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## PREVALENCE OF CHANGES DETECTED FROM THE HEEL PRICK TEST IN A MUNICIPALITY IN SOUTHERN SANTA CATARINA

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***Alícia Brighenti Bendo***

University of Southern Santa Catarina –  
UNISUL, Faculty of Medicine, Tubarão – SC  
<https://orcid.org/0009-0000-3718-2424>

***Manuela Pozza Ellwanger***

Contestado University, Master's Degree in  
Regional Development – UNC, Mafra – SC  
<https://orcid.org/0009-0003-1115-6377>

***Maurício Prätzel Ellwanger***

Contestado University, Faculty of Medicine –  
UNC, Mafra – SC  
<https://orcid.org/0009-0002-8823-2588>

***Bruna Camargo***

University of Southern Santa Catarina –  
UNISUL, Faculty of Medicine, Tubarão – SC  
<http://lattes.cnpq.br/4696607458463662>

***Gabriel Pellegrin Nicoleit***

University of Southern Santa Catarina –  
UNISUL, Faculty of Medicine, Tubarão – SC  
<http://lattes.cnpq.br/4798252088435878>

***Maria Eduarda Caetano da Rosa***

University of Southern Santa Catarina –  
UNISUL, Faculty of Medicine, Tubarão – SC  
<http://lattes.cnpq.br/0222225534233827>



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**Guilherme Luiz Marcondes**

University of Southern Santa Catarina –  
UNISUL, Faculty of Medicine, Tubarão – SC  
<https://lattes.cnpq.br/9211964481481642>

**João Vitor Marosin de Oliveira**

University of Southern Santa Catarina –  
UNISUL, Faculty of Medicine, Tubarão – SC  
<http://lattes.cnpq.br/8117435560059444>

**Debora Reinert**

Contestado University, Faculty of Medicine –  
UNC, Mafra – SC  
<https://orcid.org/0009-0006-5881-7568>

**Alex Douglas de Jesus Silva**

Albert Einstein Brazilian Israeli Charitable  
Society, Cocal, RO  
<https://orcid.org/0009-0008-8624-102X>

**Matheus Henrique Cassias de Lima**

Contestado University, Faculty of Medicine –  
UNC, Mafra, Santa Catarina  
<https://orcid.org/0009-0009-5968-2775>

**Chaiana Esmeraldino Mendes Marcon**

University of Southern Santa Catarina –  
UNISUL, Faculty of Medicine, Tubarão – SC  
<https://orcid.org/0000-0001-7031-437X>

**Abstract:** Objective: To estimate the prevalence of abnormalities detected by the heel prick test in a municipality in southern Santa Catarina, Brazil, from August 2023 to February 2024. Method: , 237 children and their mothers were analyzed, along with the test results, in the neonatal screening unit of the Municipal Health Foundation of the municipality. Results: Of the 237 newborns analyzed, 21 presented abnormalities in the heel prick test. Sick cell trait was the main abnormality detected, and prematurity and/or low birth weight were the main determinants for the need for retesting. There were no significant differences between the test results and high-risk pregnancy or mode of delivery. Conclusions: The study highlights the importance of the heel prick test in the early detection of neonatal pathologies, with hemoglobinopathies being the most prevalent. Despite limitations, the results show satisfactory adherence to the collection period and prenatal consultations, indicating improvements in maternal and child care.

**Keywords:** Neonatal Screening; Newborn; Prevalence.

## INTRODUCTION

The heel prick test is a mandatory neonatal screening test, guaranteed by law,<sup>1</sup> preferably performed between the 3rd and 5th day of life, which aims to actively diagnose congenital, metabolic, and infectious diseases while still in their asymptomatic stages, allowing for early treatment and altering the course of the disease and its complications.<sup>2,3</sup> Previously, the screening provided by the PNTN (National Neonatal Screening Program) covered six diseases: phenylketonuria, congenital hypothyroidism, sickle cell disease, biotinidase deficiency, cystic fibrosis, and congenital adrenal hyperplasia.<sup>4,5</sup> Sickle cell disease and phenylketonuria are the most prevalent in Brazil and worldwide.<sup>6,7,8</sup>

In May 2021, Law No. 14,154 sanctioned the expansion to 50 diseases, in 14 groups, to be diagnosed by the Guthrie Test in the Unified Health System,<sup>9</sup> and recently, the implementation of the Expanded Guthrie Test is scheduled in five stages.<sup>10</sup> In the first stage, the Guthrie Test will continue to detect the six diseases and include the diagnosis of congenital toxoplasmosis. In a second stage, tests for galactosemia, amino acidopathies, urea cycle disorders, and beta-oxidation disorders of fatty acids will be added. In the third stage, lysosomal diseases will be added. In stage four, primary immunodeficiencies will be tested, and in stage five, spinal muscular atrophy will be tested.<sup>(10)</sup>

Phenylketonuria (PKU), an autosomal recessive disease, is characterized by the absence or defect of the enzyme phenylalanine hydroxylase, responsible for converting phenylalanine into tyrosine. In this disease, the enzymatic action does not occur or is slowed down, causing the accumulation of phenylalanine (FAL) in the patient's blood, resulting in high levels. A new sample must be collected to guide the child's family.<sup>(1,6)</sup> Congenital hypothyroidism (CH) is mostly a primary disorder of the thyroid gland, with a consequent increase in TSH, the hormone that regulates thyroid function.<sup>(1)</sup>

Sickle cell anemia, an autosomal recessive disease, results from a mutation in the beta chain of the hemoglobin gene, in which hemoglobin S (HbS) replaces hemoglobin A (HbA), altering the shape of red blood cells to a sickle or crescent shape. Screening can differentiate between sick children (HbSS) and those with sickle cell trait (HbAS). In addition, the HbS gene may be accompanied by other changes that cause similar symptoms, called Sickle Cell Disease (SCD), which include: HbSS, HbSC, HbSD, HbS-beta thalassemia, and other combinations.<sup>1,3</sup> Cystic fibrosis (CF), presented by the classic triad of chronic lung disease, pancreatic insufficiency, and "sal-

ty sweat," is an autosomal recessive disease. The protocol consists of measuring reactive immunotrypsin (IRT), which is elevated in situations other than CF, such as infections and prematurity. Therefore, in the event of elevated IRT, a second sample should be collected within 3 to 4 weeks of the newborn's life.<sup>(1,11)</sup> Finally, Congenital Adrenal Hyperplasia (CAH), characterized by enzyme deficiency in the synthesis of adrenal steroids, and Biotinidase Deficiency (BDT), an error in biotin metabolism, both of an autosomal recessive nature, complete the list of diseases screened for in the PNTN's heel prick test.<sup>1,12,13</sup>

The possibility of early diagnosis of chronic diseases provides an opportunity to adopt immediate measures and treatments, which enable better living conditions for children and their families, reducing the potential for clinical and social complications.

Therefore, this study aimed to describe the pre l prevalence of abnormalities detected by this test and the clinical profile of its participants, , in a municipality in southern Santa Catarina, from August 2023 to February 2024.

## METHODOLOGY AND RESULTS

An observational epidemiological study with a cross-sectional, descriptive design was conducted. The study population consisted of newborns, at least 72 hours old, and postpartum women of all ages, from a municipality in southern Santa Catarina, from August 2023 to February 2024.

The study included 237 newborns, at least 72 hours old, who underwent collection of the first or second sample of the Guthrie test in a municipality in southern Santa Catarina, at the Neonatal Screening Center of the municipal polyclinic. Newborns whose parents could not read and/or write, who did not agree to participate in the study, who were unable to complete the study, or who had cognitive impairments that interfered with data collection were excluded from the study.

To achieve the study objectives, the following variables were included: Mother's age, mother's marital status, mother's color/race, maternal conditions with potential false positives, use of cigarettes/alcohol/drugs during pregnancy, high-risk pregnancy, prenatal care, number of prenatal visits, gestational age at birth, mode of delivery, biological sex of the newborn, color/race of the newborn, newborn feeding, conditions of the newborn with potential false positive, Apgar 1st minute, Apgar 5th minute, birth weight, age of the newborn, age at the time of the heel prick test, result of the first and second samples, phenylalanine (PHA) dosage, thyroid-stimulating hormone (TSH), standard hemoglobin analysis, 1st sample of reactive immunotrypsin (IRT), 2nd collection after the first result showed a change in IRT, 1st collection of 17 alpha hydroxyprogesterone (17-OHP), 17-OHP recollection, biotinidase activity.

Quantitative variables were described using measures of central tendency and data dispersion. Qualitative variables were described using absolute frequency and percentage. Differences in proportions were tested using Pearson's chi-square test, and differences and mean proportions were tested using Student's t-test or nonparametric equivalents, depending on the normality of the data. The level of statistical significance was set at 5% (p-value < 0.05).

The study was conducted in accordance with Resolution No. 466/2012 and Resolution No. 510/2016, and was approved by the Research Ethics Committee of Unisul (CEP - Unisul) on July 12, 2023, under opinion number 6.177.238.

### RESULTS

The study showed a prevalence of 8.86% in changes detected by the Guthrie Test, as shown in Figure 1. Of these, 57.14% were female and 42.85% were male, with a predomi-

nance of whites (66.66%) and blacks (19.04%) among other color/race classifications. Regarding birth, 61.90% were born with a gestational age between 32 and 36 weeks, and 38.09% were born after 37 weeks. The average birth weight was 2769.95 g, SD  $\pm$  539.764 g, ranging from 1902 g to 4070 g. Most were aged up to 10 days (66.66%).

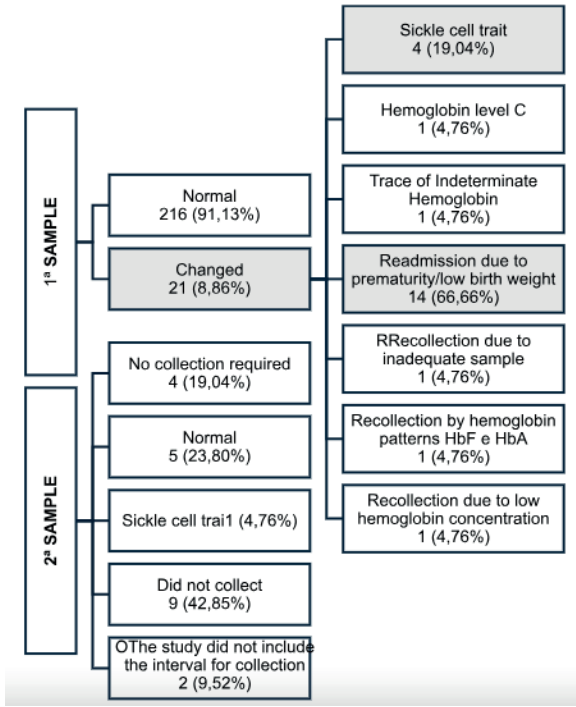


Figure 1 – Main changes detected in the Guthrie test during the period from August 2023 to February 2024 in a municipality in southern Santa Catarina. (N=237)

Source: The Authors, 2024

Of the altered results, the presence of sickle cell trait was the main alteration detected in the first and second samples, 19.04% and 4.76%, respectively. In terms of the need for retesting, prematurity and/or low birth weight were the main determinants (66.66%) of this condition. These and other results are described in Figure 1.

In total, 237 newborns (Table 1) and their respective mothers (Table 2) were surveyed and analyzed socially and clinically. Most of the newborns were delivered by cesarean section (57.38%), and the prevalence continued to be

female (53.16%) compared to male (46.83%). In contrast to those who tested positive, most were born with a gestational age equal to or ly greater than 37 weeks (91.98%), and in terms of color/race distribution, 80.16% were white, followed by 13.08% brown. The average age was 6.44 days, SD  $\pm$  9.03, ranging from 3 to 120 days. However, regarding the first sample, 33.33% were older than 5 days of life. The clinical and social variables of the newborns can be seen in Table 1.

Variables	n
<b>Mode of delivery</b>	
Cesarean	136
Vaginal	101 (42.61%)
<b>Gestational age at delivery</b>	
28 to 31 weeks	3
32 to 36 weeks	16 (6.75%)
$\geq$ 37 weeks	218 (91.98%)
<b>Biological sex of the newborn</b>	
Female	126 (53.16%)
Male	111 (46.83%)
<b>Color/Race of the newborn</b>	
White	190 (80.16%)
Black	12 (5.06)
Yellow	3 (1.26%)
Brown	31 (13.08%)
Indigenous	1 (0.42%)
<b>Birth weight</b>	
Good birth weight ( $\geq$ 2500g)	223 (94.09%)
Low birth weight (<2500g)	14 (5.90%)
<b>Apgar score at 1 minute</b>	
Severe asphyxia (0-3)	5 (2.10%)
Moderate asphyxia (4-6)	14 (5.90%)
Good vitality (7-10)	216 (91.13%)
Did not receive Apgar score	2
<b>Apgar score at 5 minutes</b>	
Severe asphyxia (0-3)	0
Moderate asphyxia (4-6)	0
Good vitality (7-10)	235 (99.15%)
Did not receive Apgar score	2 (0.84%)
<b>Feeding</b>	
Exclusive breastfeeding	215 (90.71%)
Infant formula	21 (8.86%)
Other	1 (0.42)
<b>Neonatal conditions with potential false positive</b>	

Prematurity	19 (8.01%)
Iodine deficiency	0
Red blood cell transfusion	1 (0.42%)
Parenteral nutrition	1 (0.42%)
Use of steroids	0
Use of dopamine	0
No condition	216 (91.13%)

**Table 1** – Clinical and social variables of newborns during the period from August 2023 to February 2024 in a municipality in southern Santa Catarina. (N=237)

**Source:** The authors, 2024

Regarding the variables of the mothers, it was noted that  $\frac{3}{4}$  were white and the average age was 28.5 years, with SD $\pm$  6.76 years, ranging from 14 to 45 years. Most were single (56.96%) or married/in a stable relationship (40.92%). Regarding the gestational period, most had 7 or more prenatal consultations, representing 87.76% of the sample (Table 2).

Variables	n
<b>Mother's age</b>	
<15 years	2
16-34	186 (78.48%)
$\geq$ 35 years	49 (20.67%)
<b>Mother's race</b>	
White	178 (75.10%)
Black	25 (10.54%)
Yellow	1 (0.42)
Brown	32 (13.50%)
Indigenous	1 (0.42%)
<b>Marital status of mother</b>	
Single	135 (56.96%)
Married/Common-law marriage	97 (40.92%)
Divorced/Separated	4 (1.68%)
Widowed	1 (0.42%)
<b>Number of prenatal consultations</b>	
< 6 consultations	29 (12.23%)
$\geq$ 7 consultations	208 (87.76%)
<b>Maternal conditions with potential false positives</b>	
Congenital adrenal hyperplasia	1 (0.42%)
Phenylketonuria	0
Hepatic steatosis in pregnancy	0
Vitamin B12 deficiency	20 (8.43%)



Blood transfusion	2 (0.84%)
Parenteral nutrition	3 (1.26%)
None	211 (89.02%)
<b>Use of cigarettes/alcohol/drugs during pregnancy</b>	
Yes	21 (8.86%)
No	216 (91.13%)

**Table 2** – Clinical and social variables of mothers during the period from August 2023 to February 2024 in a municipality in southern Santa Catarina. (n=237)

**Source:** The authors, 2024

The results of the first sample were cross-referenced with other variables, and the results are shown in Table 3. The Normal and Abnormal results did not differ significantly in relation to high-risk pregnancy ( $p = 0.818$ ) and mode of delivery ( $p = 0.661$ ). However, non-risk pregnancy and cesarean delivery predominated in both samples (Table 3).

	First Sample		P value
	Normal n (%)	Altered n (%)	
<b>High-risk pregnancy</b>			0.818
Yes	87 (90.62%)	9 (9.37%)	
No	129 (91.48%)	12 (8.51%)	
<b>Mode of delivery</b>			0
Vaginal	93 (92.07%)	8 (7.92%)	
Cesarean	123 (90.44%)	13 (9.55%)	

**Table 3** – Association of the results of the first sample with variables of the newborn and its gestational period, during the period from August 2023 to February 2024, in the municipality in southern Santa Catarina. (n=237)

**Source:** The authors, 2024.

### DISCUSSION

The present study showed the prevalence of abnormalities detected in the heel prick test and the clinical and social profile of newborns and their respective mothers. In this stu-

dy, 21 abnormal heel prick tests were found, meaning that, on average, one in every 11.28 newborns did not have a normal result. Most of these were diagnosed as carriers of sickle cell trait or required further testing due to prematurity and/or low birth weight. During the collection period, the interval between birth and testing was between 72 hours and 5 days, as recommended by the Ministry of Health, thus ensuring a lower chance of false positives and false negatives.<sup>1</sup>

Studies show that sickle cell disease is the most prevalent hemoglobinopathy in Brazil, with an incidence of 3.78 per 10,000 live births between 2014 and 2020. It has African origins and consequently affects black and brown people more. In this study, no cases of sickle cell disease were diagnosed, i.e., no homozygosity pattern (HbSS). A plausible explanation for this finding would be the municipality's own miscegenation, which, according to data from the 2022 demographic census (IBGE), the black and brown populations corresponded to 4.6% and 8.8%, respectively.<sup>16</sup>

The findings of this study, referring to the altered results, showed a higher frequency of samples with HbAS, indicative of sickle cell trait, with an incidence in the study of 1:47.4. Unlike newborns with a normal first sample, who were predominantly white and brown, those who received an altered first sample were predominantly white and black. Previous studies estimate, according to PNTN data, that approximately 180,000 children per year are born with sickle cell trait.<sup>15</sup> This hemoglobinopathy is not synonymous with disease, and its occurrence varies between states due to historical characteristics of miscegenation in each population, with a higher incidence in Bahia (1:17) compared to an incidence of 1:65 in Santa Catarina.<sup>15,17</sup> There is a prevalence of whites among other classifications, precisely because they are the predominant population in the municipality and in this study,

but the subsequent classification of those who received altered results for sickle cell trait is precisely black, which supports the epidemiology of hemoglobinopathy.<sup>15-17</sup>

Further supporting this finding, a study conducted in a municipality in southern Brazil<sup>18</sup> showed hemoglobinopathies as the most prevalent, with SST (Sickle Cell Trait) as the most observed pattern with 1.84%, followed by AF (Blood Transfusion) with 0.64%, and FAC (Hemoglobin C trait) at 0.15%, among other patterns.<sup>18</sup> Thus, we found a similar pattern in this sample, with five FAS patterns, one AF, and one FAC.

Hemoglobin C (HbC) is a hemoglobinopathy similar to sickle cell anemia, which results in the rigidity of red blood cells. These hardened blood cells are subsequently destroyed in the spleen, which can lead to hemolytic anemia. HbC is less common than sickle cell anemia in tests performed on newborns.<sup>19</sup> In this specific study, no homozygous conditions were found, only one case of hemoglobin C trait (HbSC) was identified.

Hemoglobin is known to be genetically inherited, and given this fact and the fact that carriers of the sickle cell trait do not show clinical manifestations throughout their lives, genetic counseling is recommended.

Another study aimed at describing the characteristics of the heel prick test in newborns treated in the intensive care unit verified maternal and neonatal conditions that could interfere with the test.<sup>20</sup> The results obtained were similar to those of this study. Regarding neonatal conditions with potential false positives, both obtained the three most prevalent: prematurity, parenteral nutrition, and red blood cell transfusion. On the other hand, consistent with maternal conditions, the findings differed in terms of sampling. While the comparative study observed, albeit in a minority of women, the use of steroids and hypothyroidism as the most prevalent

conditions, this study presented, in descending order: vitamin B12 deficiency, parenteral nutrition, red blood cell transfusion, and congenital adrenal hyperplasia.<sup>1,20</sup> Finally, another common characteristic was the recognition that the use of cigarettes, alcohol, or drugs during pregnancy is also a relevant factor, since these substances increase the chances of adverse health effects on development and pregnancy outcomes such as spontaneous abortion, preterm birth, low birth weight, and other conditions.<sup>20,21</sup>

As for the reasons for the need for collection, prematurity and/or low birth weight are closely related to changes in the Guthrie test, as they are more prone to false positive and false negative results.<sup>1,20</sup> Other reasons found in the results of this study were: inadequate sample, hemoglobin HbF and HbA patterns, low hemoglobin concentration, hemoglobin C trait, and indeterminate hemoglobin trait. However, even though they were aware of the need for and importance of the test, many did not attend the collection, at least not at the municipal referral site or during the data collection period for this study.

Exclusive breastfeeding was prominent at the time of collection, present in 90.71% of newborns, indicating that most children are receiving all the nutritional, immunological, and psychological benefits that breastfeeding provides.<sup>22,23</sup> However, breastfeeding should continue to be supported and encouraged by health professionals throughout the follow-up of these children, since early weaning is a frequent problem in Brazil and worldwide.<sup>22</sup> A literature review aimed at identifying factors that interfere with early weaning during exclusive breastfeeding also considered the need for follow-up through continuity of care in order to reduce such rates.<sup>23</sup>

Another relevant point for maternal and fetal health is prenatal care, which is essential for early identification of pregnancy compli-

cations and risk reduction.<sup>24</sup> In the sample of this study, composed of 237 postpartum women, 208 reported having had seven or more consultations, exceeding the minimum number recommended by the Ministry of Health, which is six consultations.<sup>24</sup> This data also exceeds the results of a study conducted in 2017, involving three municipalities, where most pregnant women had between four and six prenatal consultations.<sup>25</sup>

Finally, it is important to note that this study has some limitations, such as the type of study and the fact that data was not obtained from all children who underwent follow-up visits.

## CONCLUSION

The heel prick test is essential for the early detection of pathologies in the neonatal period, even while asymptomatic. The prevalen-

ce in this study was of white female newborns, with a mean age of 6.44 days, born by cesarean section and with a gestational age equal to or greater than 37 weeks. Their mothers were mostly white, single, with an average age of 28.5 years. Hemoglobinopathies were the most frequently identified anomalies, with sickle cell trait being the most prevalent. The results also showed adequate adherence to the recommended collection period, as well as a high rate of prenatal consultations, indicating an improvement in access to and quality of maternal and child care. The findings provide relevant information on neonatal health and point to the continuing need for health policies aimed at promoting the well-being of children and their families.

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