

## Title page

**Title:** Feasibility and acceptability of targeted salivary cytomegalovirus screening through universal newborn hearing screening.

**Type:** Original article

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## Acknowledgements

### Author Contributions

VS, CJ, KS and EW conceived the study. All authors and the HearS-cCMV Study Team were involved in the study design. EW and AG collected and analysed the data. EW wrote the first draft of the manuscript. All authors contributed to the manuscript and approved the final version of the manuscript.

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### **Conflict of interest statement**

The authors report no conflict of interest.

## Abstract

**Aims:** This study aimed to determine the feasibility and parental acceptability of screening for congenital cytomegalovirus (cCMV) through saliva polymerase chain reaction (PCR) in infants who did not pass their newborn hearing screening. Additionally, the utility (i.e., time to diagnosis and treatment) of this enhanced clinical pathway was evaluated.

**Methods:** The study was conducted through the Victorian Infant Hearing Screening Program (VIHSP) across four maternity hospitals in Melbourne, Australia during June 2019-March 2020. Parents were approached by VIHSP staff about obtaining a test for CMV at the time of their baby's second positive ('refer') result on the VIHSP screen. Participating parents collected a saliva swab for CMV PCR from their infants. Feasibility was determined by the proportion of 'referred' infants whose parents completed the salivary CMV screening test  $\leq 21$  days of life. Acceptability was measured through parent survey.

**Results:** 96/126 (76.0%) of all eligible families had salivary screening swabs taken  $\leq 21$  days of life. Most families (>90.0%) indicated that screening was acceptable, straightforward, and thought testing their baby for cCMV was a good idea. One infant screened positive on day 30, was diagnosed with cCMV via confirmatory testing by day 31 and commenced valganciclovir on day 32.

**Conclusions:** Obtaining a saliva sample to screen for cCMV in infants who do not pass their newborn hearing screen is feasible and appears acceptable to parents. This targeted cCMV screening method could be an option where mothers are rapidly discharged from hospital in the context of the COVID-19 pandemic.

**Key words:** Congenital cytomegalovirus, hearing loss, targeted screening, feasibility

### What is already known on this topic

- Congenital cytomegalovirus (cCMV) is the leading infectious cause of permanent hearing loss in infants;
- Accurate diagnosis of cCMV requires appropriate specimens from an infant to be obtained within 21 days of life, with timely antiviral treatment of cCMV recommended within one month of life if there is evidence of symptomatic disease;
- In Australia, cCMV is not routinely screened for, so detecting affected infants in time to commence antiviral treatment is unlikely.

### What this paper adds

- Salivary swabs taken by parents for PCR testing is a feasible method for screening for cCMV within Victoria's newborn hearing screening program;
- Parents found obtaining a saliva sample to screen their baby for cCMV to be acceptable;
- Targeted cCMV screening by parents could be an option where mothers are rapidly discharged from hospital, especially in the context of the COVID-19 pandemic.

## Introduction

Congenital cytomegalovirus infection (cCMV) is the leading cause of sensorineural hearing loss (SNHL) in infants and affects 0.3% to 0.7% of livebirths.<sup>1,2</sup> A randomised controlled trial showed that six months of oral valganciclovir, given to infants with symptomatic cCMV and commenced within 30 days of life, improved hearing and neurodevelopmental outcomes at two year follow up.<sup>3</sup> Salivary CMV polymerase-chain-reaction (PCR) testing is the preferred method of diagnosing cCMV and has high sensitivity (>97.0%) and specificity (99.9%) compared to rapid culture screening.<sup>4</sup> Samples must be obtained within 21 days to distinguish between congenital and postnatally acquired CMV infection, the latter of which does not generally impact hearing or long-term neurodevelopment in immunocompetent infants.

The benefits from early commencement of valganciclovir in symptomatic infants led to international guidelines in 2017 recommending targeted cCMV screening of newborns who do not pass their universal newborn hearing screening (UNHS).<sup>5</sup> Prospective observational cohort studies in the United Kingdom (U.K.), United States (U.S.A) and Israel have shown that targeted cCMV salivary screening of infants referred for audiology testing through UNHS is feasible and cost-effective.<sup>6-9</sup> In Queensland, Australia, feasibility and cost-effectiveness have also been reported when targeted screening is completed by clinicians.<sup>10</sup>

In Victoria, Australia, the Victorian Infant Hearing Screening Program (VIHSP) screens 99% of newborn infants for hearing loss in their first weeks of life.<sup>11</sup> Unlike in Queensland not all Victorian screeners have medical training and are not able to take saliva swabs from infants. Some Victorian maternity hospitals also require mothers to be discharged by 48 hours post-delivery. Before considering widespread targeted cCMV screening in Victoria, there is a need to test the feasibility and acceptability of parents themselves completing the saliva cCMV screen. If successful, this mechanism of screening can also be implemented where there is rapid discharge of mothers post-delivery, particularly in the context of the COVID-19 pandemic.

Our primary objective was to determine the feasibility of targeted cCMV screening by parents taking a saliva sample from their infant who did not pass newborn hearing screening. The secondary objectives were to evaluate parent acceptability and the clinical utility of the screening.

## Methods

This prospective cohort study was conducted between June 2019 and March 2020 at four large tertiary perinatal hospitals in Melbourne, Victoria. Infants were eligible to participate if they met the VIHSP inclusion criteria (i.e. born at 34 weeks' gestation or later, settled, medically stable and close to hospital discharge), received a second positive 'refer' result and were aged 21 days or less at this time. A VIHSP screener used a two-stage automated auditory brainstem response (AABR) screening protocol

(Supplementary Figure 1). If the infant did not pass the first screen (AABR1), usually completed within 48 hours of birth, a second screen (AABR2) was arranged at least 24 hours later. If the infant did not pass the AABR2, they received a 'refer' result, and the screener provided eligible families an information pack about the study. A VIHSP Area Manager arranged formal diagnostic audiology testing, discussed the study and obtained written consent from parents. All VIHSP staff members in participating hospitals undertook education and training with a study member trained in Good Clinical Practice.

Parents collected saliva specimens by swabbing the inside of their baby's mouth for one minute at least one hour after a breast-milk feed to reduce the potential for false positive results from CMV in breast-milk. The saliva swab was sent to the Royal Children's Hospital (RCH) Diagnostic Molecular Laboratory for CMV PCR testing, either by express mail post or through a hospital courier.

Before the study started, acceptable stability of CMV nucleic acid was confirmed at 21 and 37 degrees Celsius for up to 7 days with a 1 Log reduction in CMV viral load in samples held at 37°C for 7 days (unpublished internal validation data). For CMV PCR, nucleic acid extraction was performed on the MagNA Pure 96 system (Roche Applied Science, Mannheim, Germany) utilising the Universal Protocol and MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche) according to the manufacturer's protocol. Testing was performed on the LightCycler 480 real-time PCR system (Roche) using an in-house validated assay utilising previously published oligonucleotide primers and hybridisation probes.<sup>12, 13</sup>

The RCH laboratory informed the hospital study clinician of the results by fax with additional direct phone contact for positive results. In the case of a positive CMV result, each hospital's study clinician contacted the family to explain the result, and confirmed permission for the study team to organise further confirmatory testing (plasma and urine CMV PCR, and repeat saliva CMV PCR if history suggested possible contamination with breast-milk). Infants confirmed with cCMV were referred for paediatric infectious diseases assessment and consideration of treatment in line with international consensus guidelines.<sup>5</sup>

All participating families were invited to complete a short online survey providing feedback on the acceptability of the CMV screen. A subgroup of families recruited between November 2019 and March 2020 were also invited to complete an additional survey about their anxiety level, measured by the State Trait Anxiety Inventory (STAI-AD) Short Form survey (Table 1).<sup>14</sup>

We presented descriptive summary statistics using means and standard deviations, or medians and ranges for continuous data, and percentages for dichotomous data, using Stata 15.0.<sup>15</sup> The study was

approved by the Royal Children's Hospital Ethics Committee (HREC18/RCHM/273) with additional ethics and governance approvals from all four participating hospitals.

## Results

During the study period, a total of 20 102 infants were born at or admitted to the four hospitals. 19 924/20 001 (99.6%) eligible VIHSP infants completed AABR1. One hundred and fifty-seven infants (0.8%) received a 'refer' on their AABR2; 31/157 (19.7%) were ineligible due to being >21 days of age or receiving their AABR2 at a non-study participating hospital (Figure 1). Of the 126 eligible families, 96 (76.2%) agreed to participate with no withdrawals (Figure 1). Participants and those who declined had similar infant characteristics, including gestation and median age at AABR2 completion, but there was a higher proportion of female infants in the decline group. All swabs were completed  $\leq$ 21 days of life (Table 3). The majority (57/96, 59.4%) were completed in hospital at the time of the AABR2 (Table 2). Higher participation rates were observed in hospitals where the swab was completed as an in-patient (Table 2). Participation rate was lowest at Hospital B, with participants with the lowest socio-Economic Indexes for Areas (SEIFA) indicating greatest socio-economic disadvantage and greatest proportion with English as a second language (Table 2). Most parents who declined to participate (20/30, 66.7%) did so at the time of follow up from the study team for reasons outlined in Figure 1.

The median (range) age of CMV swab completion was 5(2-21) days (Table 3). The swabs were completed earlier when taken in hospital at a median of 3(2-21) days of age, in comparison to a median of 9(6-19) days of age when taken in the outpatient setting, and a median of 15(3-21) days of age when taken at home (Table 3). The median turnaround time from swab collection to receipt by the central laboratory for analysis was 3(0-11) days, with a greater and more varied turnaround time when the swab was completed at home and mailed to the central laboratory (Table 3). The overall median time from date of birth to result received was 9(3-34) days with a greater length of time observed when the swab was completed at home or as an outpatient (Figure 2), with the CMV result of one infant (n=34 days) taking 5 days for analysis due to commencement of COVID-19 swabs concurrently at the central laboratory.

Four infants had positive CMV saliva swabs (Figure 1). All four completed further CMV testing (urine and plasma CMV PCR) and had confirmatory audiological testing within 21 days of life. Three infants had false positive results (negative urine, plasma, and saliva, if completed, CMV PCR). Two families misunderstood instructions and completed the CMV swab within one hour after breastfeeding, and the third family completed the swab one-hour post-breastfeeding but observed the infant vomiting prior to completing the swab.

One of the four (25.0%) infants with positive CMV saliva swabs was diagnosed with cCMV with confirmation on urine and plasma CMV PCR. The swab was obtained at home on day 21, was received by the laboratory and results delivered on day 30, confirmatory testing on day 31, consultation with an infectious disease specialist on day 32 and commencement of valganciclovir on day 32. This infant had bilateral severe to profound hearing loss at the time of diagnostic audiology and abnormal liver function tests (increased transaminases). The infant had a normal brain magnetic resonance imaging and ophthalmology examination and completed six months of oral valganciclovir which was well tolerated with no adverse effects.

Of the 65/96 parents (67.7%) who participated in the acceptability survey, most responded 'moderately so' or 'very much so' to the three statements: 'the saliva testing for cCMV was easy to do' (63/65, 92.0%), 'testing my baby for cCMV was a good idea' (59/64, 92.0%) and 'I am glad my baby was screened for cCMV' (63/65, 96.9%) (Figure 3). Acceptability was not influenced by infant hearing status or whether English was their first language (Supplementary Figures 2 a, b and c). Parents who completed swabs at home or at outpatients reported lower ease of saliva testing, in comparison to parents who completed it as an inpatient (Supplementary Figure 2a). Of the subgroup of parents offered to complete the STAI-AD, 22/32 (68.8%) completed it, and 21/22 (95.0%) had State and Trait mean scores that fell within one standard deviation of the reported mean value for working adults.<sup>14</sup>

## Discussion

This is the first study to demonstrate parent-completed targeted cCMV screening via salivary PCR is feasible and acceptable in an urban Australian clinical setting. Considering the requirement for parents to perform the swabs themselves, the participation rate was high (76.2%) and all swabs were obtained by 21 days of life with timely return of results. All 4 infants who screened positive to salivary CMV received hearing status confirmation within 21 days. The infant with confirmed cCMV saw an infectious disease specialist and commenced valganciclovir by day 32. Most parents (>92.0%) indicated the cCMV screen was acceptable to them and did not appear to increase parental anxiety, similar to parental attitudes in the UK<sup>8</sup> and the US.<sup>18</sup>

Our study had a higher participation rate (76.2% compared with 40.0%) than a similar U.K. study where most salivary swabs were taken by parents and sent for analysis through postal recruitment. This rate is comparable with other studies where swabs were performed by screeners in hospital ((BEST-2 (203/255, 80.0%)<sup>7</sup> and BEST-Q (234/283, 83.0%))<sup>10</sup>. In two mandated targeted salivary cCMV screening programs in the U.S., the participation rates were (314/509) 61.7%<sup>16</sup> and (171/171) 100%.<sup>17</sup> The greater uptake in the latter study could be attributed to earlier AABR2 completion and mandating of swabs being completed by clinical staff prior to infant discharge.

Whilst all saliva swabs were completed within 21 days, it was clear that swabs were completed earlier if the infant was still in hospital than if performed at home. Although most results were available by 9 days of life, the range in time from day of birth to result received (apart from an outlier of 34 days due to the impact on laboratory services of the COVID-19 pandemic) was 30 days, predominantly due to the swabs being completed at home with a lag-time for parents to mail the swab to the central laboratory, suggesting this approach may delay diagnosis. This could impact on timely consultation with paediatric infectious disease specialist and the potential commencement of anti-viral treatment.

The higher false positive rate of cCMV screen in our study (3/4, 75.0%) in comparison to other studies<sup>7, 8</sup> was attributed to breastmilk contamination, mostly due to parental misunderstanding and swab taking within 1 hour of breastfeeding. This is similar to the BEST-Q study (5/8, 62.5%), where the protocol allowed the swab to be taken within 1 hour of breastfeeding.<sup>10</sup> It is likely that if cCMV screening was completed with oversight by a trained health professional, the rate of false positives would be minimised.

Our study had several limitations. The sample size was small with recruitment ceased early due to the COVID-19 pandemic. The use of postal services was a barrier for timely analysis. This was also reported in the UK.<sup>8</sup> Our study sent samples to a central laboratory for testing because it is the only laboratory in the state with validated saliva PCR methodology. Our study was conducted in four urban maternity hospitals and results may not be generalisable to rural hospitals. Only a small proportion of parents, inclusive of a parent who received a false positive screen, were given the opportunity to complete the anxiety survey due to the impact of COVID-19.

A recent study from Utah assessed the decision to use a targeted hearing cCMV screening approach using the 10 criteria from Wilson & Junger's "Principles and Practice of Screening for Disease".<sup>19</sup> This highlighted the substantial rationale and evidence to support a hearing-screen targeted approach to identify cCMV. Our study provides a sound mechanism for parent-completed cCMV screening particularly where mothers are discharged rapidly from hospital post-delivery, especially in the context of the COVID-19 pandemic. There remains, however, a need for ongoing clinical trials evaluating antiviral treatment for infants with asymptomatic or isolated SNHL to provide guidance around therapy. Further research into cCMV prevalence and long-term outcomes for children with cCMV in Australia is also required.

A health economic analysis is currently underway to inform the cost-benefit of targeted cCMV screening in Victoria, specifically the costs to the Victorian and Australian healthcare systems. The study team is also in the process of completing interviews and focus groups with parents and hearing screeners to determine the barriers and enablers of implementing targeted salivary cCMV screening. The next step is to assess whether training hearing screeners, midwives and nurses on the maternity



wards to complete the saliva swabs is feasible, would increase cCMV screen uptake and improve turnaround time for results.

## Conclusion

We have demonstrated that parent-completed salivary cCMV screening within an established universal hearing screening program is feasible and acceptable. Our study provides a sound mechanism for cCMV screening where mothers are rapidly discharged from hospital post-delivery, especially in the context of the COVID-19 pandemic. Our findings, combined with a cost-benefit analysis and further qualitative data on enablers and barriers of targeted cCMV screening, will provide the necessary evidence to train hearing screeners, midwives and nurses to complete swabs in hospital, which in turn would likely reduce false positive rates and improve the uptake and timeliness of cCMV screening.

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## Tables

Table 1: Outcome measures

Outcome	Measures
<b>Primary outcome:</b>	
Feasibility	Measured by the number of infants who completed a salivary cCMV screening test prior to or at 21 days of age as a proportion of those who received a 'refer' result on the second newborn hearing screening
<b>Secondary outcomes:</b>	
Acceptability	<p>Parent survey with three questions:</p> <ol style="list-style-type: none"> <li>1. I was glad my baby was screened for cCMV</li> <li>2. Testing my baby for cCMV was a good idea</li> <li>3. The saliva testing for cCMV was easy to do</li> </ol> <p>with responses on a 4-point Likert scale ('not at all', 'somewhat', 'moderately so', and 'very much so'). Questions 1 and 2 from BEST study.<sup>8</sup> Question 3 study-generated.</p> <p>Parental anxiety measured by the 20-item State Trait Anxiety Inventory (STAI-AD) Short Form survey.<sup>14</sup> The STAI has been used for measuring anxiety levels in parents of newborns, specifically those with neonatal disability or completing newborn hearing screening<sup>20, 21</sup></p>
Clinical utility measures	<p>Proportion of cCMV positive infants who received a 'refer' result on the second newborn hearing screening divided by the overall number of infants who received a 'refer' result</p> <p>Time to diagnosis of cCMV measured from date of birth to date of diagnosis for cCMV positive infants</p> <p>Time to paediatric infectious disease specialist appointment measured from date of birth to date of appointment for cCMV positive infants</p> <p>Time to audiological diagnosis measured from date of birth to date of audiological diagnosis for cCMV positive infants</p>

Table 2: Demographics for infants who received their second refer result at a HearS-cCMV hospital

Variable n (%)	All	Hospital A	Hospital B	Hospital C	Hospital D
Number of AABR2 performed	140	39	43	40	18
Number eligible	126 (90.0)	37 (94.9)	41 (95.4)	35 (87.5)	13 (72.2)
Number participating	96 (76.2)	30 (81.1)	29 (70.7)	27 (77.1)	10 (76.9)
Male	56 (58.3)	19 (63.3)	16 (55.2)	16 (59.3)	5 (50.0)
Gestation ( <i>weeks + days</i> , <i>standard deviation in days</i> ) <sup>†</sup>	38+4 (11.8)	38+4 (14.1)	38+5 (11.2)	38+2 (9.9)	39+3 (9.9)
Median age (range) at AABR2 ( <i>days</i> ) <sup>‡</sup>	3 (2-19)	3 (2-19)	3 (2-19)	4 (2-18)	14 (3-19)
AABR2 in hospital <sup>‡</sup>	3 (2-21)	2 (2-19)	2 (2-10)	3 (2-21)	3 (3-4)
AABR2 in the outpatient setting <sup>‡</sup>	10 (6-19)	15 (10-19)	9 (8-10)	13 (8-18)	8 (6-16)
Participant SEIFA	1003.0 (68.7)	1032.8 (72.3)	964.4 (57.2)	993.1 (58.6)	1052.6 (43.4)
English as second language	15 (11.5)	4 (13.3)	5 (17.2)	2 (7.4)	0 (0)
Location of swab completion					
Inpatient	57 (59.4)	21 (70.0)	13 (44.8)	21 (77.8)	2 (20.0)
Outpatient	10 (10.4)	2 (6.7)	3 (10.3)	2 (7.4)	3 (30.0)
Home	25 (26.0)	6 (20.0)	12 (41.4)	3 (11.1)	4 (40.0)
Unknown	4 (4.2)	1 (3.3)	1 (3.4)	1 (3.7)	1 (10.0)

AABR2 - Automated Auditory Brainstem Response 2.

SEIFA - Socio-Economic Indexes for Areas (SEIFA) Index of Relative Socio-Economic Disadvantage is a composite Census based measure summarising the social and economic conditions of Australian neighborhoods (national mean 1000, SD 100, where higher values represent less disadvantage).<sup>22</sup><sup>†</sup>(mean, SD) <sup>‡</sup>(median, range)

Table 3: Timing of saliva swab completion and analysis

<b>Variable n = median (range) in days</b>	<b>All</b>	<b>Hospital A</b>	<b>Hospital B</b>	<b>Hospital C</b>	<b>Hospital D</b>
Number of swabs completed by 21 days (n, %)	96 (100)	30 (100)	29 (100)	27 (100)	10 (100)
Child age (days) when AABR2 completed	3 (2-19)	3 (2-19)	3 (2-19)	4 (2-18)	14 (3-19)
Child age (days) when swab completed	5 (2-21)	3 (2-21)	9 (2-21)	5 (2-21)	14 (3-20)
Inpatient	3 (2-21)	2 (2-19)	3 (2-10)	3 (2-21)	3 (3-4)
Outpatient	9 (6-19)	14 (10-19)	9 (8-10)	13 (8-18)	8 (6-16)
Completed at home	15 (3-21)	9 (3-21)	16 (9-21)	11 (7-12)	19 (15-20)
Days taken for swab to arrive at lab for testing	3 (0-11)	3 (1-9)	2 (0-11)	2 (0-6)	3 (2-6)
Inpatient <sup>†</sup>	2 (0-5)	3 (1-5)	1 (0-3)	2 (0-5)	2 (2-2)
Outpatient <sup>†</sup>	3 (0-5)	2 (2-2)	3 (0-4)	3 (1-5)	3 (3-3)
Completed at home <sup>†</sup>	3 (0-11)	3 (2-9)	4 (1-11)	1 (0-6)	3 (2-6)
Days taken for swab to be analysed <sup>‡</sup>	0 (0-5)	-	-	-	-
Total number of days from birth to CMV result available	9 (3-34)	6 (3-30)	12 (3-34)	8 (3-23)	19 (5-23)

<sup>†</sup>Samples were transported to the central laboratory via Australia Post. Hospital B samples were transported through a combination of Australia Post and hospital courier

<sup>‡</sup> All swabs were analysed the central laboratory from Monday to Friday, with a Saturday run only on request

## Figures

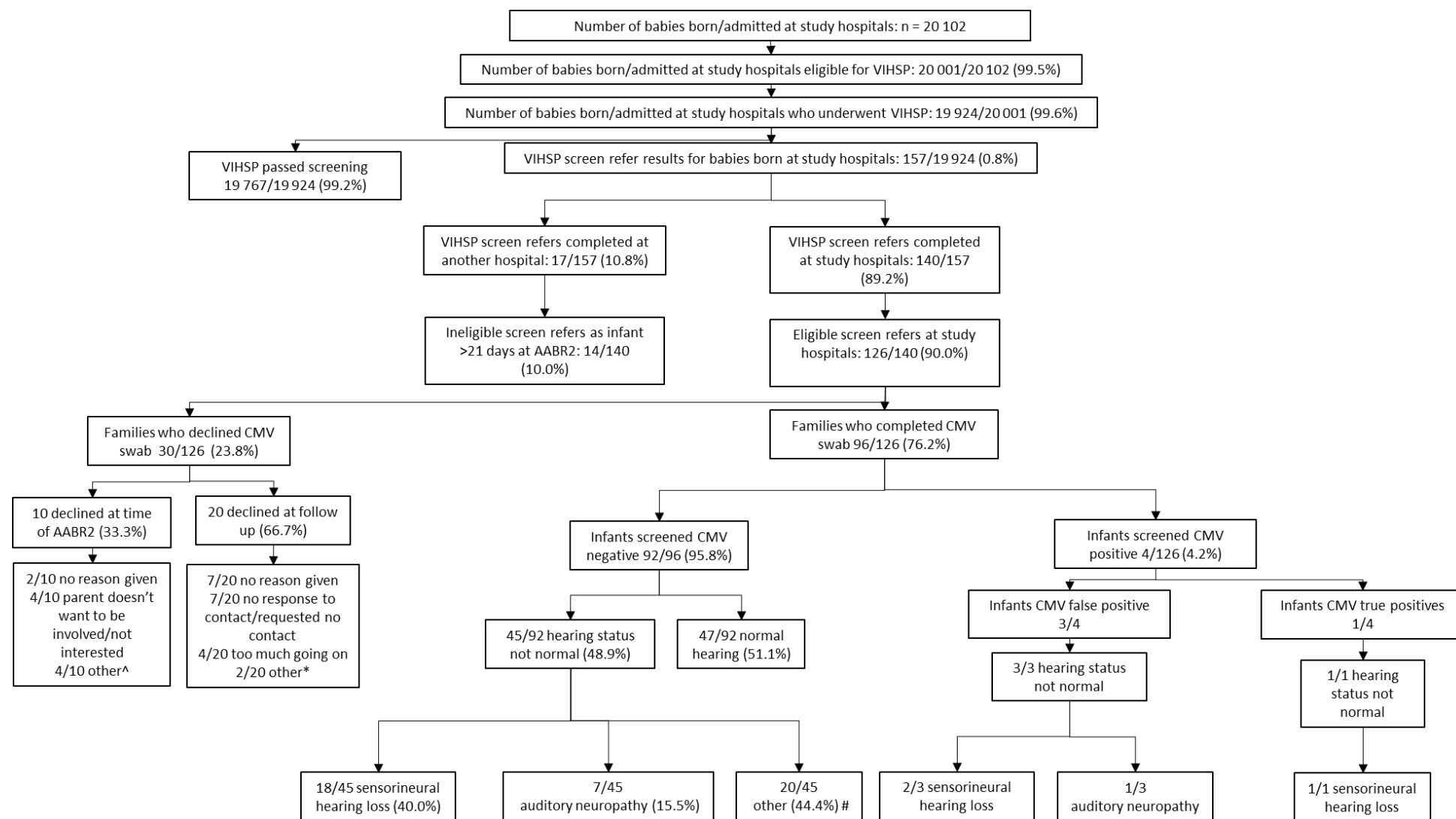


Figure 1: Recruitment flowchart



Figure 2: Number of days from birth to CMV result received dependent on location of swab completed



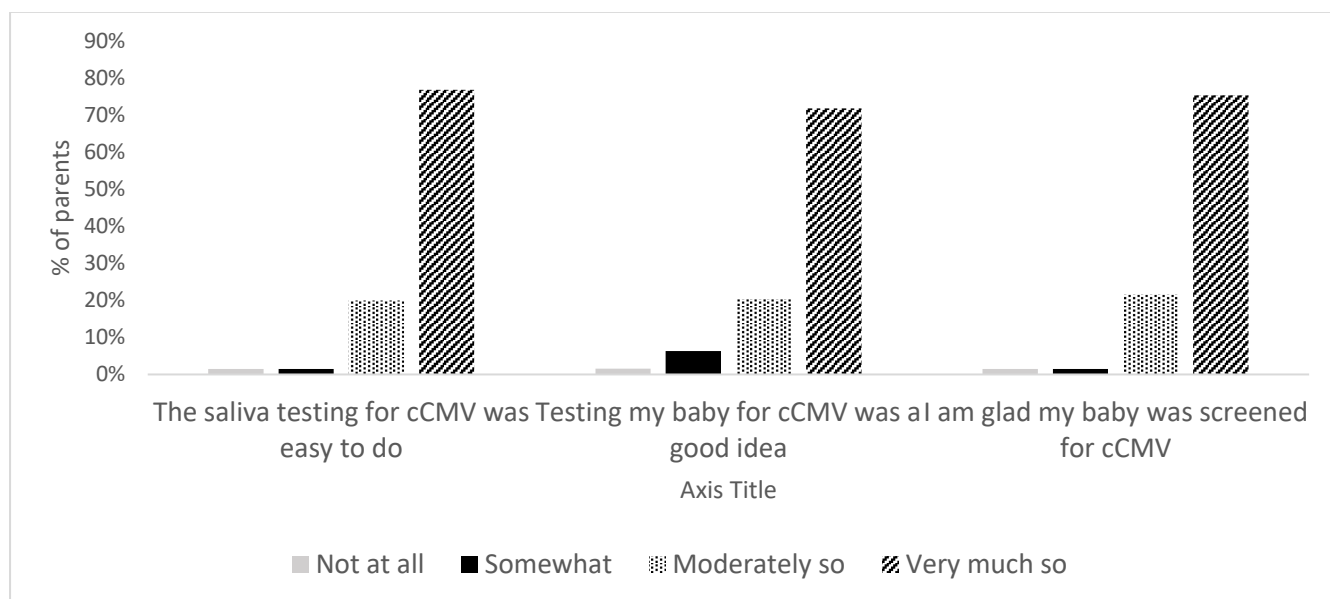
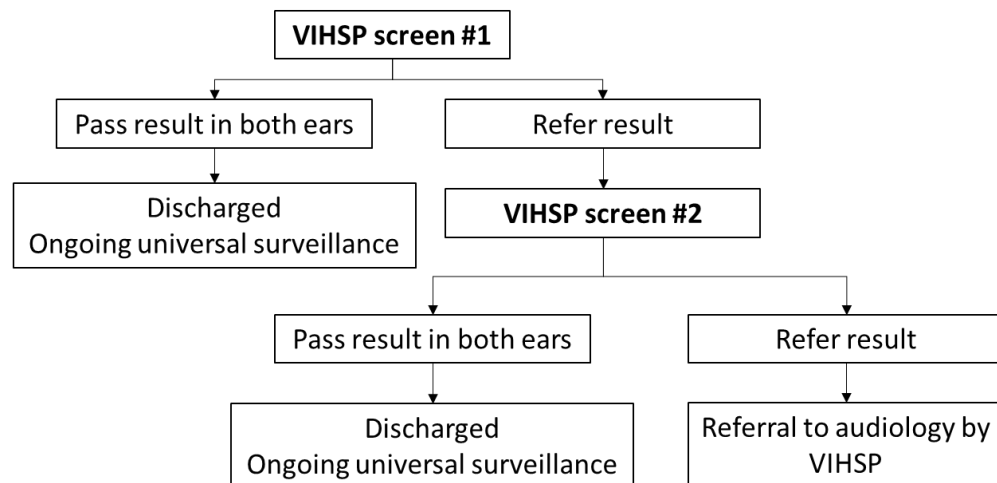


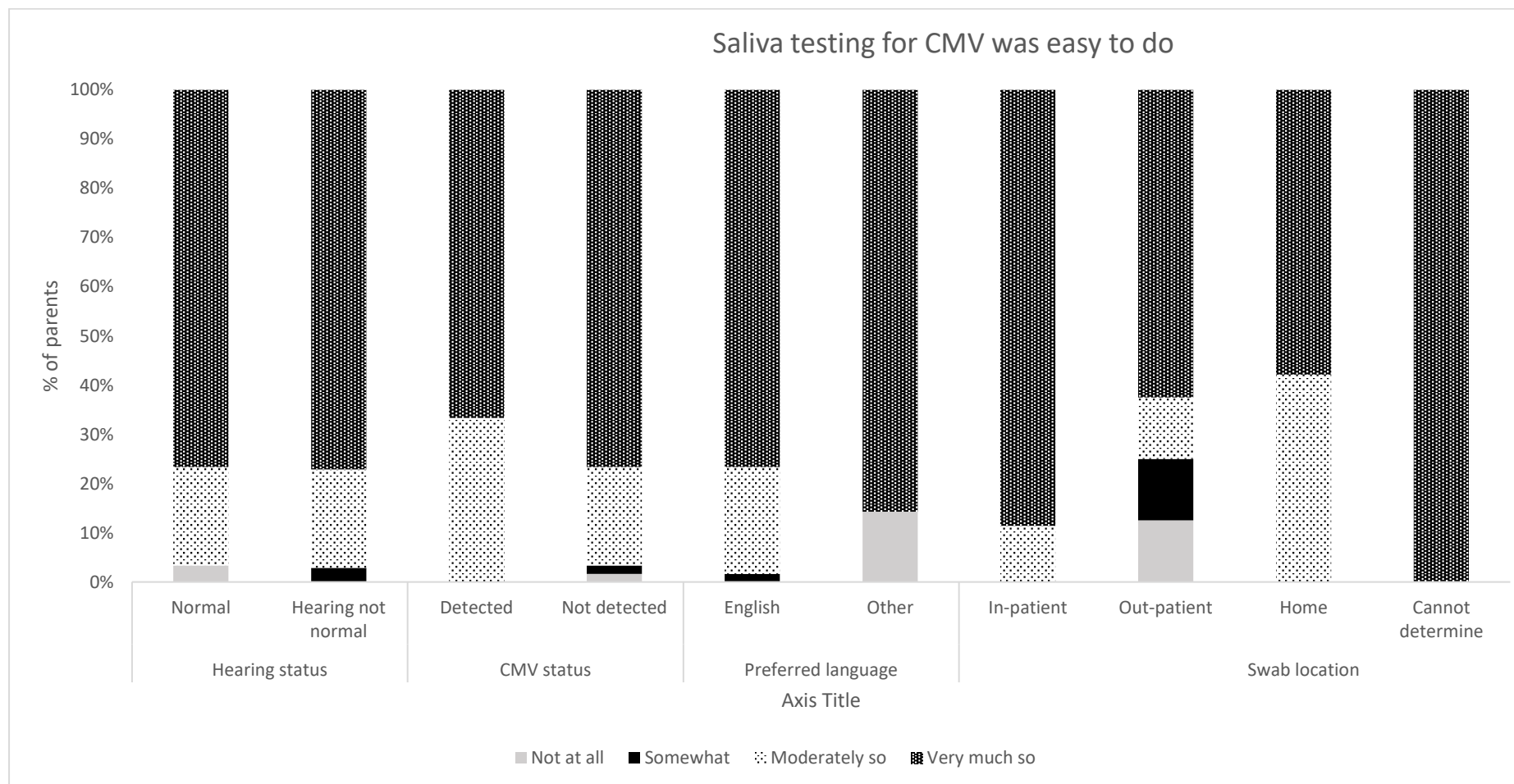
Figure 3: Parent survey responses exploring acceptability of targeted salivary cCMV screening

## Supplementary

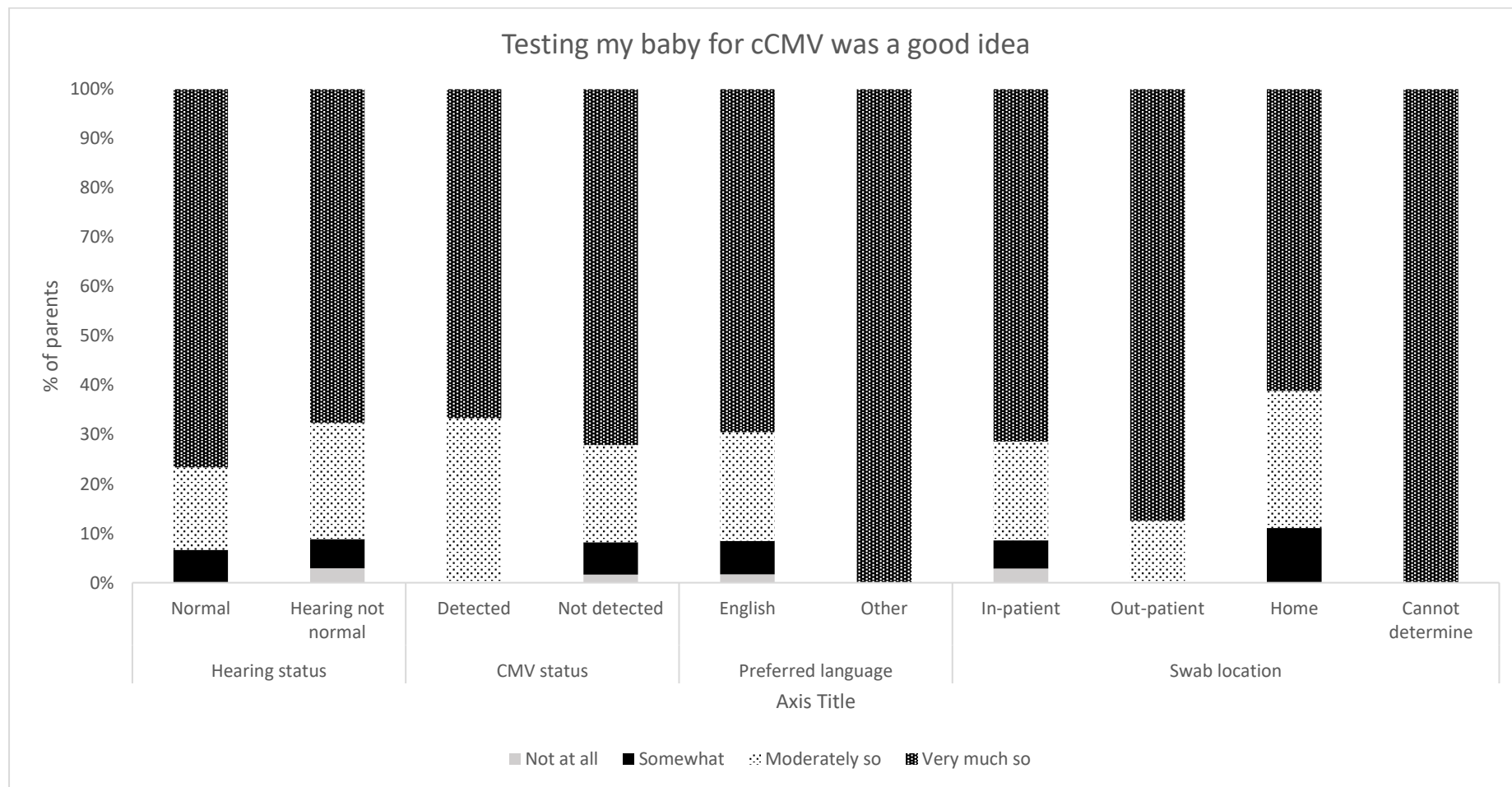
The VIHSP screening protocol



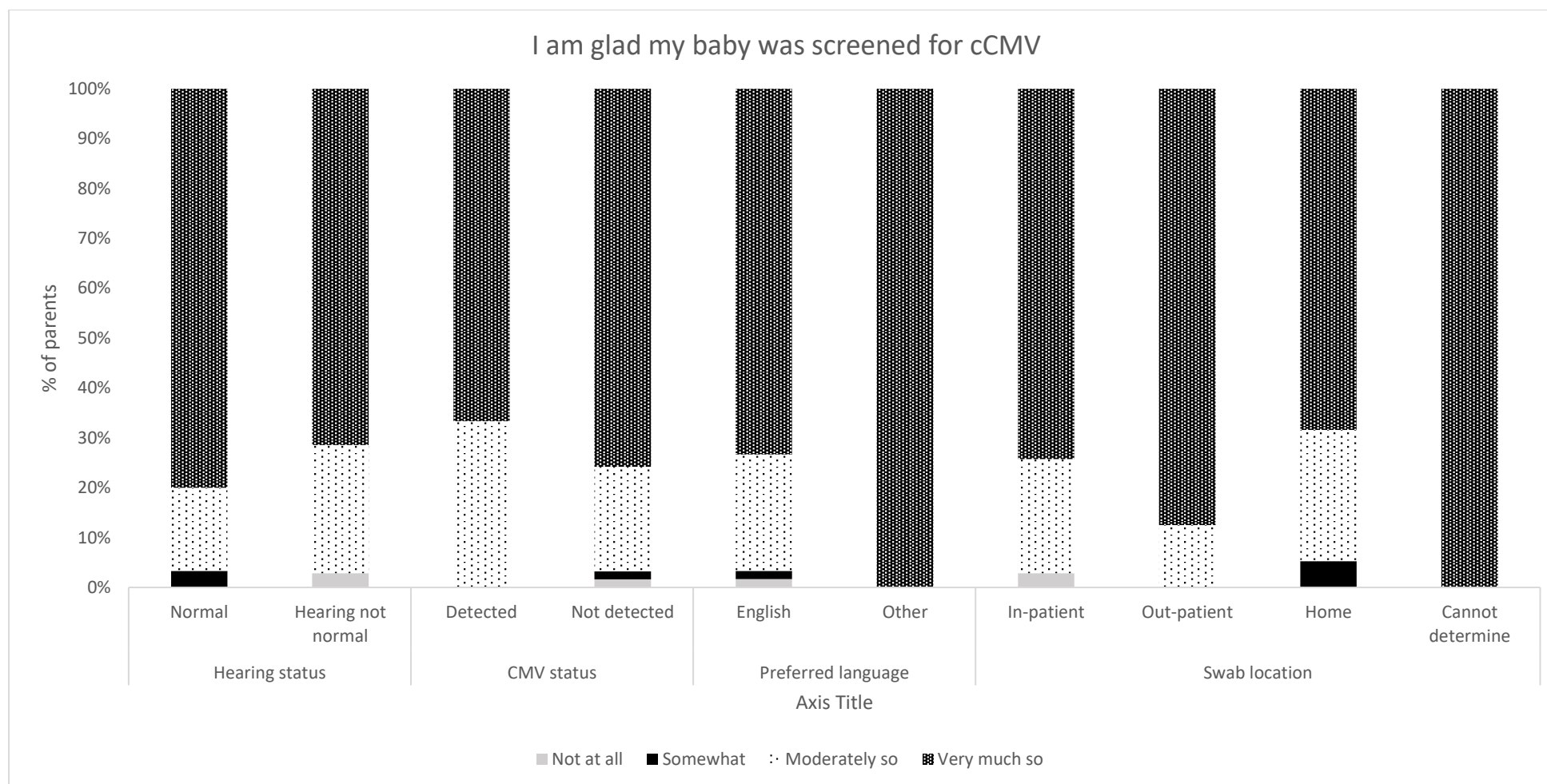
Supplementary Figure 1: VIHSP screening protocol where a 'refer result' is in one or both ears



Supplementary Figure 2a: Parental responses to acceptability survey question 1 by hearing status, CMV status, preferred language and swab location



Supplementary Figure 2b: Parental responses to acceptability survey question 2 by hearing status, CMV status, preferred language and swab location



Supplementary Figure 2c: Parental responses to acceptability survey question 3 by hearing status, CMV status, preferred language and swab location