MCAD			22	5	7			33	5	6
MSUD			57	5	12			28	6	16
PA/MMA/CblA/B			175	6	9			180	6	8
PKU			163	5	7			114	5	7
TYR1			12	5	8			17	6	8
VLCAD			247	6	8			358	5	7
Biotinidase Deficiency			156	6	8			186	6	8
Galactosemia			174	6	11			125	6	9
CAH			622	6	8			525	6	8
CH			917	5	7			915	5	7
Cystic Fibrosis			5,804	9	14			5,820	9	14
Hemoglobinopathies	5	7	116	7	10	5	7	111	7	9
SCID	7	11	979	10	17	7	11	2,377	9	12
MPS1H	5	7	198	12	22	4	6	732	9	18
SMA	7	11	86	14	20	7	11	106	17	25

The median age (4 days) at receipt did not change between 2020 and 2021. While it appears that there were more samples that required second and third tier testing for MPS1 in 2021 compared to 2020, 2021 was the first

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full year of screening for MPS1 (introduced at the end of July 2020). While the median remained similar for age at initial results, the 90th centile for age at final results decreased across many assays.

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.

Table 21. Median and 90th centile values for time from receipt to initial results, and time from receipt to final results, 2020 and 2021.

	Screening (Initial Samples) 2020 Only					Screening (Initial Samples) 2021 Only				
Category	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	25	28	52	50	78	24	26	49	50	75
CUD			178	49	54			189	49	72
FAOD			19	50	121			12	50	106
GA1			30	49	73			39	50	74
HCY			31	50	54			42	49	73
IVA			62	50	74			44	50	74
LCHAD/TFP			13	50	88			11	49	74
MCAD			22	30	67			33	49	72
MSUD			57	49	51			28	49	53
PA/MMA/CblA/B			175	50	97			180	50	98
PKU			163	49	52			114	49	52
TYR1			12	50	73			17	50	73
VLCAD			247	49	72			358	49	73
Biotinidase Deficiency			156	50	72			186	50	74
Galactosemia			174	49	50			125	49	51
CAH			622	51	97			525	51	98
CH			917	50	74			915	50	74
Cystic Fibrosis			5,804	146	244			5,820	146	248
Hemoglobinopathies	28	50	116	97	134	26	50	111	80	127
SCID	100	170	979	170	318	99	152	2,377	146	176
MPS1H	28	76	198	195	458	25	27	732	148	345
SMA	99	170	86	247	366	98	150	106	339	511

4.2 Screen Positive Infants

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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. Each disease group developed clinically meaningful benchmarks and aggressive diseases were assigned alert and non-alert benchmarks. The goal would be to have 90% of the screened population meet the benchmarks.

Comparing the data from 2016-2020 to the 2017-2021 time period, there continue to be improvements in the percentages of infants achieving benchmarks for screen positive infants throughout the screening experience. Improvements related to Age at Receipt and Age at Screening Results are likely attributed to a combination of factors including earlier age at collection, improved shipping times, and NSO expanding operations to include weekend reporting. However, despite these enhancements, challenges persist regarding the timely receipt of samples at NSO and this in turn ultimately influences the remainder of the screening process and ability to meet downstream benchmarks related to result availability. The percentage of infants meeting the benchmark regarding Age at Retrieval has made small improvements, particularly with the aggressive disorders. Regional variation in triage practices and certain clinical criteria/eligibility to pursue diagnostic investigations (e.g. GA and weight requirements for sweat chloride testing) may be influencing the disease categories where a lower % of infants are meeting this benchmark.

4.3 True Positive Infants

Overall, many factors within a screening system can impact timeliness benchmarks, and comparing and contrasting benchmarks from all screen positives alongside true positives can illuminate some of these issues. There are external and other circumstances that can increase the screen positive rate of a disorder and thus screening timeliness benchmarks as well (for example, consider delayed transit times for Galactosemia). However, when the true positive data for Galactosemia is examined the percentage meeting benchmarks improves dramatically.

4.4 Treatment Centre Deltas

To review the days from referral to different time points (which eliminates the downstream effects of age at collection, receipt and referral) screening timeliness data was reviewed looking at just treatment centre metrics. As in other analyses, DERFs that were pending and infants diagnosed prior to retrieval were excluded from the analysis. The time from referral to retrieval was ≥70% in the majority of disease groups indicating quick action on the part of the treatment centres. The majority of disease groups were meeting benchmarks for age at definitive diagnosis. However, time to initial diagnosis did not improve with this analysis with only alert Galactosemia infants being diagnosed by 1 day after referral.

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5. CCHD Screening

5.1 CCHD cards received

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 2021 was 146,804 representing 142,936 infants. Including CCHD missed screens in which a card was not received, the total number of infants is 143,297, which is lower than the estimated number of infants in Ontario that was derived from the blood spot samples, of 143,888 (figure 1).

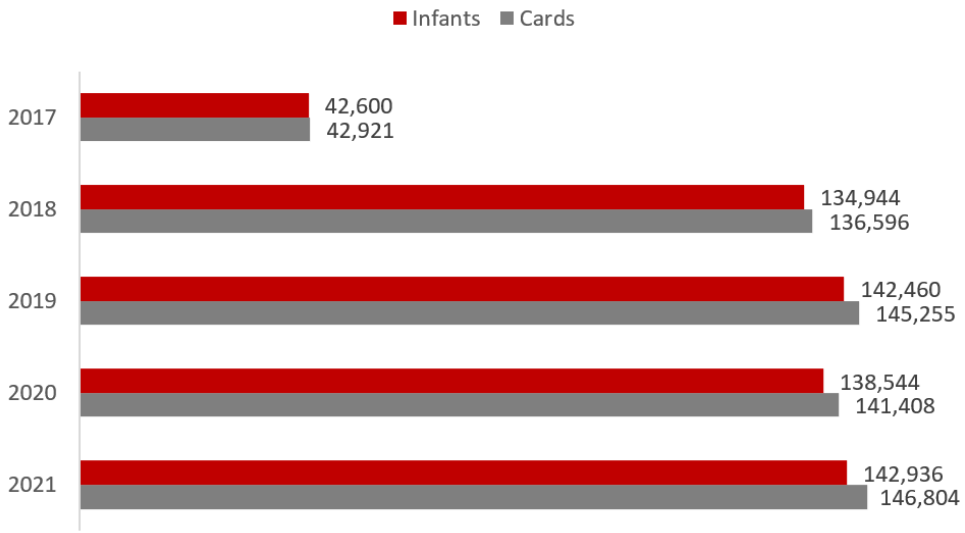

Figure 9. CCHD cards received at NSO and total number of infants between 2017-2021.

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 2021, 7,540 of the requisitions submitted were for screens not done.

Table 33. CCHD cards received.

CCHD Cards received	222020		2019		2018	
Screen Completed	134,834	95.40%	138,775	95.50%	132,134	96.70%
Screen Not Done*	6,574	4.60%	6,480	4.50%	4,462	3.30%
	141,408		145,255		136,596	

*NSO began tracking blank cards in 2019 (and continued this practice in 2020), resulting in an increase in 'Screens not Done' for between 2018 and 2019.

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5.2 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, 99.13% of the screens were resolved after just one test (most often this would be a pass, but this could also be an immediate referral). Only 0.77% required a second test and 0.11% required three tests to complete the screen.

Table 23. Tests required to complete screen between 2018-2021.

Tests Done	2021		2020		2019		2018	
1 Test	138,050	99.13%	131,592	98.80%	136,935	98.70%	129,967	98.40%
2 Tests	1,067	0.77%	1,431	1.10%	1,621	1.20%	1,948	1.50%
3 Tests	147	0.11%	222	0.20%	218	0.20%	219	0.20%
	139,264		133,245		138,775		132,134	

5.3 Screens Not Done

In 2021, CCHD screens were not done on 5.14% of the cards received. The most common reason for CCHD screen not done is because the infant is expected to be in the NICU for > 7 days.

Table 24. Reasons for CCHD Screen not done between 2018-2021.

	2021		2020		2019		2018	
'Screen Not Done' cards submitted	7,540		6,574		6,480		4,462	
Decline/deferred (back page of form not completed)	106	1.41%	95	1.40%	93	1.40%	78	1.70%
Declined	139	1.84%	66	1.00%	26	0.40%	26	0.60%
Deferred	541	7.18%	565	8.60%	542	8.40%	465	10.40%
Infant diagnosed prenatally with heart defect	178	2.36%	101	1.50%	74	1.10%	58	1.30%
Infant diagnosed with heart defect by physical exam	70	0.93%	33	0.50%	47	0.70%	58	1.30%
Infant is not appropriate for screening (e.g. NICU > 7 days, on oxygen, IV in right hand, limb anomaly, etc.)	4,745	62.93%	4,725	71.90%	4,732	73.00%	3,735	83.70%
Already done	514	6.82%	169	2.60%	17	0.30%	8	0.20%
Insufficient information provided/blank card	1,005	13.33%	671	10.20%	704	10.90%	18	0.40%
Other	242	3.21%	149	2.30%	245	3.80%	16	0.40%

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In 2019, NSO began tracking blank cards submitted. Tracking of blank cards was in preparation for launching missed screen reporting in 2020 (see Section 4.4). This was accompanied by education of submitters on the completion of the card even when the screen was not being completed and the addition of a check box for the submitter to indicate when a screen has already been submitted when a repeat DBS is required.

Part way through 2020, NSO stopped correcting blank cards where the infant was <1500g and/or <33 weeks gestational age. While these were not followed up as unsatisfactory with the submitter as the infant was inappropriate to screen, they were correctly counted as blank cards and used to educate submitters who were not filling out the screening card completely. As a result, the number of 'blank cards' increased in number compared to 2020 and 2019.

Of the decline/deferred group (106) where the back of the form was not completed – 87 had a CCHD screen completed. There were 12 infants who had the DBS screen but no CCHD screen. The declined screens are reviewed further below in the missed screen section.

5.4 CCHD Missed Screens

In January 2020, NSO began to track CCHD missed screens using a comparison of dried blood spot samples received to CCHD screening cards. Alerts were received for infants born >14 days ago for which no CCHD screening card had been received and for infants who were >33 weeks gestation AND >1500g birth weight. Infants at the Hospital for Sick Children were also excluded as this was not a birth hospital and infants transferred to this location are generally unwell and closely monitored.

In 2021, 562 potential missed screens were identified, significantly fewer than in 2020 when there were 1,297. The majority of the alerts were from hospitals (512). The majority of these alerts were due to improper documentation – either the infant was screened but documentation was not sent to NSO (274) or the infant was not suitable for screening and documentation was not sent to NSO (206). There were 5 families who declined CCHD screening where documentation was not sent prior to the missed screen alert. There were 59 CCHD screens that were missed for eligible infants (again a significant drop from 2020 when 134 were missed). 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.

There were more CCHD decline forms than in previous years. Submitters sent in 139 declined CCHD forms and 106 forms that didn't specify if the family was declining or deferring. In total, 52 families declined CCHD screening (28 from the decline forms received, 19 from the defer/decline forms, and 5 from the missed screen notifications). Of the 139 decline forms received, 110 did have CCHD screening suggesting that the decline form was completed in error and should have either indicated a deferral of screening or that a completed screening card had already been submitted. There were 24 families that declined both the CCHD screen and the DBS screen. There were 28 families who consented to the DBS screen but declined the CCHD screen.

5.5 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 (93.8%) of screening has been done in the recommended range which is an increase from last year (91.3%).

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Table 25. Age at time of CCHD Screen from 2018-2021

Age at time of CCHD screen	2021		2020		2019		2018	
	Number of screens	%	Number of screens	%	Number of screens	%	Number of screens	%
Less than 24 hours	2,212	1.6	2,247	1.7	6,265	4.5	5,978	4.5
24-48 hours (1-2 days)	130,562	93.8	123,135	91.3	122,051	87.9	116,035	87.8
>48-72 hours (2-3 days)	1,721	1.2	1,706	1.3	2,571	1.9	3,178	2.4
>72-168 hours (3-7 days)	940	0.7	928	0.7	1,144	0.8	1,147	0.9
Greater than 168 hours (> 7 days)	197	0.1	255	0.2	352	0.3	300	0.2
Not specified	3,632	2.6	6,289	4.7	6,391	4.6	5,496	4.2

The percentage of screens done at less than 24 hours is 1.6% overall which is a slight reduction from the 1.7% observed in 2020. During the 2020 data review we identified potential data entry errors with date of screen being entered as the same as date of birth. The 2020 data was reviewed and approximately 25% of records where the DOB = DOC was entered incorrectly. These records were corrected. It is anticipated that a similar rate of error would be found in the 2019 and 2018 data. A process was implemented to identify these potential errors so that they can be reviewed and corrected where needed in a timely manner.

5.6 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 2021 was 1,179, which was 0.80% of the cards received. The most frequent error was incomplete documentation – either of a repeat test done after 1 hour or missing screening values (Table 26). The number of unsatisfactory screens increased in 2019 as NSO started to contact submitters where cards were received with demographic information but no CCHD screening values recorded. With increased submitter education, the unsatisfactory rate decreased in 2020 and remained below 1% in 2021.

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Table 26. Outcomes from unsatisfactory CCHD screen notifications.

	2021	2020	2019	2018
Unsatisfactory Screens	**1,179**	**1,069**	**1,855**	**615**
Baby >7days, no rescreen recommended	39 (3.3%)	65 (6.1%)	49 (2.6%)	31 (5.0%)
Baby in hospital, no screen recommended	203 (17.2%)	253 (23.7%)	566 (30.5%)	33 (5.4%)
Documentation inaccurate or incomplete	723 (61.3%)	574 (53.7%)	865 (46.6%)	297 (48.3%)
Family Declined	0	0	<5	0
No action needed	57 (4.8%)	38 (3.6%)	51 (2.7%)	0
Physical exam recommended (screen positive)	<5	<5	<5	<5
Missed - baby >7 days, no screening recommended	6 (0.5%)	9 (0.8%)	5 (0.3%)	251 (40.8%) (only recorded as rescreen)
Missed - screening recommended	65 (5.5%)	54 (5.1%)	119 (6.4%)	
Rescreen recommended	83 (7.0%)	76 (7.1%)	195 (10.5%)	
Total Screening Forms Submitted	**146,804**	**141,408**	**145,255**	**136,596**
Unsatisfactory Rate	**0.80%**	**0.76%**	**1.28%**	**0.45%**

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 1,179 unsatisfactory screens, and in 61.3% of follow up cases the result was amended by the submitter due to incorrect completion of the form. In 7.0% of cases a rescreen was recommended. Through the follow up of unsatisfactory screens NSO was able to follow up with submitters for 148 infants that had not received a proper CCHD screen and needed to be screened (missed) or rescreened.

Missed screens specifically were not captured prior to 2019 but if an infant was identified as missed at <8 days of age the recommendation was to screen the infant and if identified >7 days the recommendation was made to contact the infant's primary care provider. Potential missed CCHD screen notifications to submitters started in January 2020.

5.7 CCHD Screen Positives – 2021 data

There were 167 CCHD screen positives in 2021, most of which were screened within 24-48 hours. There was one screen performed at 23 hours of age where the infant was found to have right ventricular hypertrophy. Of the true positives, eight were screened between 24-26 hours of age and one was screened at 51 hours of age

Table 27. Age at time of screen positive

Age at Screen Positive	Total No.
< 24 hours	<5
24-48 hours	163
> 48 hours	<5
Not available	<5
Grand Total	**167**

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Table 28. Definitive diagnosis for CCHD Screen Positives (individual years and cumulative)

Definitive Diagnosis Categorization	Cumulative
Primary target	**47**
Tetralogy of Fallot	8
Total anomalous pulmonary venous return	16
Transposition of the great arteries	10
Tricuspid atresia	<5
Truncus arteriosus	<5
Hypoplastic left heart syndrome	<5
Pulmonary atresia w/ intact septum	6
Secondary target	**216**
Coarctation of the aorta	7
Ebstein anomaly	<5
Interrupted aortic arch	<5
Infection	36
Persistent fetal circulation *(including pulmonary hypertension and delayed transition)*	43
PPHN*	26
Pulmonary disease *(non-infectious)*	99
Double outlet right ventricle	<5
Incidental Finding	**184**
CHD *arrhythmia*	6
CHD *structural*	46
CHD *Other*	25
Other	40
No disease, no definitive diagnosis	67
Not affected	**355**
Lost to follow up	**<5**
Grand Total	**803**

*Please note PPHN was previously included in persistent fetal circulation but has since been separated out.

Of the 167 screen positives received in 2021, 9 were diagnosed with a critical congenital heart defect, 107 had a secondary CHD target or were diagnosed with an incidental finding such as pulmonary disease or infection, and 51 were found to be not affected.

5.8 CCHD Definitive Diagnosis Data and Positive Predictive Values

In 2021, the Positive Predictive Value (PPV) for CCHD screening was 5.4% for primary targets and 46.7% for primary and classical secondary target diseases (table 44). Cumulatively since the beginning of the program, the PPV is 5.9% for primary targets, and 32.8% for primary and classical secondary target diseases. Of the 802

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screen positives since the initiation of CCHD screening (the lost to follow up DERF has been excluded from analysis), 355 (44.3%) have been determined to be not affected after diagnostic follow up.

Table 29. PPV calculations for CCHD Screen Positives (yearly and cumulative)

Data set	PPV (Primary)	PPV (Primary + Secondary)	Total No. Screen Positive	Outcome Classification				
				Primary Targets	Secondary Targets	Incidental Findings	Not Affected	Lost to follow up
2017-18	4.4%	27.6%	272	12	63	55	142	<5
2019	9.0%	30.5%	167	15	36	44	72	<5
2020	5.6%	30.1%	197	11	48	47	90	<5
2021	5.4%	46.7%	167	9	69	38	51	<5
Cumulative	5.9%	32.8%	803	47	216	184	355	<5

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6. Risk Factor Screening for Permanent Hearing Loss

6.1 Introduction

The Ministry of Children, Community and Social Services' (MCCSS) Infant Hearing Program (IHP) is a well-established program that provides universal newborn hearing screening in hospital or community settings, diagnostic audiology assessments to identify PHL, monitoring of children at risk of developing PHL and language development services. The IHP and NSO began offering dried bloodspot (DBS) risk factor screening for Permanent Hearing Loss (PHL) for babies born on or after July 29, 2019, as a complement to newborn hearing screening. Risk factor screening for PHL uses the newborn DBS to look for Cytomegalovirus (CMV) infection and DFNB1 and DFNB4-associated PHL (variants in the genes *GJB2/6* and *SLC26A4*). These are the most common causes of childhood PHL and children with these risk factors are at risk of congenital or early onset PHL.

6.2 Consent

When risk factor screening for PHL launched, parents/guardians were approached for consent as part of the infant hearing screening process. When the COVID-19 pandemic began in 2020, and all non-essential services were discontinued temporarily, the IHP postponed all audiometric hearing screening and was no longer able to obtain consent for risk factor screening. After careful review and options-analysis with the Ontario Ministry of Health and Ministry of Children Community and Social Services, a decision was made to continue with the risk factor screening without the need for additional consent from the IHP until it became feasible again. This decision was made due to high rate at which approached parents had been consenting and so that babies at high risk for PHL would continue to be identified. All DBS from babies born on or after March 26, 2020, were screened for CMV and genetic risk factors for PHL. This continued throughout 2021.NSO and the IHP are working on an improved workflow and electronic system for when consent is reinstated.

Table 42 shows the number of infants screened for CMV and genetic risk factors for PHL.
NSO screened 143, 749 infants in 2021. Risk factor screening for PHL (CMV and genetics) was completed for 142,239 infants in 2021. Not all babies were screened for hearing loss risk factors as some had specimens that were unsatisfactory for analysis or had unsatisfactory results specifically for CMV or genetic risk factors.

Table 30. Number of babies screened for risk factors for PHL

	2021
Infants screened at NSO	143,749
IHP Screening Form received	N/A
Consent for risk factor screening	N/A
Babies screened for CMV and genetic risk factors	142,239
Babies screened for CMV	143,344
Babies screened for genetic risk factors	142,936

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6.3 Screen Positive Results

CMV screening is performed using a real-time PCR assay and specimens where CMV is detected are reported as screen positive. Genetic screening is performed using mass array technology for a panel of selected mutations in the genes *GJB2/6* and *SLC26A4*, and infants with 2 or more mutations in the same gene are considered screen positive. The referral care pathways are summarized in the 2020 NSO Annual Report.

Table 31. Number of risk factor screen positive babies in 2021

Risk Factor	2020 # screen positives (% rate)	2021 # screen positives (% rate)
CMV	159 (0.12)	140 (0.097)
Genetics	22(0.016)	32 (0.022)

Table 31 shows the number of risk factor screen positive infants. In 2021, there were 140 CMV screen positive infants. The CMV screen positive rate was 0.097%. While this is lower than we originally anticipated based on suspected population prevalence, the positivity rate has remained stable since the start of risk factor screening in 2019 and has not changed considerably throughout the COVID-19 pandemic. We have been made aware of 6 cases of cCMV that were ascertained clinically but missed through screening (i.e. false negatives) from 2020-2021. NSO is continuously evaluating and considering ways to increase the sensitivity of the CMV screening assay.

There were 32 infants with genetic screen positive results in 2021. This increase was expected due to the introduction of reflexive testing for the *GJB2* p.(V37I) mutation in October 2020. The *GJB2* p.(V37I) mutation is only screened reflexively when another mutation is detected on the common mutation panel used for risk factor screening. Most of the mutations included in screening are highly penetrant, truncating mutations that confer a high risk for congenital PHL. The p.(V37I) variant is non-truncating and has reduced penetrance. While not all infants with this mutation will have or develop PHL, part of the rationale to include it in screening was to help identify infants at risk for non-congenital pre-school PHL who may be missed by audiometric hearing screening alone.

We are not aware of any missed cases of PHL involving the mutations included on the screening panel. We continue to evaluate the frequency of mutations screened in our population and they are as expected.

6.4 Screen positive referrals

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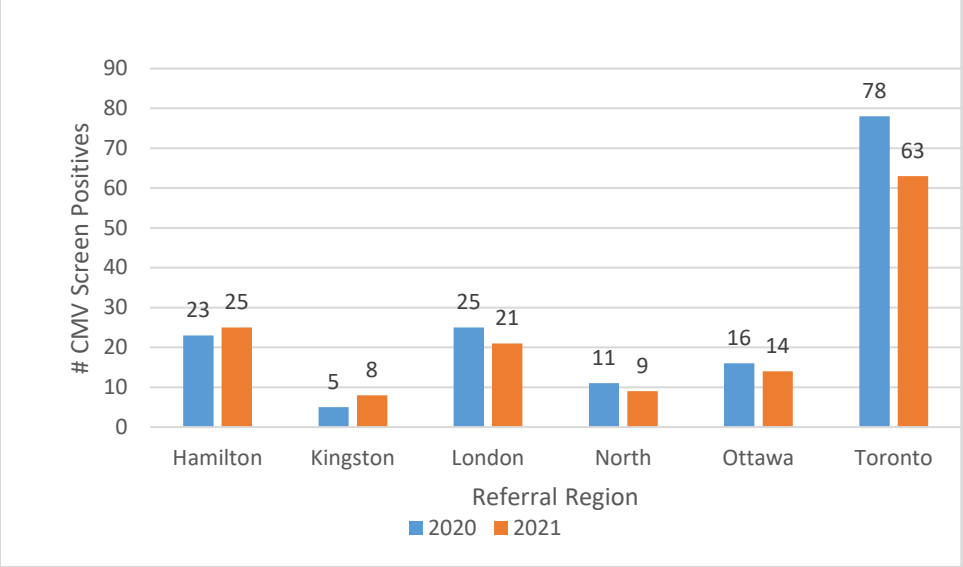

Figure 10. CMV screen positives by referral region

Figure 10 shows the breakdown of CMV screen positive referrals by region. As expected, Toronto received the largest number of CMV referrals (63/140, 45%), followed by Hamilton (25/140, 18%), London (21/140, 15%), Ottawa (14/140, 10%), Northern Ontario (9/140, 6%), and Kingston (8/140, 6%). This distribution is similar to that observed in 2020 and in keeping with what typically observed for other newborn screen positive diseases.

The majority of CMV screen positive infants were referred to a community pediatrician for their initial assessment (113/140, 81%). The remaining infants were referred directly to ID for their initial assessment (27/140, 19%). This proportion was similar to that observed in 2020, where 84% were seen for their initial assessment by community pediatricians and 16% by ID. Reasons for a direct referral to ID were geographical/travel related, due to logistical factors related to the COVID-19 pandemic, or because possible symptoms were noted at retrieval.

6.5 CMV screen positive outcomes

Table 32. Confirmatory urine CMV PCR results for CMV screen positive infants

	Confirmatory Urine CMV PCR Results						
	Results available			Results not available			
DBS Screening Result	Detected	Not Detected	TOTAL available	Not Done	Pending	Total not available	GRAND TOTAL
Detected	103 (92%)	9 (8%)	112	<5	5	8	120
Borderline	15 (83%)	3 (17%)	18	<5			20
TOTAL	118	12	130	5	5	10	140

Table 32 summarizes the urine CMV PCR results in 2021. Urine CMV PCR results are available for 130 (93%) of the screen positive infants. Of these, 118 (91%) had positive/detected results. There were 12 cases (9%) where the DBS was positive, but the confirmatory urine CMV PCR results were negative/not detected. These infants were referred to ID for further testing and interpretation of results. To date, false positive newborn screening results, false negative urine diagnostic lab results, and contaminated blood spot cards have all been observed in such cases, and they have been difficult to resolve.

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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 2021 show that 17% of borderline screen positives cases had negative urine CMV PCR results as compared to 8% of robust screen positives. This suggests that a borderline result at NSO is more likely to result in a negative urine CMV PCR result but is not entirely predictive on its own. NSO is working to further streamline the evaluation of cases with negative urine CMV PCR to help support the more rapid resolution of these cases.

Table 33. Definitive diagnoses for CMV screen positive infants

Definitive Diagnosis	Positive Urine CMV Results	Negative Urine CMV PCR Results	Urine CMV PCR not done	Urine CMV PCR Pending	Total
Asymptomatic cCMV	96	<5	<5	<5	**97**
Symptomatic cCMV	18	<5	<5	<5	**18**
Indeterminate/inconclusive	<5	<5	<5	<5	<5
cCMV excluded (false positive)	0	7	0	0	**7**
LTFU	<5	<5	<5	<5	**5**
Pending	<5	<5	<5	5	**11**
TOTAL	**118**	**12**	**5**	**5**	**140**

Of the CMV screen positive infants with positive confirmatory urine CMV PCR results, 81% (96/118) were deemed to have asymptomatic cCMV infection and 14% (17/118) were classified as symptomatic, with the remainder being lost to follow-up or pending (Table 33). Based on the literature, we would expect that approximately 10-15% of babies with cCMV would be symptomatic. Our observations are at the upper limit of what has been reported, this could mean that the DBS assay is better at detecting infants with higher viral load infection and may be symptomatic, or be a result of the comprehensive assessment infants are receiving to discover symptoms that would be difficult/impossible to ascertain clinically without screening (e.g. isolated HUS findings).

Seven of the eighteen (39%) 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, 17% of infants had PHL identified at the initial diagnostic audiology assessment. There were no infants with isolated PHL. The importance of ongoing hearing surveillance must be underscored for all CMV screen positive infants as there is there is risk of developing PHL for both asymptomatic (~10%) and symptomatic (~30%) cases. It will be important to review IHP outcome information from audiologic surveillance to learn what proportion of infants develop non-congenital PHL and at what age to better understand any predictors.

Table 33 shows that definitive diagnoses of "indeterminate/inconclusive" and "cCMV excluded (false positive)" were only given to infants with negative urine CMV PCR results. As mentioned above, some of these cases were resolved and determined to be confirmed asymptomatic infection or proven false positives; however, there were some cases that were considered inconclusive/indeterminate because a diagnosis was difficult to establish conclusively.

6.6 Change to the CMV screen positive assessment and treatment guidelines

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Data from the first two years of screening (July 29, 2019-July 28, 2021) were reviewed with our Infectious Diseases and Ophthalmology partners in early 2022, and no cCMV-related eye findings were noted in the group of children deemed to have asymptomatic CMV. The decision was made that eye exams no longer need to be arranged routinely for all cCMV screen positive infants. All infants with suspected symptomatic cCMV will continue to be referred to ID and ID Clinics will assess whether and how urgently an eye exam is required.

6.7 Genetic screen positive outcomes

Table 34. Genetic screen positive results and PHL interventions

	Genotype Class		
Intervention	T/T genotype	T/NT genotype	TOTAL
CI candidate	8	<5	8
Amplification	<5	5	9
Monitoring+	<5	8	9
Surveillance**	<5	6	6
TOTAL	13	19	32

+ Infants with minimal hearing loss are offered close audiologic monitoring
** Infants with normal hearing were offered audiologic surveillance in accordance with IHP protocols

There were 13 infants with truncating/truncating genotypes. As expected, these infants were all found to have some degree of PHL at their initial diagnostic audiology assessment and some had a family history of a first-degree relative with PHL.

There were 19 infants with truncating/non-truncating gentoypes. About 70% of these infants had some degree of PHL noted at their initial diagnostic audiology assessment and the degree of hearing loss observed was never in the severe-profound range. More commonly, infants would have a minimal PHL identified that would warrant close monitoring or be found to have hearing loss in the mild-moderate range. There were a few children with T/NT genotypes who had a family history of a first-degree relative with PHL. It will be important to collect audiology outcome data over time to see if hearing loss develops in the children with normal hearing at birth, or if there is progression in the group of children with PHL.

6.8 Future directions

In summary, risk factor screening for PHL has been successful to date at identifying babies who have or are at risk for PHL.

As risk factor screening for PHL continues, we will focus our efforts in the following areas.

- Development of an improved workflow for obtaining consent for risk factor screening, with electronic transfer of information between NSO and the IHP.
- Updating the screen positive care pathway to get answers more quickly for CMV screen positives with negative urine CMV PCR results.
- Evaluation of the genetic screening panel and consideration of the addition of the *GJB2* p.(V37I) mutation on the first-tier screening panel.

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Appendix A: Detailed Screening Timeliness Data

Table 1A: Median, 70th and 90th Centile for All Screen Positive Samples by Disease Category, 2017-2021.

					Alert Confirmation				Routine Confirmation				ALERT				Non-Alert				ALERT				Non-Alert			
Category	ACMG Code	Age at Receipt			Age at Alert Screening Result				Age At Screening Result				Age at retrieval (contact with family)								Age at Definitive Diagnosis and Disposition [2]							
		Median	70th Centile	90th Centile	# of Samples Prioritized	Median	70th Centile	90th Centile	# of Samples Confirmed	Median	70th Centile	90th Centile	# of Samples Prioritized	Median	70th Centile	90th Centile	# of Samples Confirmed	Median	70th Centile	90th Centile	# of Samples Prioritized	Median	70th Centile	90th Centile	# of Samples Confirmed	Median	70th Centile	90th Centile
Benchmark (days of age)		4			5				7				5				8				90							
Congenital Adrenal Hyperplasia	CAH	3	4	5	17	4	5	6	592	6	7	8	13	4	5	6	548	6	7	8	14	12	46	93	544	14	18	42
Aggressive Organic and Amino Acidemias	PROP, MUT, Cbl A,B, IVA, ASA, CIT, MSUD, TYR1	4	5	11	41	4	5	8	461	6	7	17	35	4	5	8	397	6	7	16	35	6	15	39	385	25	32	66
Galactosemia	GALT	6	10	26	30	6	8	12	26	9	26	32	23	6	8	14	23	12	26	33	21	28	45	94	21	37	58	92
Benchmark (days of age)		4			5				7				5				8				90							
Aggressive Fatty Acidopathies	MCAD, VLCAD, LCHAD, TFP	4	5	7	57	4	6	7	306	6	7	13	46	5	6	7	274	6	7	14	47	13	22	52	274	27	42	71
Benchmark (days of age)		4							7								8				90							
Fatty Acid Oxidation Diseases	CUD, CPT1, CPT2, FADD Other	4	5	15	No Type 1				162	6	7	18	No Type 1				134	7	8	13	No Type 1				130	17	24	50
Benchmark (days of age)		4							9								10				27							
Spinal Muscular Atrophy	SMA	4	5	7	No Type 1				14	10	10	14	No Type 1				13	10	12	15	No Type 1				13	15	18	35
Benchmark (days of age)		4							10								12				90							
Organic and Amino Acidemias	GA1, HCY, PKU	4	4	6	No Type 1				499	6	7	9	No Type 1				428	6	7	10	No Type 1				409	24	27	49
Biotinidase Deficiency	BIOT	3	4	6	No Type 1				269	6	7	8	No Type 1				239	7	8	11	No Type 1				234	24	33	73
Congenital Hypothyroidism	CH	3	4	5	No Type 1				1391	6	7	8	No Type 1				1299	6	7	9	No Type 1				1294	11	17	42
Benchmark (days of age)		4							14								21				90							
Severe Combined Immune Deficiencies	SCID	3	4	6	No Type 1				146	10	11	15	No Type 1				123	11	13	17	No Type 1				111	40	79	139
	Benchmark (days of age)	25							35								42				111							
	SCID PREM	23	25	37	No Type 1				42	29	31	46	No Type 1				30	31	33	35	No Type 1				25	98	109	220
Benchmark (days of age)		4							14								21				90							
Cystic Fibrosis	Category A, B, C	3	4	6	No Type 1				1203	10	11	14	No Type 1				1147	17	21	30	No Type 1				1119	29	39	75
	Type 1 and 3	3	4	6	No Type 1				91	22	28	33	No Type 1				49	25	30	39	No Type 1				49	34	56	88
	Benchmark (days of age)	4							21								28				90							
	Type 2	4	4	5	No Type 1				44	24	28	37	No Type 1				27	28	34	43	No Type 1				27	52	62	100
Mucopolysaccharidosis type 1 Hurler	MPS1H	3	3	9	No Type 1				11	24	27	29	No Type 1				4	25	N/A	N/A	No Type 1				4	43	N/A	N/A
Benchmark (days of age)		4							14								30				90							
Hemoglobinopathies	Hb SS, Hb S/βTh, Hb SC, Hb S/HPFH	3	4	5	No Type 1				448	7	9	12	No Type 1				333	22	34	45	No Type 1				337	60	79	113

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Appendix B: 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 1B. 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.

Table 2B. 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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