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

Annual Report to the Newborn Screening Ontario Advisory Council Calendar Year 2020

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Contents

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Executive Summary

The year 2020 will not be soon forgotten. The COVID-19 pandemic has brought about many changes in every element of society and has required innovative and creative solutions to new problems. As the broader healthcare industry pivoted over the year towards reducing non-essential services to support critical care, testing and vaccination needs, the newborn screening community has been faced with maintaining services in a period of unforeseen barriers and uncertainty. Newborn Screening Ontario (NSO) has been very successful in adjusting workflows and introducing new strategies to reduce the impact of the pandemic on the provision on services, while continuing to innovate and expand.

Beginning in March 2020, as the initial emergency orders were put in place, NSO was able to immediately facilitate work-from-home options for all non-laboratory staff. This was made possible, without significant workflow impacts, due to the transition to the new web-based Screening Information System in July 2019. For the safety of laboratory staff, and the protection of laboratory capacity in the event of an outbreak, the lab staff were split into two non-overlapping shifts. The NSO staff have been exceptional at adapting to rapid change, maintaining a safe environment, and identifying efficiencies to be gained in these new environments.

In addition to staffing changes, a number of safety mechanisms were required to reduce barriers in the screening system. Due to the unprecedented demand on courier services caused by online shopping, NSO began experiencing shipping delays and an increase in related missed screen alerts. Troubleshooting with Purolator led to new bright red packaging for sample shipments for better visibility at sorting stations for quicker transitions. NSO's Track Kit software has allowed for rapid identification of delays for immediate follow up with the courier and/or submitter. This, along with other COVID-19 impacts, are highlighted in grey text boxes throughout this year's report.

Like all laboratories, NSO has faced significant challenges this year in the procurement of supplies, particularly plastics and pipette tips. Through advocacy with the vendors, and validation of alternative options where available, a stable supply has been achieved so as not to impact testing. These procurement issues and shortages are expected to continue throughout 2021, and NSO will be implementing an inventory management tool to help with this area of operations.

Despite the challenges of a global pandemic, NSO had many big achievements in 2020. NSO began screening for Hurler Disease (MPS1H) on July 27, 2020. Spinal Muscular Atrophy (SMA) was added as a pilot to the newborn screening panel on Jan 13, 2020 and officially added to the panel on July 27, 2020. NSO participated in the first ever virtual accreditation visit and received a perfect report with no non-conformances.

The number of screen positives in 2020 decreased significantly from the previous year, mostly due to the introduction of third tier sequencing for CF screening in March 2020. There was a general reduction in the number of referrals for metabolic disease, due in part to the disorder logic changes implemented in mid 2019 but also due to the introduction of a TPN hold initiative in NICUs which has led to a reduction in the false positive referrals in this population.

In January 2020, NSO began to track Critical Congenital Heart Disease (CCHD) screens using a comparison of dried blood spot samples received to CCHD screening cards. 1297 potential missed screens were investigated;

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134 eligible infants were identified as having missed CCHD screening, and their health care providers were notified.

NSO continued its collaboration with Ontario's Infant Hearing Screening Program in 2020. In consultation with the ministries involved, NSO shifted to a waiver of consent model during the pandemic, as there were barriers to accessing hearing screening and obtaining consent. In total, 96.33% of babies born in 2020 were screened for risk factors for permanent hearing loss (PHL). 159 babies screened positive for cCMV and 22 screened positive for genetic risk factors. Interestingly, even with the almost complete screened population, the incidence of CMV in the population continues to be lower than expected and may be due to the public health measures in place for COVID-19. This will be monitored as these measures are lifted in 2021.

While significant change has been forced upon us in 2020, the NSO team has responded with agility and innovation to overcome barriers. This is evident in the data contained in this year's Annual Report, showing this year's numbers and the consistency across years that the program has maintained exceptional service.

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1. Screening Samples in 2020

Table 1. Screening sample volumes between 2016-2020.

Sample Type	2020	2019	2018	2017	2016
Satisfactory	141,548	146,099	145,724	145,405	145,018
Unsatisfactory*	1,785	1,356	1,365	2,248	1,755
Routine Screening – Total	**143,333**	**147,455**	**147,089**	**147,653**	**146,773**

*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 2020 is slightly lower than previous years, although the unsatisfactory rate is higher than in previous years.

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.

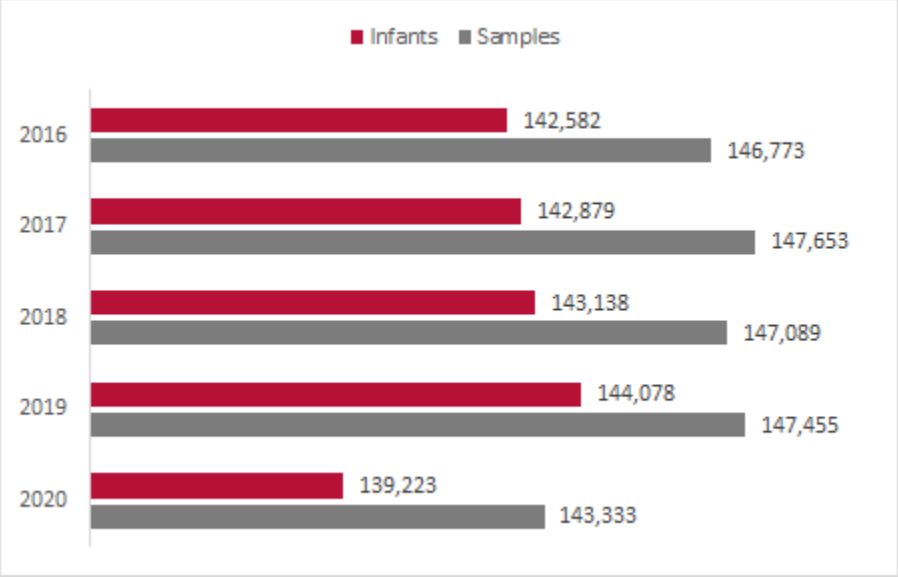

Figure 1: Total number of infants and samples screened between 2016-2020.

The overall number of infants tested has decreased over the last year; this corresponds with a decrease in the NSO-estimated number of infants born in Ontario in 2020 compared to previous years. Based on defers/ declines (Section 1.1.2), missed screen alerts and deceased infants from BORN (Section 1.1.3), and newborn

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screening sample counts (Table 1), NSO estimates the total number of infants in Ontario as 139,750 and the rate of screening uptake in 2020 as 99.6% (compared to 144,278 infants and a screening uptake of 99.8% in 2019).

COVID-19 Impact

Lower overall birth rates seen in 2020 are an anticipated effect of the COVID-19 pandemic, but monthly comparisons of 2019 and 2020 do not show any significant decline in the later months due to fewer pregnancies. However, the lower monthly totals throughout 2020 may relate to less immigration into Ontario due to travel restrictions.

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 2020, NSO received 713 completed decline/defer forms (Table 2), a continued increase from previous years. The number of declines documented using this form has increased slightly with 76 declines in 2020 compared with 68 in 2019. The remaining 637 forms received indicated a parent's desire to defer screening, and samples were eventually received for all but 13 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 2016-2020.

Case Type	2020	2019	2018	2017	2016
Declined/deferred form received	713	607	603	499	396
Decline	76	68	62	50	28
Deferral	637	539	541	449	368

Table 3. Overall declined screens between 2016-2020.

Infants with declined newborn screening test				
2020	2019	2018	2017	2016
136	131	120	127	116

An additional 65 declined screens were also identified via missed screen alerts. In total there were 136 infants with declined newborn screening tests (Table 3).

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1.1.3 Missed Screens

COVID -19 Impact
There was unprecedented demand on courier services which caused some shipping delays and an increase in related missed screen alerts.

In 2020, there was an increase in potential missed screen alerts investigated where the sample was received after the alert but was collected before (157 in 2020 compared to 76 and 117 in 2019 and 2018 respectively).

In 2020, there were 144 potential missed newborn screen alerts that required follow up by NSO. Hospitals were the responsible facility in 76% of the missed screen alerts and midwives were involved in roughly 24% of the cases. Action on the part of NSO resulted in 99 of the 144 (69%) truly missed screens being completed. This is comparable to the rate in 2019, where 71% truly missed screens were completed.

1.1.4 Hemoglobin Carriers

Table 4. Hemoglobin carrier requests between 2016-2020.

	2020	2019	2018	2017	2016
Requests from high risk population	23	35	46	61	28
Total Requests	**32**	**40**	**55**	**69**	**45**
Number of carriers	12	16	18	18	11

Table 5. Carriers identified in 2020.

HGB Pattern	Carriers Identified
FAC	373
FAD	208
FAE	265
FAS	1374
FAX	86
Grand Total	**2306**

In 2020, approximately 0.5% of carriers requested their results. The number of hemoglobin carrier requests has decreased over the last year.

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.

1.1.5 Age at Collection

Table 6. Age at collection for 2020, initial samples only.

Age at Collection	Number of Initial Samples (2020)	% of Initial Samples (2020)	% of Initial Samples (2019)	% of Initial Samples (2018)
Less than 24 hours	916	0.66%	0.69%	0.56%
24-47 hours (1-2 days)	135699	97.48%	96.36%	95.20%
48-72 hours (2-3 days)	1862	1.34%	1.99%	2.79%
73-168 hours (3-7 days)	533	0.38%	0.50%	0.81%
Greater than 168 hours (7 days)	192	0.14%	0.46%	0.58%

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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 2016-2020.

			2020	2019	2018	2017	2016
SAMPLES	Satisfactory Samples		143,333	146,099	145,045	144,717	144,359
	Unsatisfactory Samples		2332	2044	2,044	2,936	2,414
	Unsatisfactory Rate		**1.63%**	**1.40%**	**1.41%**	**1.99%**	**1.64%**
	Samples Collected at <24hrs		547	697	575	577	518
	Unsatisfactory Samples excluding <24hr samples		1785	1347	1,469	2,359	1,896
	Unsatisfactory Rate excluding <24hr samples		**1.25%**	**0.90%**	**1.01%**	**1.60%**	**1.30%**
REASONS	Lab Unsat Reasons	Quantity of blood insufficient	1297	919	710	1471	1094
		Blood spots appear scratched or abraded	94	118	292	531	421
		Blood spots are supersaturated	42	97	176	185	193
		Blood spots appear clotted or layered	155	202	403	639	491
		Blood spots appear diluted	<5	<5	<5	5	17
		Blood spots exhibits serum rings	70	82	168	200	95
		Blood spots are wet and/or discolored	14	10	38	<5	5
		Other	25	50	88	62	35
	Data Unsat Reasons	Blood dot collection paper is expired	38	14	12	77	95
		Insufficient data provided	11	9	11	29	14
		Damaged or delayed in transit	5	5	45	8	1
		Delivered to lab > 14 days after collection	33	19	8	23	4
		Sample collected at <24hrs	547	697	575	577	518
		Other/Mislabel	27	6	90	47	46

There were 27 samples that were deemed unsatisfactory for both a lab and a data unsat reason.

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 7). 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 anticipation of this change, an assessment was done in the sample-receiving area of the lab, confirming that we could expect an increase in the number of unsats due to insufficient quantity of blood (as well as an increase in the number of priority panels ordered) with the addition of the new assay. In October 2020, the first tier assay of the MPS1H screen was updated to be run without replication rather than in duplicate, reducing the minimum number of punches required to complete a full screen. See further discussion about MPS1H in section 3.4.4.

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The number of unsatisfactory samples collected at <24 hours in 2020 decreased to 547, compared to 697 in 2019 (Table 7).

2.2 Repeat Rates for Unsatisfactory Specimens

The majority (74%) of repeat samples are received within 2 weeks of the initial sample (Table 8). By 6 weeks, 86.1% of unsatisfactory samples have had screening completed via a repeat sample.

Table 8. Repeats received on unsatisfactory samples, 2020 data only.

Time to receipt of unsatisfactory repeat sample		
Total unsatisfactory samples 2020	**2332**	
< 1 week	1314	56.3%
1 - <2 weeks	410	17.7%
2 - <3 weeks	155	6.6%
3 - <6 weeks	128	5.5%
≥ 6 weeks	33	1.4%
Not received	292	12.5%

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 one of the most common disorders screened by NSO and the most aggressive, early onset diseases and include Metabolic diseases (AAAC platform), galactosemia, CH (TSH) and CAH (17OHP).

In 2020, NSO performed 1255 priority panels (70.3% 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.

Table 9. Repeat samples for priority panel unsats.

Time to receipt of priority panel repeat sample		
Total priority panels 2020	**1255**	
< 1 week	682	54.3%
1 - <2 weeks	278	22.2%
2 - <3 weeks	87	6.9%
3 - <6 weeks	68	5.4%
≥ 6 weeks	15	1.2%
Not received	125	10.0%

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There were 14 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 2020 there were 9 TLU where a repeat was not received. Table 10 summarizes shows the time to receipt of repeat samples after a TLU.

Table 10. Repeat samples for TLU.

Time to receipt of TLU repeat sample		
Total Test Level Unsats – Routine	**74**	
< 1 week	28	37.8%
1 - <2 weeks	19	25.7%
2 - <3 weeks	7	9.5%
≥3 weeks	12	16.3%
Not received	8	10.8%
Total Test Level Unsats - Urgent	**50**	
< 1 week	29	58.0%
1 - <2 weeks	12	24.0%
≥2 weeks	8	16.0%

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.

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COVID -19 Impact

Pandemic online ordering and shipping demands also resulted in additional unsats related to delays in shipping. This is reflected in the increase in DBS samples delivered to the lab > 14 days after collection (Table 7), as well as in this year's sample turnaround time data (see Section 4, below). NSO closely monitored for lost or delayed packages using Track-Kit and implemented new packaging to help with priority sorting.

There were 38 unsatisfactory samples due to expired filter paper, up from 14 in 2019 (Table 7). Expired cards can fluctuate year to year, depending on when the lots of cards expire. There were three lots of cards that expired in 2020, in March, May and September. 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 2020, there were 881 screen positive referrals (Figure 2). This represents ~0.63% of the total number of infants screened by NSO.

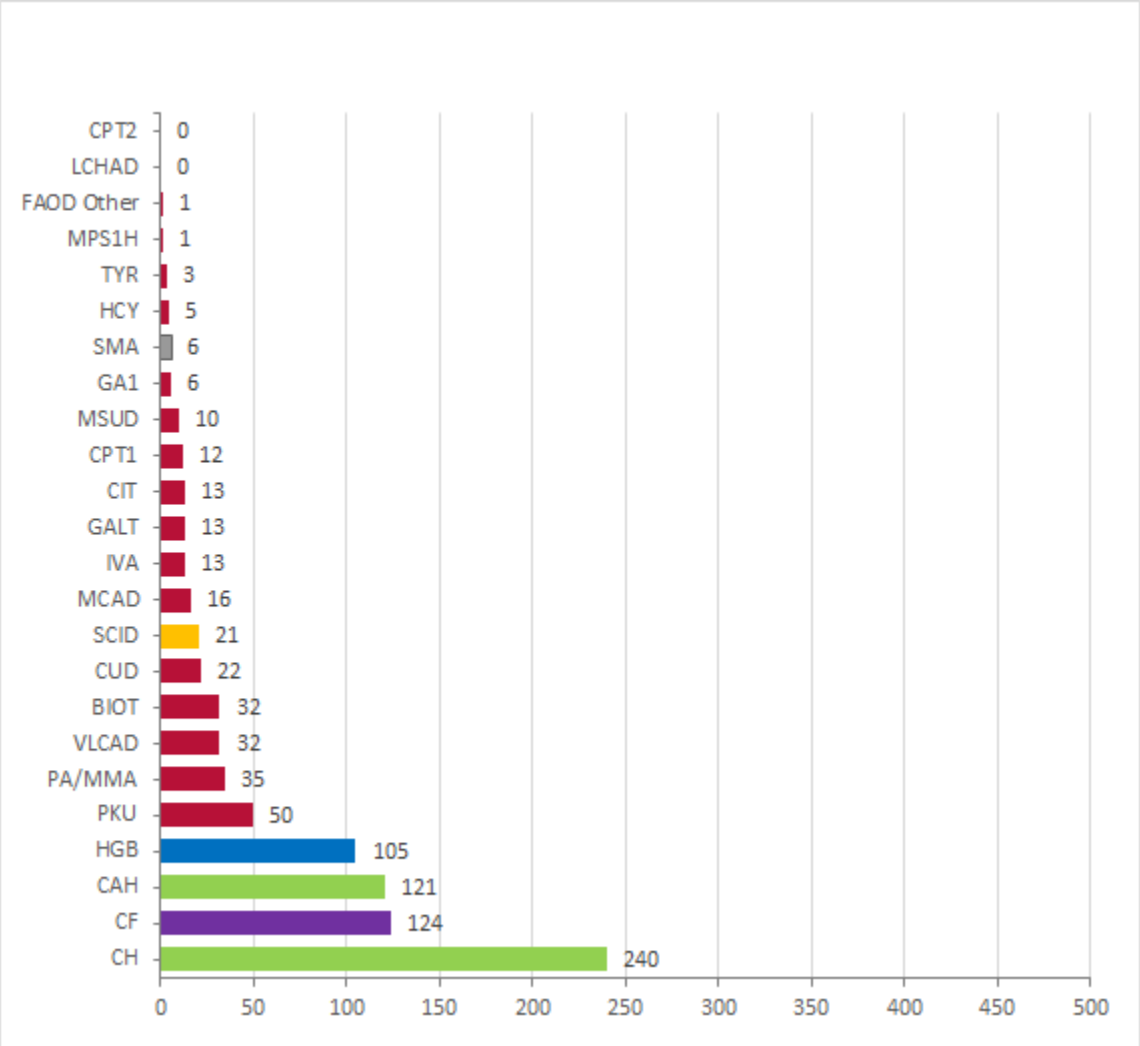

Figure 2. Total number of screen positive referrals by disease in 2020

The number of screen positive infants referred in 2020 decreased significantly from 2019 (1503 vs. 881). This is discussed further in Section 3.4.

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3.1 Referrals by Treatment Centre

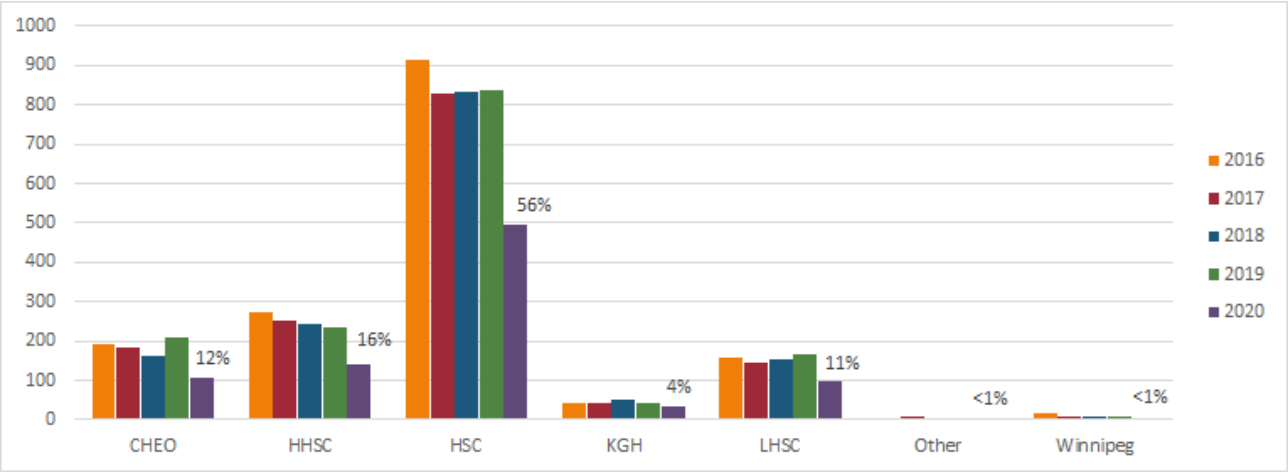

Figure 3. The total number of referrals by treatment centre between 2016-2020.

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 3). '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. Although the number of referrals decreased in 2020, the proportion of referrals received by each of the five Ontario regional treatment centres was similar between 2019 and 2020.

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3.2 Screen Positive Referrals by Disorder Group

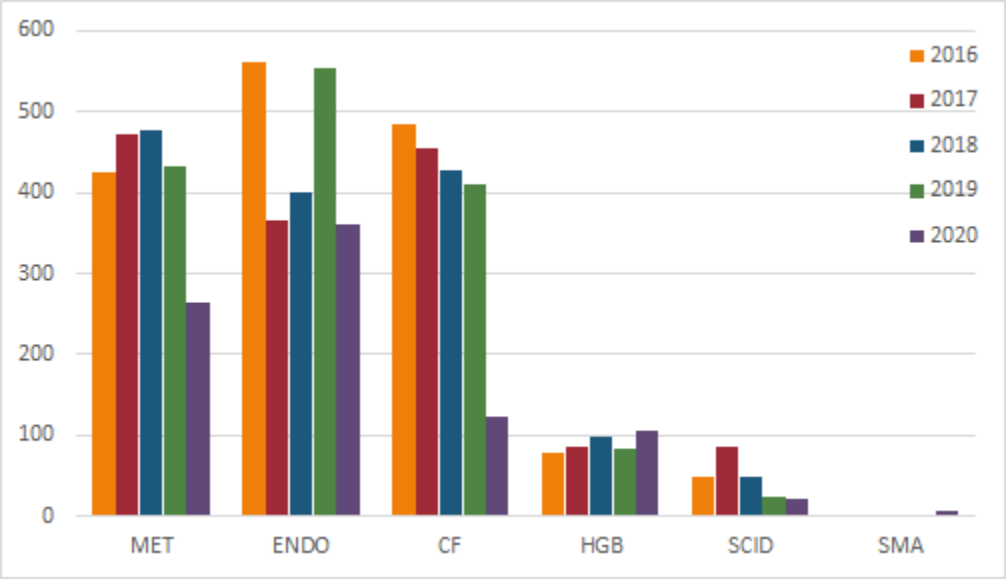

Figure 4. The total number of screen positives by disease grouping between 2016-2020.

The number of screen positive referrals per disease grouping increased slightly for hemoglobinopathies (Figure 4). Numbers remained relatively constant for SCID, whereas they decreased for Metabolic disorders, Cystic Fibrosis, and endocrinopathies. This is the first year that spinal muscular atrophy (SMA) and Mucopolysaccharidosis type 1 Hurler disease (MPS1H) were added to the newborn screening panel. These details are discussed further in section 3.4.

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3.2.1 Percentage of Screen Positive Referrals by Disorder in 2020

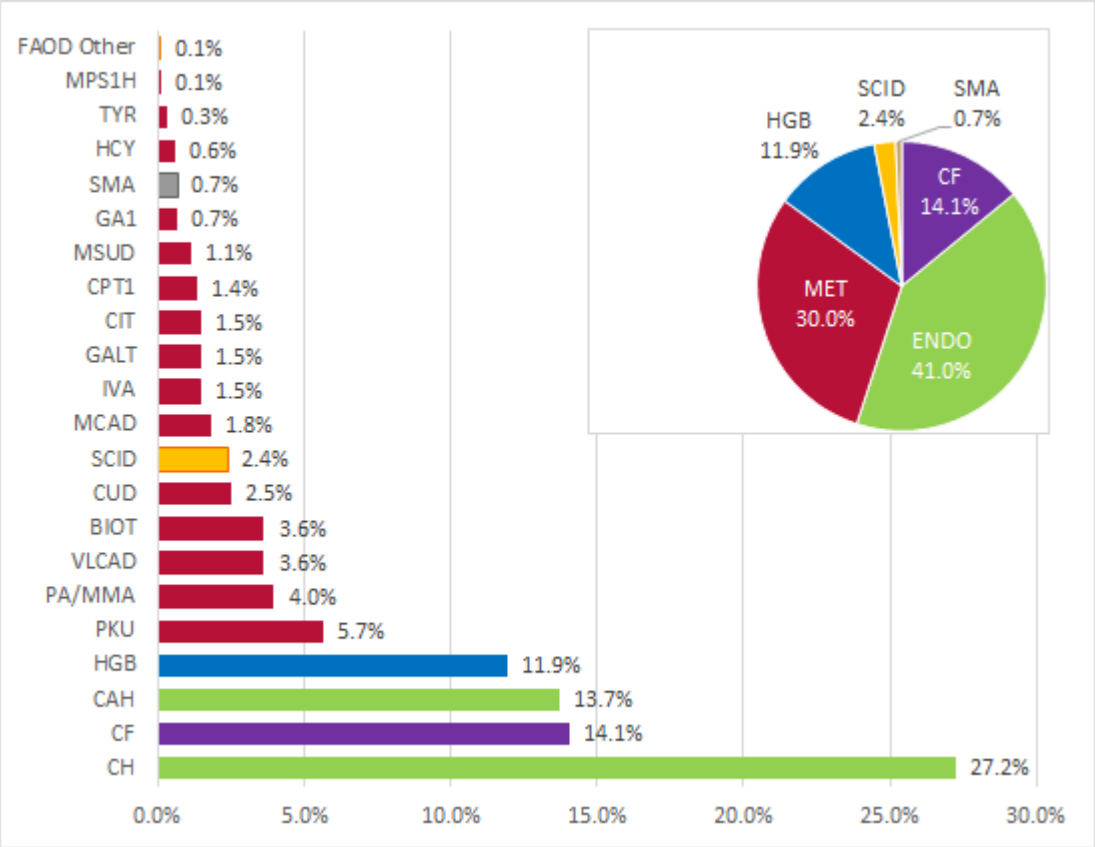

Figure 5. The percentage of screen positive referrals by disorder in 2020.

Endocrinopathies and Metabolics represent 41% and 30% of screen positives respectively (Figure 4). SCID screen positive referrals decreased in 2020 but due to overall lower referral numbers, now represent 2.4% of total screen positive referrals. The number of Cystic Fibrosis referrals decreased dramatically in 2020 and now represent 14.1% of total screen positive referrals (see Section 3.4.2 for discussion). Hemoglobinopathies represent approximately 11.9% of screen positive referrals. SMA represents 0.7% of referrals.

3.3 Diagnostic Feedback

Approximately 24.9% (219 cases) of diagnostic evaluation report forms (DERFs) remain pending for the referrals made in 2020 as of April 1, 2021. While the number of pending DERFs is similar to last year, in terms of the percentage of outstanding (compared to the total number of referrals) it has almost doubled.

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3.4 Definitive Diagnosis Data and Positive Predictive Values

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 A.

3.4.1 Hemoglobinopathies

The number of screen positives in 2020 increased by 22 referrals from 2019.

Table 11. The PPV calculations for the current and past screening algorithms.

Disease	Additional information	PPV (Yes)	PPV (Yes + Variant)	PPV (Yes + 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, 2020)	**58.9%**	**59.6%**	**90.4%**	**9.4%**

3.4.2 Cystic Fibrosis

The number of screen positives in 2020 decreased significantly with the introduction of third tier sequencing of the *CFTR* gene in March 2020. There were 124 referrals this year compared to 410 in 2019. Prior to the introduction of sequencing, CF referrals were Category A (elevated IRT with 2 *CFTR* variants identified), Category B (elevated IRT with 1 *CFTR* variant identified) or Category C (IRT >99.9% centile with no *CFTR* variants identified). The new algorithm for CF is the same for the first and second tiers of testing. Samples with 2 *CFTR* panel variants are referred immediately as Type 1 (or occasionally as Type 2, depending on the genotype). The former Category B and C groupings would then proceed to third tier sequencing of the *CFTR* gene. Results from this final stage are referred if 2 or more variants (VUS, likely pathogenic or pathogenic) are identified in the *CFTR* gene and are categorized as follows: Type 1 – genotypes consistent with a high risk of a diagnosis of CF; Type 2 – genotypes consistent with a high risk for a *CFTR* -related disorder NOT meeting CF diagnostic criteria; and Type 3 – genotypes of uncertain clinical significance. Prior to the implementation of sequencing there were 63 referrals (January – March 18) and 61 for the remainder of 2020.

Table 12. The PPV calculations for the current and past screening algorithms.

Disease	Additional information	PPV (Yes)	PPV (Yes + Variant)	PPV (Yes + Variant + Secondary)	% of DERFs Pending
Cystic Fibrosis	Past (Jul 28, 2019 - Mar 18, 2020) Cat A	75.0%	100.0%	100.0%	52.9%
	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	4.5%	10.0%	10.0%	10.1%
	Current (Mar 19 - Dec 31, 2020) Type 1	100.0%	100.0%	100.0%	50.0%
	Current (Mar 19 - Dec 31, 2020) Type 2	0.0%	100.0%	100.0%	65.5%
	Current (Mar 19 - Dec 31, 2020) Type 3	0.0%	100.0%	100.0%	75.0%
	Current (Mar 19 - Dec 31, 2020) ALL	**43.5%**	**100.0%**	**100.0%**	**62.3%**

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

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3.4.3 Endocrinopathies

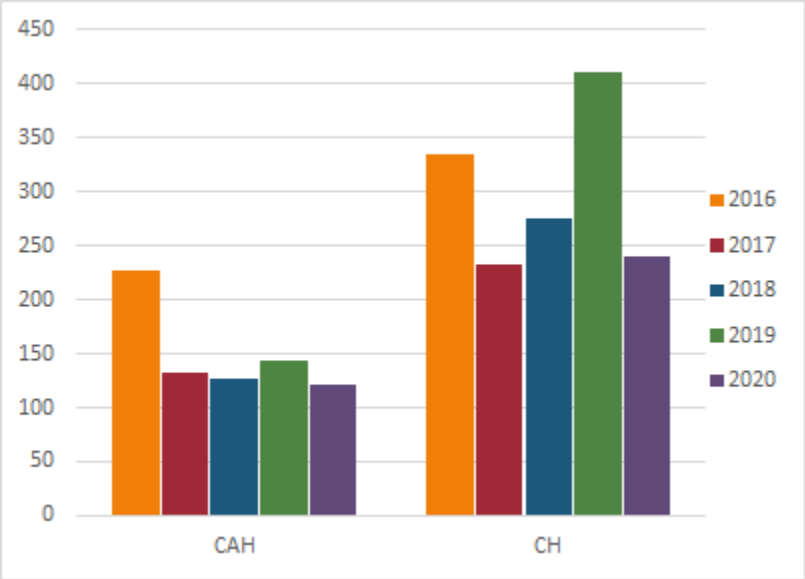

Figure 6. The total number of congenital adrenal hyperplasia and congenital hypothyroidism screen positives between 2016-2020.

The number of screen positives for CAH remained consistent for the last 4 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 decreased in 2020. This is due to the cutoff change implemented in July 2019, which brought the TSH cutoff from 15 mIU/L to 17 mIU/L. This is the first full year of referrals with this cutoff and has brought CH referrals back to levels seen in 2017 and 2018.

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

Disease	Additional information	PPV (Yes)	PPV (Yes + Variant)	PPV (Yes + 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, 2020)	**20.4%**	**34.7%**	**34.7%**	**10.5%**
Congenital Adrenal Hyperplasia	Past (Sept 2, 2016 - Jun 11, 2018)	7.0%	7.0%	7.0%	3.0%
	Current (Jun 12, 2018 - Dec 31, 2020)	**4.1%**	**4.1%**	**4.4%**	**7.7%**

*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

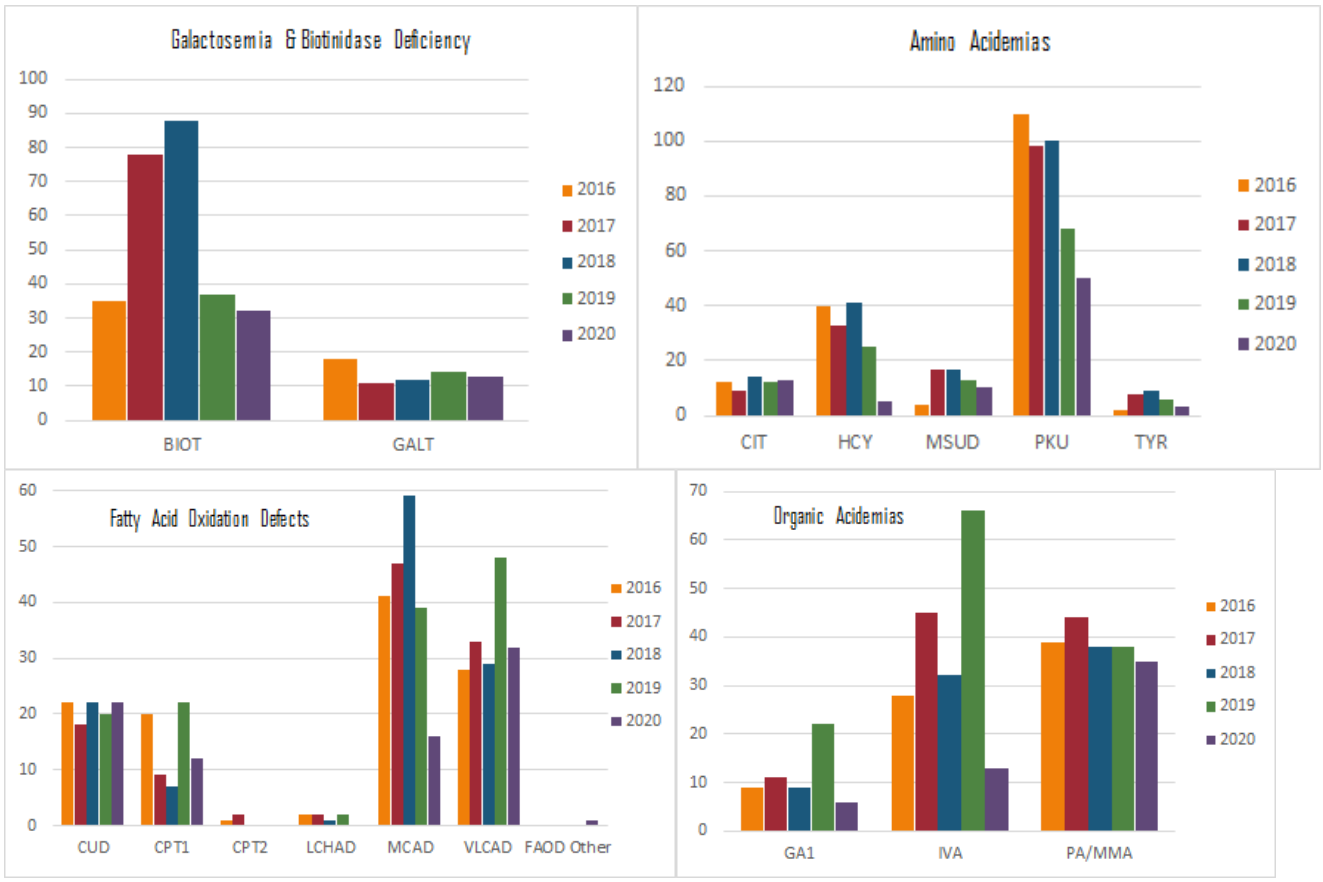

Figure 7. The number of metabolic screen positives between 2016-2020 by disease

NSO began screening for MPS1H on July 27, 2020. The screening algorithm consists of a first tier screen for iduronidase (IDUA) through a fluorometric analysis. If the enzyme is below the cutoff, IDUA is measured again through an MS/MS method. If the enzyme is again below the cutoff, IDUA sequencing is performed as a third tier. Infants with 2 or more *IDUA* variants of interest (either VUS, pathogenic or likely pathogenic) are referred as screen positive; infants with IDUA activity below the failsafe and no pseudodeficiency alleles are also reported. Carriers and infants with no *IDUA* variants of interest and activities above the failsafe threshold are not reported.

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 also due to the TPN hold initiative underway across some of the NICUs in the province. Holding TPN for 3 hours prior to obtaining the newborn screening sample has led to a reduction in false positive referrals. In 2020, 6 hospitals were participating and 137 requisitions were received indicating TPN was held. In the first 3 months of 2021, 172 requisitions have been received indicating TPN was held.

MCAD had a disorder logic change in 2019 which saw a reduction in referrals the latter part of that year and all of 2020. VLCAD has returned to referral rates observed in 2016-2018, although no disorder logic change was implemented.

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There was only 1 metabolic disorder logic change made in 2020. 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.

Table 14. The PPV calculations for the current and past (where applicable) screening algorithms.

Disease	Additional information	PPV (Yes)	PPV (Yes + Variant)	PPV (Yes + Variant + Secondary)	% of DERFs Pending
Glutaric Aciduria type 1		**9.2%**	**9.2%**	**25.4%**	**1.1%**
Isovaleric Acidemia	Past (until Feb 17, 2020)	3.0%	4.2%	4.2%	0.5%
	Current (Feb 18 - Dec 31, 2020)	**33.3%**	**33.3%**	**33.3%**	**57.1%**
PA/MMA	Past (Feb 16, 2010 - Apr 21, 2013)	3.7%	3.7%	8.3%	0.9%
	Current (Apr 22, 2013 - Dec 31, 2020)	**4.3%**	**4.7%**	**9.1%**	**10.0%**
CPTI		**4.2%**	**60.1%**	**60.1%**	**4.0%**
CPTII		**12.1%**	**12.1%**	**12.1%**	**0.0%**
LCHAD		**80.0%**	**80.0%**	**93.3%**	**6.3%**
VLCAD		**8.0%**	**13.1%**	**15.1%**	**3.8%**
CUD	Past (until Mar 4, 2014)	5.5%	5.5%	5.5%	0.0%
	Current (Mar 5, 2014 - Dec 31, 2020)	**4.5%**	**4.5%**	**4.5%**	**7.9%**
MCAD	Past (Sep 1, 2016 - Jul 28, 2019))	18.9%	20.3%	21.6%	1.3%
	Current (Jul 29, 2019 - Dec 31, 2020)	**75.0%**	**81.3%**	**81.3%**	**30.4%**
Citrullinemia		**17.9%**	**20.4%**	**20.4%**	**5.1%**
Homocystinuria	Past (until Jul 28, 2019)	**0.4%**	**0.4%**	**4.0%**	**3.1%**
	Current (Jul 29, 2019 - Dec 31, 2020)	**0.0%**	**0.0%**	**0.0%**	**50.0%**
Phenylketonuria	Past (until Jul 28, 2019)	**14.2%**	**27.4%**	**27.4%**	**1.3%**
	Current (Jul 29, 2019 - Dec 31, 2020)	**18.0%**	**26.0%**	**26.0%**	**21.4%**
MSUD	Past (until Nov 14, 2011)	3.8%	3.8%	3.8%	0.0%
	Current (Nov 15, 2011 - Dec 31, 2020)	**7.9%**	**8.9%**	**8.9%**	**3.7%**
Tyrosinemia	Past (Sep 14, 2009 - Sep 19, 2011)	1.4%	1.4%	1.4%	0.0%
	Current (Sep 20, 2011 - Dec 31, 2020)	**12.3%**	**12.3%**	**15.8%**	**7.7%**
Galactosemia	Past (until Jan 12, 2014)	35.7%	41.4%	41.4%	1.4%
	Current (Jan 13, 2014 - Dec 31, 2020)	**16.7%**	**28.9%**	**28.9%**	**8.8%**
Biotinidase Deficiency	Past (Jan 13, 2014 - Jul 2, 2014)	2.1%	37.5%	37.5%	0.0%
	Current (Jul 3, 2014 - Dec 31, 2020)	**4.7%**	**37.2%**	**37.2%**	**5.0%**
MPS1H					100.0%

*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

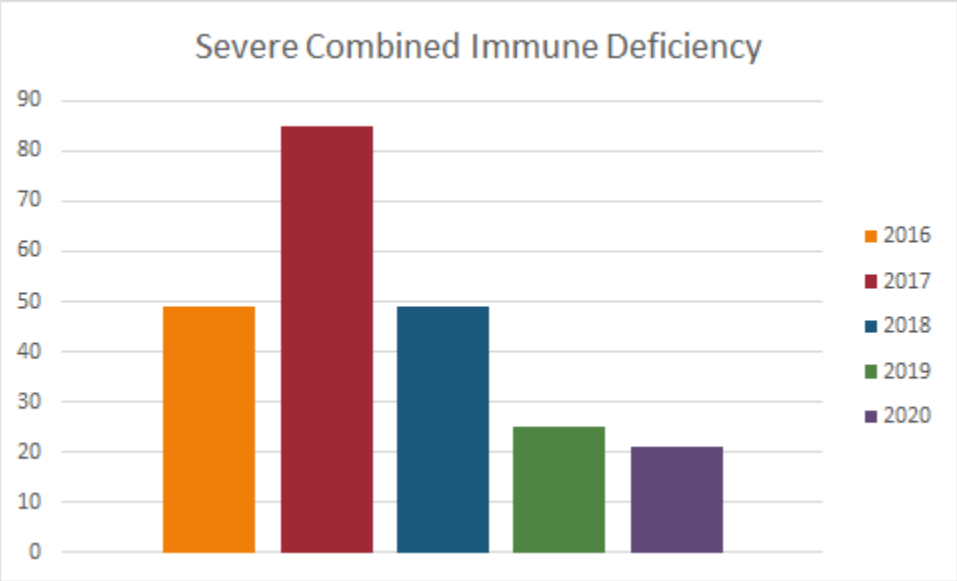

Figure 8. The number of SCID screen positives between 2016-2020.

The overall number of screen positive results for SCID decreased slightly in 2020. SCID screening changed to a fixed curve with intercepts at 41.5 (confirm) and initial (39.25). This change was implemented on Jan 6, 2020.

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

Disease	Additional information	PPV (Yes)	PPV (Yes + Variant)	PPV (Yes + Variant + Secondary)	% of DERFs Pending
Severe Combined Immune Deficiency	Past (Jul 29, 2019 - Jan 5, 2020)	0.0%	0.0%	0.0%	0.0%
	Current (Jan 6 - Dec 31, 2020)	**37.5%**	**37.5%**	**37.5%**	**47.6%**

*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

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 4 or less copies of the *SMN2* gene are screen positive (*SMN2* copy number >4 are screen negative). Carriers are not identified through this screening methodology. Since screening began 6 infants were identified.

Table 16. The PPV calculations for the current screening algorithm.

Disease	PPV (Yes)	PPV (Yes + Variant)	PPV (Yes + Variant + Secondary)	% of DERFs Pending
Spinal Muscular Atrophy	**100.0%**	**100.0%**	**100.0%**	**16.7%**

*Cells are highlighted in red when >10% of DERFs are outstanding for a particular disorder or group of disorders. Note in this case only 1 DERF was pending.

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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 the 1 MPS1H referral of 2020 so an incidence was not calculated.

Table 17. 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	2.3%	1 in 2,069	88%
Congenital Adrenal Hyperplasia (CAH)	14-May-07	1.6%	1 in 22,398	27%
Sickle Cell Disease	24-Nov-06	4.1%	1 in 2,894	97%
Cystic Fibrosis (CF)	9-Apr-08	1.9%	1 in 4,950	95%
Severe Combined Immune Deficiency (SCID)	12-Aug-13	6.3%	1 in 62,762	33%
Glutaric Aciduria type 1 (GA1)	9-Aug-06	1.1%	1 in 129,902	100%
Isovaleric Acidemia (IVA)	9-Aug-06	1.4%	1 in 159,879	56%
Propionic Acidemia (PA)/ Methylmalonic Acidemia (MMA)/ Cobalamin A & B Defects	9-Aug-06	4.3%	1 in 83,137	35%
Long-chain 3-Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD)/ Trifunctional Protein Deficiency (TFP)	9-Aug-06	6.3%	1 in 173,203	86%
Very-long-chain Acyl-CoA Dehydrogenase Deficiency (VLCAD)	9-Aug-06	3.8%	1 in 74,230	60%
Carnitine Uptake Defect (CUD)	9-Aug-06	2.7%	1 in 94,474	20%
Medium-chain Acyl-CoA Dehydrogenase Deficiency (MCADD)	4-Apr-06	1.8%	1 in 15,996	92%
Citrullinemia (CIT)/Argininosuccinic Acid Lyase Deficiency (ASA)	9-Aug-06	5.1%	1 in 71,670	54%
Homocystinuria (HCY)	9-Aug-06	4.4%	1 in 2,078,432	Unknown
Phenylketonuria (PKU)	4-Apr-06	2.7%	1 in 16,240	65%
Maple Syrup Urine Disease (MSUD)	9-Aug-06	2.0%	1 in 188,948	26%
Tyrosinemia type 1	9-Aug-06	3.1%	1 in 230,937	71%
Galactosemia (GALT)	19-Feb-07	5.7%	1 in 50,084	17%
Biotinidase Deficiency (BIOT)	19-Feb-07	2.7%	1 in 71,549	11%
Mucopolysaccharidosis type 1 Hurler (MPS1H)	27-Jul-20	100.0%	Unknown	Unknown
Spinal Muscular Atrophy (SMA)	13-Jan-20	16.7%	1 in 27,672	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. CCHD Screening

4.1 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 2020 was 141,408 representing 138,544 infants. This is lower than the estimated number of infants in Ontario that was derived from the blood spot samples, of 139,910 (Figure 9).

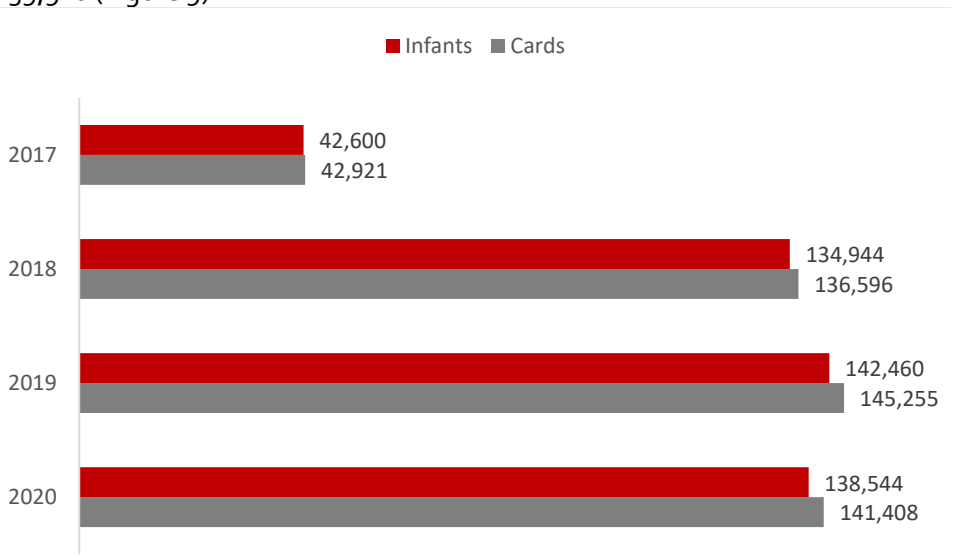

Figure 9. CCHD cards received at NSO and total number of infants between 2017-2020.

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 2020, 6,574 of the requisitions submitted were for screens not done.

Table 18. CCHD cards received.

CCHD Cards received	2020		2019		2018	
Screen Completed	134,834	95.4%	138,775	95.5%	132,134	96.7%
Screen Not Done*	6,574	4.6%	6,480	4.5%	4,462	3.3%
	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 2019 and 2020.

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4.2 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, 98.8% 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 1.1% required a second test and 0.2% required three tests to complete the screen.

Table 19. Tests required to complete screen between 2018-2020.

Tests Done	2020		2019		2018	
1 Test	131,592	98.8%	136,935	98.7%	129,967	98.4%
2 Tests	1,431	1.1%	1,621	1.2%	1,948	1.5%
3 Tests	222	0.2%	218	0.2%	219	0.2%
	133,245		138,775		132,134	

4.3 Screens Not Done

In 2020, CCHD screens were not done on 4.6% 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 20. Reasons for CCHD Screen not done between 2018-2020.

	2020		2019		2018	
'Screen Not Done' cards submitted	6574		6,480		4,462	
Decline/deferred (back page of form not completed)	95	1.4%	93	1.4%	78	1.7%
Declined	66	1.0%	26	0.4%	26	0.6%
Deferred	565	8.6%	542	8.4%	465	10.4%
Infant diagnosed prenatally with heart defect	101	1.5%	74	1.1%	58	1.3%
Infant diagnosed with heart defect by physical exam	33	0.5%	47	0.7%	58	1.3%
Infant is not appropriate for screening (e.g. NICU > 7 days, on oxygen, IV in right hand, limb anomaly, etc.)	4725	71.9%	4732	73.0%	3735	83.7%
Already done	169	2.6%	17	0.3%	8	0.2%
Insufficient information provided/blank card	671	10.2%	704*	10.9%	18	0.4%
Other	149	2.3%	245	3.8%	16	0.4%

*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.

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Of the decline/deferred group where the back of the form was not completed – 81 had a CCHD screen completed (19 of these infants did not have a DBS screen). The 66 declined screens are reviewed further below in the missed screen section.

4.4 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 2020, 1297 potential missed screens were identified. The majority of the alerts were from hospitals (1105). The majority of these alerts were due to improper documentation – either the infant was screened but documentation was not sent to NSO (754) or the infant was not suitable for screening and documentation was not sent to NSO (290). There were 23 families who declined CCHD screening where documentation was not sent prior to the missed screen alert. There were 134 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.

There were more CCHD declined screens than in previous years. In total 89 families declined CCHD screening. Of the declines, 41 did go on to have CCHD screening suggesting that the decline form was completed in error and should have indicated a deferral of screening. There were 39 who had the DBS screen performed (5 of these had no CCHD screening record).

4.5 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 (91%) of screening has been done in the recommended range which is an increase from last year (87.9%).

Table 21. Age at time of CCHD Screen from 2018-2020

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

The percentage of screens done at less than 24 hours is 1.7% overall which is a large reduction from the 4.5% observed in 2019 and 2018. During our 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

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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. Going forward we have created a workflow to identify these potential errors so that they can be reviewed and corrected where needed in a timely manner.

4.6 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 done in 2020 was 1,069, which was 0.76% of the cards received. The most frequent error was incomplete documentation – either of a repeat test done after 1 hour or missing screening values. 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, these numbers decreased in 2020.

Table 22. Outcomes from unsatisfactory CCHD screen notifications.

	2020	2019	2018
Unsatisfactory Screens	**1,069**	**1855**	**615**
Baby >7days, no rescreen recommended	65 (6.1%)	49 (2.6%)	31 (5.0%)
Baby in hospital, no screen recommended	253 (23.7%)	566 (30.5%)	33 (5.4%)
Documentation inaccurate or incomplete	574 (53.7%)	865 (46.6%)	297 (48.3%)
Family Declined	0	<5	0
No action needed	38 (3.6%)	51 (2.7%)	0
Physical exam recommended (screen positive)	0	<5	<5
Missed - baby >7 days, no screening recommended	9 (0.8%)	5 (0.3%)	251 (40.8%) (only recorded as rescreen)
Missed - screening recommended	54 (5.1%)	119 (6.4%)	
Rescreen recommended	76 (7.1%)	195 (10.5%)	
Total Screening Forms Submitted	**141,408**	**145,255**	**136,596**
Unsatisfactory Rate	**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 by the dried blood spot card) or a satisfactory CCHD screen located that was previously unlinked to infant.

NSO performed follow up on the 1,069 unsatisfactory screens, and in 53.7% of follow up cases the result was amended by the submitter due to incorrect completion of the form. In 7.1% of cases a rescreen was recommended. Through the follow up of unsatisfactory screens NSO was able to follow up with submitters for 130 infants that had not received a proper CCHD screen and needed to be screened (missed) or rescreened.

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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.

4.7 CCHD Screen Positives – 2020 data

There were 197 CCHD screen positives in 2020, most of which were screened within 24-48 hours. There was 7 screen positive identified after an early screen at less than 24 hours.

Table 23. Age at time of screen positive

Age at Screen Positive	Total No.
< 24 hours	7
24-48 hours	172
> 48 hours	5
Not available	13
Grand Total	**197**

Of the 197 screen positives received in 2020, 11 were diagnosed with a critical congenital heart defect, 94 had a secondary CHD target or were diagnosed with an incidental finding such as pulmonary disease or infection, and 88 were found to be not affected.

4.8 CCHD Definitive Diagnosis Data and Positive Predictive Values

In 2020, the Positive Predictive Value (PPV) for CCHD screening was 5.7% for primary targets and 30.1% for primary and classical secondary target diseases. Cumulatively since the beginning of the program, the PPV is 6.0% for primary targets, and 29.1% for primary and classical secondary target diseases. Of the 637 screen positives since the initiation of CCHD screening, 302 (47.4%) have been determined to be not affected after diagnostic follow up.

Table 24. PPV calculations for CCHD Screen Positives (2020 and cumulative)

Data set	PPV (Primary)	PPV (Primary + Secondary)	Total No. Screen Positive	Outcome Classification				
				Primary Targets	Secondary Targets	Incidental Findings	Not Affected	DERF Pending
2020 only	5.7%	30.1%	197	11	47	47	88	4
Cumulative	6.0%	29.1%	637	38	146	146	302	5

Table 25. Definitive diagnosis for CCHD Screen Positives (2020 and cumulative)

Definitive Diagnosis Categorization	2020	Cumulative
Primary target	11	38
Secondary target	47	146
Incidental Finding	47	146
Not affected	88	302
DERF pending	4	5
Grand Total	**197**	**637**

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5. Risk Factor Screening for Permanent Hearing Loss

5.1 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 Permanent Hearing Loss (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 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-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.

5.2 Consent

In the first quarter of 2020, families were offered the option to consent to risk factor screening on the DBS at the time of the infant hearing screen, or when the appointment for the infant hearing screen was booked. Consent was obtained by the IHP, using a standardized consent process that was put in place at the onset of the risk factor screening program.

COVID-19 Impact

When the COVID-19 pandemic began and all non-essential services were discontinued, 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 (98.95%) 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.

The section below summarizes the number of babies screened before and after the change, as well as the consent metrics in the first period. Note that the inclusion criteria for Period 1 and Period 2 is based on date of birth.

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Table 26. Babies consented and screened for risk factor screening for PHL

	Period 1- Consent through IHP DOB 2020-01-01 to 2020-03-25	Period 2- Waived consent DOB 2020-03-26 to 2020-12-31	TOTAL
Births*	31,802	107,672	139,474
IHP Screening Form received	27,757 (87.28%)		
Consent for risk factor screening	27,465 (86.36%)		
Babies screened for CMV and genetic risk factors	27,314 (85.89%)	107,052 (99.42%)	134,366 (96.33%)
Babies screened for CMV	27,426 (86.23%)	107,177 (99.54%)	134,603 (96.50%)
Babies screened for genetic risk factors	27,334 (85.95%)	107,224 (99.58%)	134,558 (96.47%)

*Estimate based on samples received, missed and declined DBS screens

NSO received IHP screening forms on 86.36% of babies born between January 1, 2020 and March 25, 2020 (Period 1). Note that access to hearing screening was restricted beginning in mid-March 2020 due to the COVID-19 pandemic, therefore the proportion of screening forms received for babies born in this period was slightly lower than earlier in the program (screening forms were received for 90.03% of babies born July 29, 2019 to December 31, 2019). 98.95% of families who were offered hearing screening and from whom NSO received a hearing screening form consented to risk factor screening for PHL.

85.89% of babies born in Period 1 were screened for CMV and genetic risk factors. This is slightly lower than the consent rate due to samples that were unsatisfactory to complete risk factor screening for PHL and missed and declined DBS screens.

99.42% of babies born between March 26, 2020 and December 31, 2020 (Period 2) were screened for CMV and genetic risk factors for PHL. This is lower than the total births due to the same reasons as listed for Period 1.

In total, 96.33% of babies born in 2020 were screened for risk factors for PHL.

5.3 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 variants in the *GJB2/6* and *SLC26A4* genes, and infants with 2 or more variants in the same gene are considered screen positive.

All risk factor screen positive infants (CMV and genetics) have a diagnostic audiology assessment arranged by the regional IHP Lead Agency. If PHL is identified, a referral to ENT is made for further evaluation, and interventions, supports and services are provided. If hearing is within normal limits, the infant is enrolled in the appropriate IHP surveillance program for audiologic monitoring.

CMV screen positive infants are retrieved by dedicated nurse practitioners working in Infectious Diseases Clinics at the Regional Treatment Centres and then referred to rostered community pediatricians in their community

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for an initial assessment. The initial assessment includes a physical exam, confirmatory urine CMV PCR, blood work (CBC, liver function tests), head ultrasound, and ophthalmology assessment. Infants with symptomatic cCMV infection are referred to ID for further assessment and treatment decision-making. All infants with confirmed cCMV infection are offered developmental surveillance by a community pediatrician or ID specialist until age 6. Diagnostic evaluation report forms are completed by the community pediatricians and ID specialists and the collection of this information is managed by the nurse practitioners.

Genetic screen positive infants are retrieved centrally by a genetic counsellor or audiologist working with NSO and the IHP. Recommendations for further follow-up are made to the infant's primary care provider following the diagnostic audiology assessment. NSO sends a referral to ENT for infants with confirmed PHL, and diagnostic evaluation report forms are completed. Families interested in additional genetic counselling and/or cascade testing can be referred to their local Genetics Clinic by their PCP, ENT, or NSO.

5.4 Screen positive rate

Table 27. Number of risk factor screen positive babies in 2020

Risk Factor	# Screen Positive			% Positive		
	Period 1	Period 2	Cumulative	Period 1	Period 2	Cumulative
CMV	29	130	159	0.11	0.12	0.12
GJB2/6	<5	19	21	0.007	0.017	0.016
SLC26A4	<5	<5	<5			

In 2020, there were 159 CMV screen positive infants. The CMV screen positive rate was 0.12%, which is lower than anticipated based on suspected population prevalence. During consent period 1, we wondered whether CMV could be overrepresented in the population of babies who were not consented through the IHP, perhaps due to common shared barriers. Interestingly, however, the screen positive rate remained stable throughout period 2 where consent was waived. Whether or not heightened hygiene measures related to the COVID-19 pandemic could have impacted the prevalence of cCMV infection remains unknown. When consent is reinstated following the COVID-19 pandemic, NSO and the IHP are working on an improved system of consent to improve timeliness, including a mechanism to report and follow-up on missed audiometric screens. NSO is continuously evaluating and considering ways to increase the sensitivity of the CMV screening assay.

With respect to genetic risk factor screening, most of the variants included in screening are highly penetrant, truncating mutations that confer a high risk for congenital PHL. On October 28, 2020, reflexive screening for the *GJB2* variant p.(V37I) began for specimens where a single *GJB2/6* variant was detected on the panel. The p.(V37I) variant is non-truncating and has reduced penetrance. While not all infants with this variant will have or develop PHL, including it in screening will help identify infants who would benefit from audiologic surveillance. The number of genetic risk factor screen positive infants is expected to increase with the introduction of reflexive screening for *GJB2* p.(V37I).

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5.5 Screen positive referrals

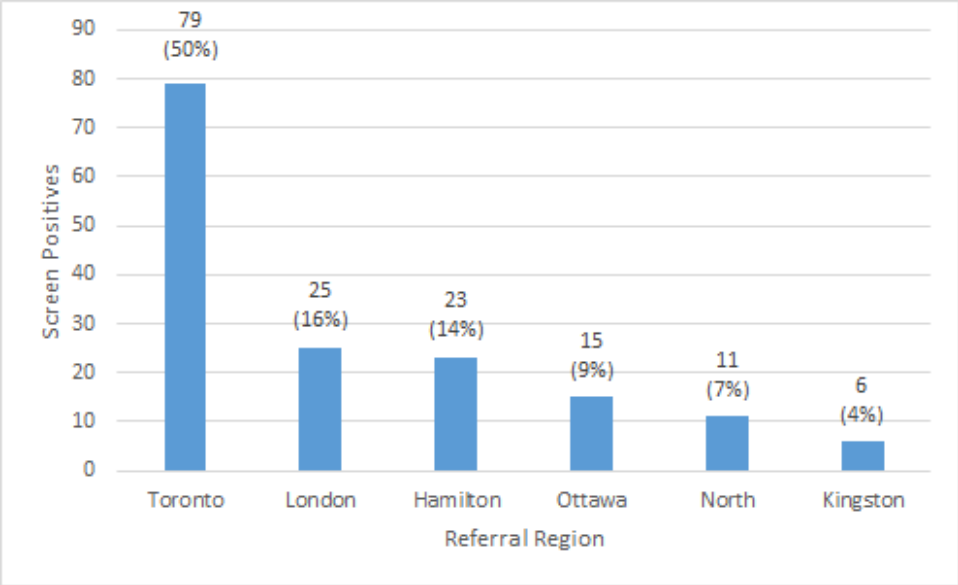

Figure 10. CMV screen positives by referral region

Figure 10 shows the breakdown of CMV screen positive referrals by region. Of note, the majority of CMV screen positive infants were referred to a rostered community pediatrician for their initial assessment (134/159, 84%). The remaining infants were referred directly to ID for their initial assessment (25/159, 16%). Reasons for a direct referral to ID were geographical/travel related, based on symptoms noted at retrieval (e.g. refer result on hearing screen) or due to factors related to the COVID-19 pandemic.

5.6 CMV screen positive outcomes

Urine CMV PCR results were available for 91% of the screen positive infants and 136 (94%) had positive/detected results. Of these, 79% were deemed to have asymptomatic cCMV infection and 17% were classified as symptomatic. Screening identified slightly more babies with symptomatic cCMV than we would expect based on the literature. 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 head ultrasound findings). Of note, very few 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. There were <10 cases 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. NSO is currently looking into ways to help resolve these cases more quickly.

5.7 Genetic screen positive outcomes

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Most of the genetic risk factor screen positive infants had PHL identified at the diagnostic ABR assessment. All infants with hearing that was within normal limits had genotypes that included a non-truncating mutation (e.g. GJB2 p.(L90P) or GJB2 p.(V37I). These infants were enrolled in audiologic surveillance through the IHP.

5.8 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. Looking forward, we will be focussing efforts on program evaluation and optimization.

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6. 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
Yes	confirmed diagnosis of a targeted condition	Classical PKU
No	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 2A. The true positive categories.

True Positive Categories	
Generic	Detailed
No	Not Affected
Yes	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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