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COMPARATIVE ANALYSIS OF TWO TREC/KREC QUANTIFICATION METHODS FOR NEWBORN SCREENING OF PRIMARY IMMUNODEFICIENCY WITH T- AND B-LYMPHOPENIA USED IN UKRAINE

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Background. Primary immunodeficiency (PID) compromises the immune system, leaving newborns highly vulnerable to infections. Severe combined immunodeficiency (SCID) is the most severe form, characterized by the absence or dysfunction of T and B cells. Without early treatment, most infants with SCID do not survive their first year. In Ukraine, after a successful pilot project, newborn screening for SCID and other types of PID is now part of Advanced Neonatal Screening, using T-cell receptor excision circles (TREC) and kappa-deleting recombination excision circles (KREC) measurements. Since the pilot project used a different method than the current screening program, the purpose of this study was to compare these two methods, evaluating their benefits and downsides, aiming to optimize screening for early, effective treatment.

Materials and Methods. In the pilot project method (Method 1), TREC and KREC quantification was performed using a custom real-time PCR assay with melting curve analysis. Method 1 included standards with known TREC and KREC copy numbers, no-template controls (NTCs), and positive controls to ensure reliable results. The method currently employed in the Advanced Neonatal Screening (Method 2) uses the Biocore® SMA/TKID PLUS Diagnostic Kit, a commercial kit, for TREC, KREC and *SMN1* quantification via real-time PCR. Measurements for both methods are reported per one million cells.

Results and Discussion. While there are differences in the general parameters of DNA extraction, PCR, and result analysis and interpretation, both Method 1 and Method 2 showed a significant difference in Cq values. Despite these differences, both methods demonstrated the capability of inentifying abnormal TREC/KREC values, enabling the detection of SCID and some PID cases.

Conclusion. The pilot project demonstrated the effectiveness of TREC/KREC quantification for SCID screening and led to its implementation in Advanced Neonatal Screening in Ukraine. Over 121,000 newborns were tested, confirming six positive cases. Method 1 provides higher precision and versatility, while Method 2 is faster, simpler, and capable of automation but lacks precise quantification. Adding standards to Method 2 could enhance its utility for widespread SCID screening.

Keywords: PCR, Severe combined immunodeficiency (SCID), T-cell receptor excision circles (TRECs), kappa-deleting recombinant excision circles (KRECs), inborn errors of immunity, gene, DNA, genetics

INTRODUCTION

Primary immunodeficiency (PID) including severe combined immunodeficiency (SCID) are life-threatening conditions in which infants lack functional immune cells, leaving them highly susceptible to infections. Without early intervention, PID significantly increases infection risks, while SCID is typically fatal (Gizewska e*t al.*, 2020; Aranda *et al.*, 2020; Argudo-Ramírez *et al.*, 2019). However, newborn screening using T-cell receptor excision circles (TRECs) and kappa-deleting recombination excision circles (KRECs) enables the early detection of T- and B-cell lymphopenia. This allows for timely, life-saving treatments such as stem cell transplants, intravenous immunoglobulin (IVIg) therapy, or gene therapy (Kumarasamy *et al.*, 2024; Aranda *et al.*, 2020).

Newborn screening programs for SCID and certain types of PID are implemented in various countries using diverse methodologies. These programs differ in their choice of DNA extraction kits for dried blood spots (DBS) and real-time PCR assays. Some assays quantify only TREC copy numbers (Verbsky *et al.*, 2011; Argudo-Ramírez *et al.*, 2019; Chien *et al.*, 2015; Al Ghamdi *et al.*, 2024), while others include both TREC and KREC measurements, enabling broader screening for T- and B-cell deficiencies (Göngrich e*t al.*, 2021; Barbaro e*t al.*, 2017; Gizewska *et al.*, 2020; de Felipe *et. al.*, 2016). Additionally, normalization methods differ significantly: some report TREC or TREC+KREC copies per microliter of blood (Gizewska *et al.*, 2020; Argudo-Ramírez *et al.*, 2019; Chien *et al.*, 2015), others per one million cells (Göngrich *et al.*, 2021), and some base measurements on copies per punch from the DBS sample. In the latter approach, β-actin quantification is used to assess the efficiency of DNA extraction from DBS samples (Barbaro *et al.*, 2017; de Felipe *et. al.*, 2016).

Using DBS punches without proper normalization introduces inaccuracies due to variability in blood spot composition, such as differences in lymphocyte-to-red blood cell ratios and plasma distribution. The lack of consistency in cell counts across punches can lead to significant over- or underestimation of TREC and KREC levels. This, in turn, increases the risk of misdiagnosis, potentially impacting the timely identification and treatment of affected newborns. For reliable results, normalization to cell counts or a control gene like β-actin is essential to ensure the efficacy of DNA extraction and the accuracy of TREC/KREC quantification. (Kwok e*t al.*, 2020; Baillargeon & Mace, 2023)

Both the pilot project previously conducted in Ukraine (Boyarchuk *et al.*, 2022) and the current method used in Advanced Neonatal Screening (Ministry of Health of Ukraine, 2021) employ real-time PCR to measure TREC and KREC copy numbers per one million cells in newborns. However, these methods differ in their DNA extraction protocols, real-time PCR assays, and normalization approaches. This study aims to compare the two methods, validate their performance, and recommend improvements to enhance the accuracy and effectiveness of newborn screening.

MATERIALS AND METHODS

Method 1. DNA isolation and purification from dried blood spots (DBS) on filter paper were performed using the DNA-SorbB nucleic acid isolation kit (AmplySens). The DNA samples were dissolved in 75 µL of TE buffer, and their concentration and optical characteristics were measured using the DENOVIX instrument.

TREC and KREC quantities were analyzed by RT-PCR. The primer sequences used for TREC amplification were (F: 5′-CCATGCTGACACCTCTGGT-3′, R: 5′-TCGTGAGA-ACGGTGAATGAAG-3′), for KREC (F: 5′-TCAGCGCCCATTACGTTTCT-3′, R: 5′-GTGA-GGGACACGCAGCC-3′), and for the albumin gene as an internal control (F: 5′-TGAACA-GGCGACCATGCTT-3′, R: 5′-CTCTCCTTCTCAGAAAGTGTGCATAT-3′) according to O. Boyarchuk *et al.* (2017) following protocol. RT-PCR reactions were set up for each primer pair and included 6 µL of ddH_2O, 1.5 µL of 5xHOT FIREPol EvaGreen Mix Plus (Solis BioDyne, Estonia), 0.3 µL of each primer, and 2 µL of DNA sample (10–25 ng/µL).

Plasmids with known Albumin, TREC and KREC copy numbers were used as standards, while deionized water served as a no-template control. A patient DNA sample with newborn screening (NBS) showing low TREC and KREC levels was used as a positive control. Real-time PCR was performed with the following settings: polymerase activation (50 °C, 2 min), initial denaturation (95 °C, 10 min) followed by 50 cycles of denaturation (95 °C, 15 sec), annealing and extension (60 °C, 60 sec). To enhance specificity, an additional analysis of PCR products was performed using melting curve analysis from 50 °C to 90 °C, in 0.5 °C increments. The RT-PCR was run on the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, USA). The calculation of TREC/KREC per 1 million blood cells was performed using the formula: TREC/KREC copies = (TREC/KREC Cq×1000000) / (Albumin Cq/2). This ensures accurate normalization relative to albumin levels (Boyarchuk *et al.*, 2022).

Method 2 (Sample Processing and Real-Time PCR). DNA was isolated and purified from dried blood spots (DBS) on filter paper using the Biocore® Nucleo-M Plus (100 extraction) kit. TREC, KREC, and SMN1 quantifications were conducted using the BIOCORE® SMA/TKID assay on a QuantStudio Real-Time PCR (qPCR) system. This workflow enables the detection of TREC, KREC, *SMN1* and the internal control gene *RNase P* in a single reaction tube. Primer sequences are proprietary to the commercial assay and are therefore unavailable.

Statistical analysis. All statistical analyses were conducted using Microsoft Excel, including the “Data Analysis” Toolpak.

- **Averages**: All average values were calculated using the Excel formula **= AVERAGE**.
- **Standard errors, standard deviations, and sample variances**: these were determined using the “Descriptive Statistics” feature from the “Data Analysis” Toolpak.

- **Comparison of Cq values**: to assess significant differences in Cq values for Albumin, TREC, and KREC between the two methods, we performed a ***t*-test: paired two sample for means** (available in the "Data Analysis" Toolpak). The *t*-test was chosen because the data followed a normal distribution.
- **Normalization and additional comparisons**: for further comparison of TREC and KREC values between methods, we normalized the values relative to Albumin. Two normalization approaches were used:
  1. **Division normalization**: Normalized Value = TREC or KREC/Albumin.
  2. **Subtraction normalization**: Normalized Value = TREC or KREC−Albumin These normalized values were also compared using the ***t*-test: paired two sample for means**.

RESULTS AND DISCUSSION

Conducted between May 2020 and January 2022, the pilot project covered 21 months and included DBS samples from 15 maternity hospitals in Ternopil region, with additional samples from neonatal intensive care units in neighboring Ivano-Frankivsk and Lviv regions. Of the 10,350 newborns screened, three had abnormal TREC or KREC levels:

- Case 1: Zero TREC copies, later confirmed as combined immunodeficiency (CID) with low T-cell numbers.
- Case 2: Decreased TREC levels due to prematurity (GA: 33 weeks, BW: 2,300 g), resulting in transient lymphopenia.
- Case 3: Reduced KREC levels associated with maternal complications (threatened abortion).

The pilot study employed the same method as described in Boyarchuk *et al.* (2022), incorporating melting curve analysis to enhance specificity. Given the low copy number of TREC and KREC relative to nuclear DNA, this additional step improved the reliability of the results. The TREC and KREC copy numbers per one million white blood cells were measured using standards with known Albumin, TREC and KREC quantities. The study established a diagnostic threshold of 2,000 copies for TREC and KREC. Once the method was fully adapted and the final cutoff values were established, the retest rate was 4.1 %, and the proportion of abnormal results was 0.5 % (Boyarchuk *et al.*, 2022).

Following the successful pilot, TREC/KREC measurement was incorporated into Advanced Neonatal Screening in Ukraine in October 2022. Over two years (October 2022–October 2024), 121,014 newborns from 12 regions of Western Ukraine, were screened. In 2024, the retest rate was 0.15 %, which is 27 times lower than that of the pilot project. Among the retested patients, four cases (0.008 %) with abnormal TREC or KREC values were confirmed:

- Two had decreased TREC levels.
- One had decreased KREC levels.
- One exhibited low levels of both TREC and KREC.

The screening employs a test system manufactured by Biocore Technologies LTD, Ukraine. This system simultaneously amplifies TREC, KREC, *SMN1*, and *RNase P* regions as an internal control. Since the method does not include standards for precise quantification of TREC/KREC copy numbers, the cutoff was determined based on Cq (quantification cycle) values, which ranged between 16 and 36 cycles.

We initiated a comparison between the two methods – **Method 1** from the pilot project and **Method 2** from Advanced Neonatal Screening – focusing on their general parameters in DNA extraction and PCR analysis (**Table 1**).

Table 1. **Description of general parameters for methods of TREC KREC measurement: DNA extraction and PCR analysis**

Testing stages	Criteria	Method 1	Method 2
DNA extraction	Duration of sample preparation, min	60	25
	DNA extraction duration, min	33	20
	Automation capability (Yes/No)	No	Yes
	Average DNA yield, ng/DBS	50 ng	70 ng
PCR	PCR duration, min	140	33
	Analytical sensitivity (limit of detection (LOD))	100 copies	10 copies
	Internal control gene	Albumin	*RNAse P*
	Target genes/sequences	TREC, KREC	TREC, KREC, *SMN1*
	Melting	Yes	No
	PCR platform versatility	Versatile (Biorad, Qiegen, Applied Biosystems)	Quant studio
	PCR reaction volume, µL	10	10
	Number of tubes and reactions per sample, N	3	1
	DNA input volume, µL	2	5
	DNA input amount, ng	20	100
	Detection channels	FAM (Albumin, TREC, KREC)	FAM (SMN1), JUN (TREC), ABY (KREC), VIC (RNase P)
Analysis	Analysis of results	Based on melting curve and absolute number of TREC/ KREC copy number per 1 million WBS	Based on Ct value

Data presented in **Table 1** indicate that **Method 2** is faster and allows for automation, making it efficient for high-throughput settings. Additionally, the program includes an extra target sequence, *SMN1*, which expands its screening capability. However, the PCR component lacks versatility, as the kit is compatible only with specific real-time PCR thermocyclers, limiting its flexibility across different platforms. The PCR method used in the pilot project incorporates an additional analysis of melting products, enhancing

specificity. The use of an intercalator allows analysis of melting curves, which ensures reliable verification of low copies of TREC, KREC. Analysis of melting curves, as one of the stages of this method, is our own development, which allows us to maximize the accuracy and specificity of detection of low copies of target molecules.

Table 2 compares TREC and KREC quantification levels obtained by real-time PCR in two methods, highlighting differences in quantification cycle (Cq) values and the use of copy number standards. In **Method 1** (pilot project), a larger ΔCq (difference in Cq) is observed between the control gene and TREC, with overall higher Cq values for the control gene, TREC, and KREC than in **Method 2** (Advanced Neonatal Screening). Notably, Method 2 lacks copy number standards for the control gene, leading to TREC and KREC measurements with levels exceeding expected ranges (e.g., 1.00E+08 for TREC and 1.00E+07 for KREC per 1 million cells). These results are inconsistent with anticipated values, as TREC and KREC levels should be below 1.00E+06 per 1 million cells.

Table 2. **Comparison of TREC and KREC quantification via real-time PCR in the pilot project (Method 1) and Advanced Neonatal Screening (Method 2)**

Clinical testing overview	Pilot project method	Advanced Neonatal Screening
General number of patient	**10,350**	**121,014**
Number of positive patients	**1**	**6**
Avarage Cq of control gene	25.80	23.50
Average Cq of TREC into negative sample	32.80	28.50
Average Cq of KREC into negative sample	32.72	32.50
Average ΔCq of TREC-Control Gene into negative sample	7.00	5.00
Average ΔCq of KREC-Control Gene into negative sample	6.92	6.50
Average copy number of TREC per 1 million cells into negative sample	6.24E+04	1.00E+08*
Average copy number of KREC per 1 million cells into negative sample	9.22E+04	1.00E+07*
Cut-off, copy number for TREC	2000	100.00
Cut-off, copy number for KREC	2000	100.00
Testing year	05.2020–01.2023	10.2022–10.2024
Age of patients	1–30 days	1–30 days
Geographic region	Ternopil region	7 regions of west of Ukraine (Lviv, Ternopil, Ivano-Frankivsk, Khmelnytskyi, Zakarpattia, Chernivtsi, Volyn regions)

Note: * Abnormally high value – exceeds expected biological range (>10^6 molecules per 1 million cells), possibly due to technical artifacts or measurement errors

Since **Method 2**, unlike **Method 1**, does not include standards (plasmids containing control genes, TREC, and KREC) to determine precise copy numbers, it is not possible to determine TREC/KREC copy numbers per 1 million cells or μL of blood. As a result, a direct comparison of TREC/KREC copy numbers between **Method 1** and **Method 2** is not feasible. Therefore, further analysis focused on the comparison of Cq values. Negative sample Cq values were compared using a *t*-test (**Table 3**), revealing a significant difference between the two methods. Additionally, normalization was performed using a control gene (calculated as either TREC/KREC Cq minus control gene Cq, or TREC/KREC Cq divided by control gene Cq). Comparative *t*-tests of these optimized values also demonstrated significant differences between the methods.

It is worth mentioning that **Method 1** uses the EvaGreen intercalator, which is a bright dye, and Cq as a quantification of the amplification efficiency, which may be slightly underestimated, due to the specificity of this dye, compared to the results obtained in **Method 2** using TaqMan PCR with four different dyes: FAM (*SMN1*), JUN (TREC), ABY (KREC), VIC (RNase P)

Table 3. **Comparison of Cq values for TREC, KREC, and control genes in negative samples using two methods**

Method comparison metrics	Albumin	TREC	KREC
Method1_Average ± Standard Deviation	24.3993±0.619498	31.81333±0.683475	30.538±0.5266226
Method2_Average ± Standard Deviation	23.6732667±0.296558	29.4768667±0.756909	31.7560667±1.080274
t Stat*	4.056813306	13.2777797	-5.478194067
P(T<=t) two-tail**	0.00117745	2.52593E-09	8.13434E-05
t critical two-tail***	2.144786688	2.144786688	2.144786688

Notes: * – test statistic, measures the size of the difference between the paired means in relation to the variability of the data; ** – two-tailed p-value, the probability of observing a *t*-statistic as extreme as (or more extreme than) the one calculated, assuming the null hypothesis is true; *** – the cutoff value of the *t*-statistic for a two-tailed test at a given significance level (0.05)

In **Method 1**, the PCR Cq values for Albumin and TREC are higher, while the Cq for KREC is unexpectedly lower. This discrepancy is unusual since all values should typically shift in the same direction (either higher or lower) under consistent conditions. Therefore, there is a possibility that one of the two methods may be inaccurate in quantifying the copy number of TREC or KREC.

Since the primary goal of the screening program is to identify patients with abnormal TREC and KREC values for the diagnosis of severe combined immunodeficiencies, we evaluated whether both methods effectively detect such cases. Our comparison demonstrated that both methods successfully identified positive cases with low TREC or low KREC values (**Table 4**).

Table 4. **Performance of two methods in positive sample identification**

Number of samples	Method 1 (TREC/KREC assessment)	Method 2 (TREC/KREC assessment)
SAVR	Normal TREC, Low KREC	Normal TREC, Low KREC
SHAF	Low TREC, Normal KREC	Low TREC, Normal KREC
SYNY	Low TREC, Normal KREC	Low TREC, Normal KREC
BUDR	Low TREC, Low KREC	Low TREC, 0 KREC

The results demonstrate that both methods reliably identify abnormal TREC or KREC values, confirming their effectiveness for detecting such conditions.

CONCLUSION

After the successful pilot project involving 10,350 newborns and 65 patients from a control group with confirmed inborn errors of immunity, TREC/KREC quantification was incorporated into Advanced Neonatal Screening for SCID in Ukraine. During the program, 121,014 newborns were tested, and six positive cases were confirmed.

Advantages of **Method 1** include the use of standards, enabling precise quantification of TREC/KREC copy numbers per 1 million white blood cells, melting curve analysis to distinguish target PCR products from nonspecific ones, and compatibility with multiple PCR platforms.

Method 2, on the other hand, lacks standards for accurate copy number quantification and is compatible with only one PCR platform. However, it is faster, simpler, and automatable, and includes additional screening for SMA (via *SMN1* gene analysis).

Nevertheless, the methods for TREC/KREC quantification used in the pilot project and Advanced Neonatal Screening differ significantly; both methods are specific for the main purpose of NBS – the identification of cases of newborns with lymphopenia.

To enhance **Method 2** for SCID and other PID screening Advanced Neonatal Screening, incorporating standards with known plasmid copy numbers for *RNase P*, TREC, and KREC sequences could make it a comprehensive and highly effective approach. Our study could not identify the sensitivity of both methods as no clinical confirmation of pseudo negative NBS results was detected. Further evaluation of the results of NBS and monitoring of Primary immunodeficiency (PID) case reports is necessary.

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COMPLIANCE WITH ETHICAL STANDARDS

Authors V. Kravets, I. Shymanska, N. Matiytsiv, and H. Makukh were employed by Scientific Medical Genetic Center LeoGENE, LTD, Lviv, Ukraine.

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, [H.M.; N.M.]; methodology, [S.I.; K.V.]; validation, [S.I.; K.V.; A.O.]; formal analysis, [K.V.; S.I.].; investigation, [S.I.; K.V.; A.O.]; resources, [B.O.]; data curation, [H.M.; N.M.]; writing – original draft preparation, [K.V.; S.I.]; writing – review and editing, [K.V.; H.M.; S.I.]; visualization, [K.V.; S.I.] supervision, [H.M.; N.M.]; project administration, [H.M.; B.O.]; funding acquisition, [authors initials].

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REFERENCES

Al Ghamdi, A., Pachul, J. W., Al Shaqaq, A., Fraser, M., Watts-Dickens, A., Yang, N., Vong, L., Kim, V. H. D., Siu, V. M., Pham-Huy, A., Brager, R., Reid, B., & Roifman, C. M. (2024). A unique comprehensive model to screen newborns for severe combined immunodeficiency – an Ontario single-centre experience spanning 2013–2023. *Genes*, 15(7), 920. doi:10.3390/genes15070920
Crossref ● PubMed ● PMC ● Google Scholar

Aranda, C. S., Guimarães, R. R., & de Gouveia-Pereira Pimentel, M. (2021). Combined immunodeficiencies. *Jornal de Pediatria*, 97, S39–S48. doi:10.1016/j.jped.2020.10.014
Crossref ● PubMed ● PMC ● Google Scholar

Argudo-Ramírez, A., Martín-Nalda, A., Marín-Soria, J. L., López-Galera, R. M., Pajares-García, S., González de Aledo-Castillo, J. M., Martínez-Gallo, M., García-Prat, M., Colobran, R., Riviere, J. G., Quintero, Y., Collado, T., García-Villoria, J., Ribes, A., & Soler-Palacín, P. (2019). First universal newborn screening program for severe combined immunodeficiency in Europe. Two-years' experience in Catalonia (Spain). *Frontiers in Immunology*, 10, 2406. doi:10.3389/fimmu.2019.02406
Crossref ● PubMed ● PMC ● Google Scholar

Baillargeon, K. R., & Mace, C. R. (2023). Microsampling tools for collecting, processing, and storing blood at the point-of-care. *Bioengineering & Translational Medicine*, 8(2). do:10.1002/btm2.10476
Crossref ● PubMed ● PMC ● Google Scholar

Barbaro, M., Ohlsson, A., Borte, S., Jonsson, S., Zetterström, R. H., King, J., Winiarski, J., von Döbeln, U., & Hammarström, L. (2017). Newborn screening for severe primary immunodeficiency diseases in Sweden – a 2-year pilot TREC and KREC screening study. *Journal of Clinical Immunology*, 37(1), 51–60. doi:10.1007/s10875-016-0347-5
Crossref ● PubMed ● PMC ● Google Scholar

Boyarchuk, O., Yarema, N., Kravets, V., Shulhai, O., Shymanska, I., Chornomydz, I., Hariyan, T., Volianska, L., Kinash, M., & Makukh, H. (2022). Newborn screening for severe combined immunodeficiency: the results of the first pilot TREC and KREC study in Ukraine with involving of 10,350 neonates. *Frontiers in Immunology*, 13, 999664. doi:10.3389/fimmu.2022.999664
Crossref ● PubMed ● PMC ● Google Scholar

Chien, Y.-H., Chiang, S.-C., Chang, K.-L., Yu, H.-H., Lee, W.-I., Tsai, L.-P., Hsu, L.-W., Hu, M.-H., & Hwu, W.-L. (2015). Incidence of severe combined immunodeficiency through newborn screening in a Chinese population. *Journal of the Formosan Medical Association*, 114(1), 12–16. doi:10.1016/j.jfma.2012.10.020
Crossref ● PubMed ● Google Scholar

de Felipe, B., Olbrich, P., Lucenas, J. M., Delgado-Pecellin, C., Pavon-Delgado, A., Marquez, J., Salamanca, C., Soler-Palacin, P., Gonzalez-Granado, L. I., Antolin, L. F., Borte, S., & Neth, O.

(2015). Prospective neonatal screening for severe T- and B-lymphocyte deficiencies in Seville. *Pediatric Allergy and Immunology*, 27(1), 70–77. doi:10.1111/pai.12501
Crossref ● PubMed ● Google Scholar

Giżewska, M., Durda, K., Winter, T., Ostrowska, I., Ołtarzewski, M., Klein, J., Blankenstein, O., Romanowska, H., Krzywińska-Zdeb, E., Patalan, M. F., Bartkowiak, E., Szczerba, N., Seiberling, S., Birkenfeld, B., Nauck, M., von Bernuth, H., Meisel, C., Bernatowska, E. A., Walczak, M., & Pac, M. (2020). Newborn screening for SCID and other severe primary immunodeficiency in the Polish–German transborder area: experience from the first 14 months of collaboration. *Frontiers in Immunology*, 11, 1948. doi:10.3389/fimmu.2020.01948
Crossref ● PubMed ● PMC ● Google Scholar

Göngrich, C., Ekwall, O., Sundin, M., Brodszki, N., Fasth, A., Marits, P., Dysting, S., Jonsson, S., Barbaro, M., Wedell, A., von Döbeln, U., & Zetterström, R. H. (2021). First year of TREC-based national SCID screening in Sweden. *International Journal of Neonatal Screening*, 7(3), 59. doi:10.3390/ijns7030059
Crossref ● PubMed ● PMC ● Google Scholar

Kumarasamy, G., Khairiz, K., Chang, W. L., Aye, T. T., & Ali, A. (2024). Paving the way in implementation of SCID newborn screening in developing nations: feasibility study and strategies to move forward in Malaysia. *Frontiers in Immunology*, 15, 1400247. doi:10.3389/fimmu.2024.1400247
Crossref ● PubMed ● PMC ● Google Scholar

Kwok, J. S. Y., Cheung, S. K. F., Ho, J. C. Y., Tang, I. W. H., Chu, P. W. K., Leung, E. Y. S., Lee, P. P. W., Cheuk, D. K. L., Lee, V., Ip, P., & Lau, Y. L. (2020). Establishing simultaneous T cell receptor excision circles (TREC) and K-deleting recombination excision circles (KREC) quantification assays and laboratory reference intervals in healthy individuals of different age groups in Hong Kong. *Frontiers in Immunology*, 11, 1411. doi:10.3389/fimmu.2020.01411
Crossref ● PubMed ● PMC ● Google Scholar

Ministry of Health of Ukraine. (2021). Order No. 2142. On the provision of extended neonatal screening in Ukraine. Registered with the Ministry of Justice of Ukraine on October 29, 2021, under No. 1403/37025. Retrieved from https://zakon.rada.gov.ua/laws/show/z1403-21#Text (In Ukrainian)

Şentürk, G., Ng, Y. Y., Eltan, S. B., Başer, D., Ogulur, I., Altındirek, D., Fırtına, S., Yılmaz, H., Kocamış, B., Kıykım, A., Camcıoğlu, Y., Ar, M. C., Sudutan, T., Beken, S., Temel, Ş. G., Alanay, Y., Karakoc-Aydiner, E., Barış, S., Özen, A., … Hatırnaz Ng, Ö. (2022). Determining T and B cell development by TREC/KREC analysis in primary immunodeficiency patients and healthy controls. *Scandinavian Journal of Immunology*, 95(3), e13130. doi:10.1111/sji.13130
Crossref ● PubMed ● Google Scholar

Verbsky, J. W., Baker, M. W., Grossman, W. J., Hintermeyer, M., Dasu, T., Bonacci, B., Reddy, S., Margolis, D., Casper, J., Gries, M., DeSantes, K., Hoffman, G. L., Brokopp, C. D., Seroogy, C. M., & Routes, J. M. (2011). Newborn screening for severe combined immunodeficiency; the Wisconsin experience (2008–2011). *Journal of Clinical Immunology,* 32(1), 82–88. doi:10.1007/s10875-011-9609-4
Crossref ● PubMed ● Google Scholar

ПОРІВНЯЛЬНИЙ АНАЛІЗ ДВОХ МЕТОДІВ КІЛЬКІСНОГО ВИЗНАЧЕННЯ TREC/KREC ДЛЯ СКРИНІНГУ ПЕРВИННИХ ІМУНОДЕФІЦИТІВ З Т- І В-ЛІМФОПЕНІЄЮ У НОВОНАРОДЖЕНИХ В УКРАЇНІ

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Вступ. Первинні імунодефіцити (ПІД) пов'язані з порушенням функції імунної системи, через що новонароджені є вразливими до інфекцій. Тяжкий комбінований імунодефіцит (ТКІД) є найтяжчою формою, що характеризується відсутністю або дисфункцією Т- та В-клітин. Без раннього лікування більшість немовлят із ТКІД не переживають першого року життя. В Україні після успішного пілотного проєкту скринінг новонароджених на ТКІД і деяких ПІД став частиною програми неонатального скринінгу, з використанням вимірювання TREC (Т-клітинні рецепторні ексцизійні кільця) та KREC (каппа-делетуючі ексцизійні кільця). Оскільки під час пілотного проєкту використовували інший метод, ніж у поточній програмі скринінгу, наше завдання полягає в порівнянні цих двох методів, оцінці їхніх переваг і недоліків з метою оптимізації скринінгу для раннього й ефективного лікування.

Матеріали та методи. У методиці пілотного проєкту (Метод 1) кількісне визначення TREC і KREC проводили за допомогою кастомізованого аналізу методом ПЛР у реальному часі з аналізом кривої плавлення. Метод 1 включав стандарти з відомою кількістю копій TREC і KREC, контрольно-негативні зразки (NTC) та позитивні контролі для забезпечення надійних результатів. У методиці, яку тепер використовують у програмі неонатального скринінгу (Метод 2), застосовується комерційний набір Biocore® SMA/TKID PLUS Diagnostic Kit для кількісного визначення TREC, KREC та SMN1 методом ПЛР у реальному часі. Результати вимірювань обох методів подаються у перерахунку на один мільйон клітин.

Результати. Загальні параметри екстракції ДНК, ПЛР, а також аналізу й інтерпретації результатів різняться між методами; показано істотну різницю у значеннях Cq. Незважаючи на ці відмінності, обидва методи продемонстрували здатність виявляти аномальні значення TREC/KREC, що дає змогу ідентифікувати ТКІД і деякі випадки ПІД.

Висновки. Пілотний проєкт довів ефективність кількісного визначення TREC/KREC для скринінгу ТКІД, що привело до його впровадження в національну програму скринінгу новонароджених в Україні. Було протестовано понад 121 000 новонароджених, з яких підтверджено шість позитивних випадків. Метод 1 забезпечує точність кількості копій та універсальність, тоді як Метод 2 є швидшим, простішим і може бути автоматизованим, але не забезпечує точної оцінки кількості копій.

Додавання стандартів внутрішнього контролю до Методу 2 може підвищити його показовість для широкого скринінгу SCID.

Ключові слова: ПЛР, тяжкий комбінований імунодефіцит (ТКІД), Т-клітинні рецепторні ексцизійні кільця (TRECs), каппа-делетуючі ексцизійні кільця (KRECs), вроджені помилки імунітету, ген, ДНК, генетика

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