Supplementary File 1

Serum 25(OH)D analysis

The quantitative determination of the serum 25(OH)D concentration was carried out on samples collected in red-top Vacutainer® tubes by a cadre of certified laboratory technicians. An Elecsys® vitamin D total assay (Roche Diagnostics, USA) based on an electrochemiluminescence binding immunoassay (ECLIA) was used. This method has been standardized against LC MS/MS, which in turn has been standardized to the US National Institute of Standards and Technology (NIST) standard (standard reference material SRM 2972). The test had a repeatability coefficient of variation (CV) ≤ 7.8%, a functional sensitivity of 4.01 ng/ml, and a CV of 18.5% [1].

The analysis was carried out on a Cobas® e 411 analyser by Roche Diagnostics (USA); the instrument underwent regular maintenance once a day and calibration two times a week. Quality control was performed by using PreciControl (PreciControl Vitamin D Total G2 Elecsys®); the control was run once every 24 hours when the test was in use or once per reagent kit following each calibration. The following batches of the reagent vitamin D total G3 Elecsys® were used: lot n. 71891503 (exp. date 28.02.2024), lot n. 71891505 (exp. date 29.02.2024), lot n. 71891507 (exp. date 29.02.2024), lot n. 74343101 (exp. date 31.05.2024) and lot n. 74343105 (exp. date 31.05.2024). The analyser automatically calculated the analyte concentration of each sample, in ng/ml, with a measuring range of 3.00 70.0 ng/ml. Maternal serum 25(OH)D status was classified according to the cut-off values reported in the Operational Definitions.

1. Roche Diagnostics - *Elecsys Vitamin D total.* 2022 [Internet, cited 2024 May 26]. Available from: https://elabdoc-prod.roche.com/eLD/api/downloads/24f313f7-be72-ec11-0d91-005056a772fd?countryIsoCode=pi