Utilizing environmental sampling, blanks and assay controls for rapid troubleshooting in genetic laboratories

Laboratoriji za medicinsku biokemiju, mikrobiologiju i citologiju

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INTRODUCTION AND GOALS

Next-generation sequencing (NGS) laboratories demand stringent cleanliness levels to avoid contamination events. Performing Non-Invasive Prenatal Testing (NIPT) on approximately 400 samples per week, the lab issued over 75,000 NIPT reports from January 2019 to January 2024. Strict environment and procedural regulations were needed to avoid contamination, maintain the integrity and accuracy of the test results.

METHODS

NIPT procedure

Maternal plasma for NIPT was separated from mothers' peripheral blood (**Figure 1**). Cell-free DNA (cfDNA) isolated using an automated liquid handler machine underwent DNA barcoding and PCR amplification to prepare DNA libraries. After purification, DNA was used as a template to create DNA NanoBalls (DNBs) using a rolling circle amplification technique. DNBs were placed on the patterned chip, and whole genome sequencing was performed in conjunction with on-site bioinformatics and report validation (**1**). Pre-PCR and post-PCR procedures are marked on **Figure 1**.

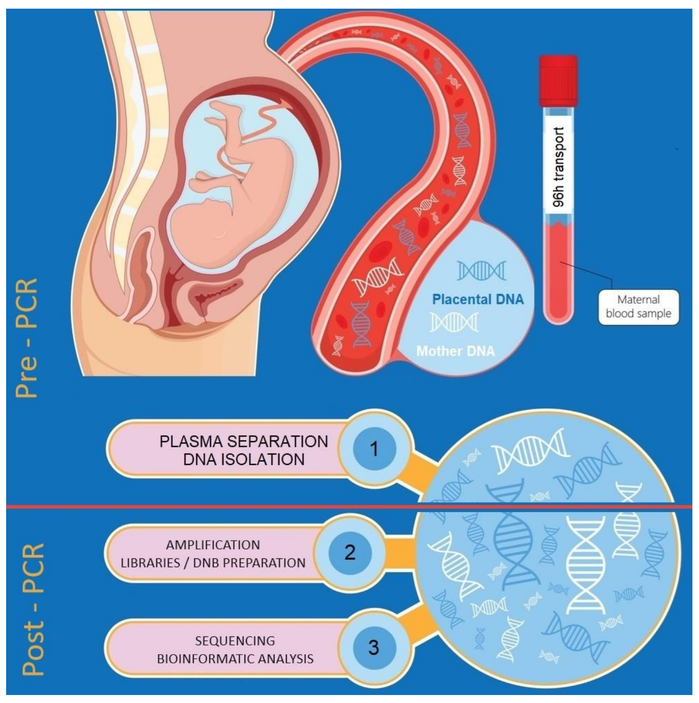

Figure 1. CfDNA fragments found in maternal blood originate from placenta. Pre-PCR and post-PCR processes in NGS NIPT laboratory are separated using a red line. Pre - PCR steps include sampling, plasma separation and DNA isolation. The second part of laboratory process includes post - PCR operations: DNA amplification in order to prepare sufficient amount of libraries and DNBs for sequencing.

Procedural quality controls and environmental monitoring

Each NGS analysis, conducted in a 96-well plate format, includes blanks, positive, and negative controls as indicated in **Figure 2**. All controls are treated as regular patient samples. While positive and negative controls are provided by the manufacturer, for blanks, instead of maternal plasma, fresh ultrapure water is pipetted into the well (**2**).

Positive and negative controls are artificial DNA with high-risk and low-risk results, respectively and have designated gender, characteristic graphs, as well as expected percent of fetal fraction.

According to the manufacturer, concentrations of the blanks should be under 0,7 ng/μL. If concentrations are elevated after several rounds of cleaning, an investigation of the contamination source is initiated. Blanks higher than 2,0 ng/μL are sequenced in order to determine the origin of DNA contamination.

Additionally, as a standard laboratory practice, each new reagent lot ordered from the manufacturer is tested upon arrival to check for potential factory defects that could affect the results.

Regular environmental monitoring, involving air and surface sampling every 1,5 months, was implemented. Any sampling area with concentration values above 0,7 ng/μL undergoes deep cleaning and is subsequently re-tested.

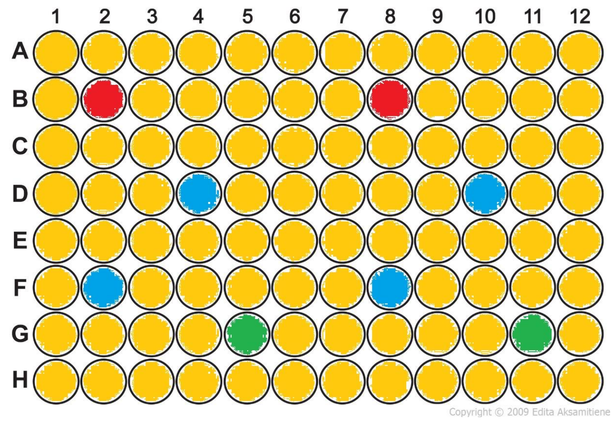

Figure 2. Schematic representation of 96 well plate with colour coded wells. Yellow represents wells filled with maternal plasma, blue wells are blanks, red wells are positive controls, green wells are negative controls. Equal types and numbers of controls are placed in the left part (columns 1-6) and right part (columns 7-12) of the plate. Controls are never placed in the outer wells or wells adjacent to other controls.

RESULTS

Blank controls and environment sampling

Increased concentrations of blanks (> 0,7 ng/μL) were observed during testing and with every subsequent use of LOT 1, even after repeated laboratory cleaning (**Figure 3**). Reagent LOT 2 was used intermittently and showed appropriate blank values, averaging 0,05 ng/μL. The investigation prompted by the laboratory amongother included analysis of environmental samples with the suspicious LOT 1, resulting in all values exceeding 0,7 ng/μL, further confirming the factory defect of the LOT 1. LOT 1 blanks with values over 2,0 ng/μL were traced to human DNA contamination after sequencing, leading to the immediate discontinuation of reagents by the manufacturer.

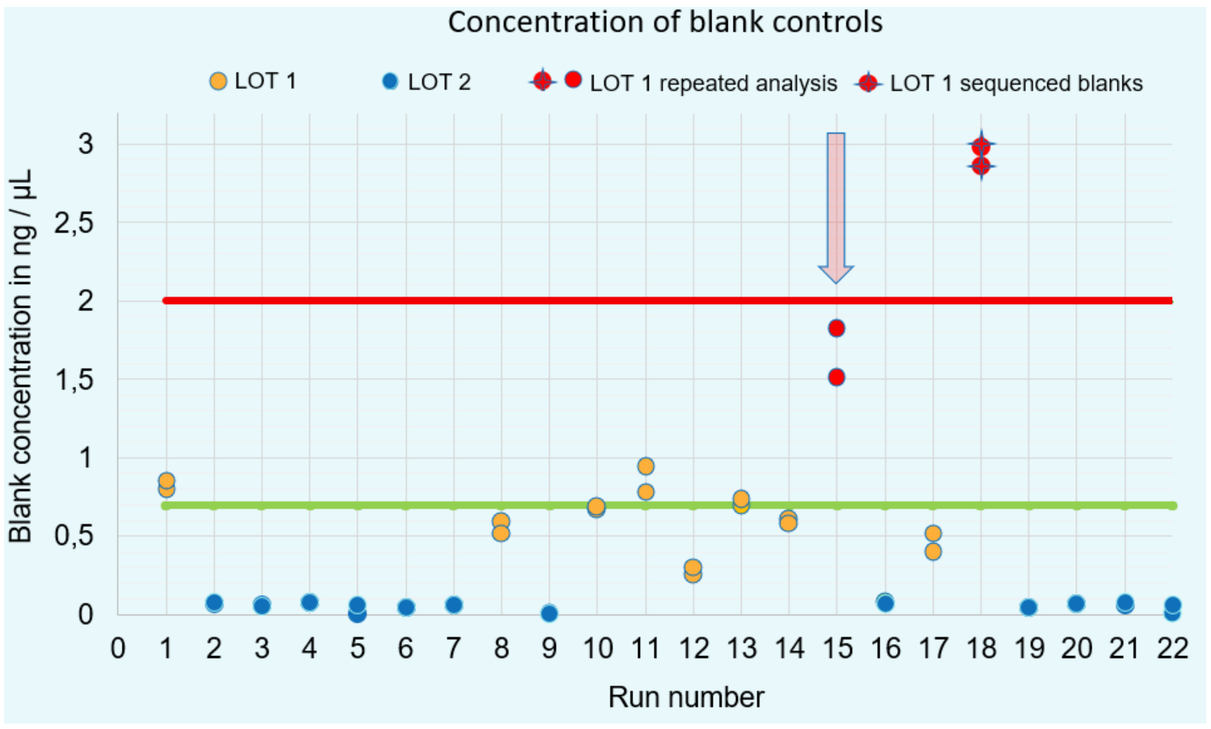

Figure 3. Concentrations of blank controls in ng/μL for 22 consecutive runs. Levels of two blanks per run for two different reagent lots are marked with color-coded dots according to the legend on the graph. Lines represent manufacturer-determined thresholds: "warning" at 0,7 ng/μL and "run reanalysis" at 2,0 ng/μL. Blanks marked with red stars were sequenced. Environmental samples present in run 15 are marked with a red arrow.

Positive controls

The laboratory noticed elevated values of fetal fraction for positive control PC 2 (**Figure 4**) and manufacturer was immediately notified. The laboratory was instructed to pause the use of PC 2 until the factory could perform an internal investigation. Subsequently, the laboratory was allowed to continue using the PC 2 reagent lot, as the manufacturer confirmed that higher fetal fraction of the positive control does not affect the results of patient samples. Furthermore, the laboratory was notified that an increase in average values of fetal fraction can be expected for each subsequent reagent lot due to changes in the production process.

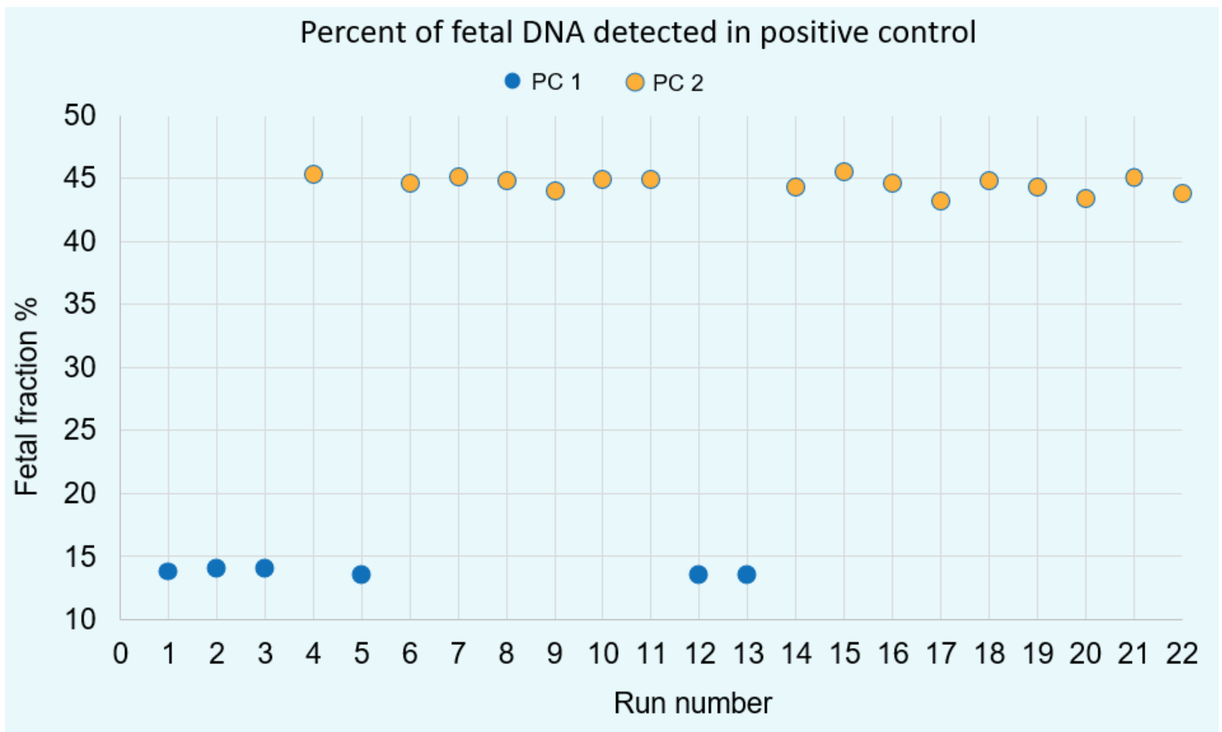

Figure 4. Positive control values in ng/μL for 22 consecutive runs. Two different reagent lots are marked with color-coded dots according to the legend on the graph.

CONCLUSIONS

Regular monitoring of environmental cleanliness and reagent quality enables prompt and informed decision-making, saving time and mitigating potential expenses.

LITERATURE

1 - Li et al, (2018) 'The application of NIPT using combinatorial probe-anchor synthesis to identify sex chromosomal aneuploidies (SCAs) in a cohort of 570 pregnancies', Molecular Cytogenetics, 11:59. DOI: 10.1016/j.cell.2018.08.016

2 - Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs). Licence: CC BY-NC-SA 3.0 IGO.

ABBREVIATIONS

cfDNA - cell-free DNA
DNA – DeoxyribouNucleic Acid
DNBs – DNA NanoBalls
NGS - Next Generation Sequencing
NIPT – Non-Invasive Prenatal Testing
PCR - Polymerase Chain Reaction
SOP – Standard Operating Procedure

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