Letter to the Editor

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Recent decline in patient serum folate test levels using Roche Diagnostics Folate III assay

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To the Editor,

Folate deficiency is clinically significant, potentially leading to megaloblastic anemia and neuropathies. Serum folate testing remains a frequently ordered laboratory investigation in Sweden and other European countries where mandatory folic acid fortification is not in place. In non-fortified settings like Sweden, substantial subgroups (e.g., the elderly, malabsorptive conditions, and patients with anemia, pregnancy, or anticonvulsant medications) are at increased risk of folate deficiency, with prevalence estimates between 8 and 25 %. Therefore, appropriate serum folate testing is warranted in individuals with related clinical signs or risk factors [1]. In contrast, in North America and other regions that have implemented folic acid fortification of grain products, the utility of serum folate testing has been questioned due to its low cost-effectiveness – given the marked decline in folate deficiency prevalence to 0.1–1% [1,2].

The Elecsys® Folate III assay from Roche Diagnostics is widely used in clinical laboratories. In 2016, the assay was restandardized against the WHO International Standard (NIBSC code 03/178), resulting in significantly lower patient results [3]. According to the manufacturer, the restandardization could produce a reduction of approximately −20 %, and up to −50 % at the lower end of the measuring range. Several authors have reported even lower recoveries and argued that the revised 2.5th percentile of Roche's European population-based reference interval – from 10.4 nmol/L (4.6 µg/L (conversion factor 1 nmol/L=0.44 µg/L)) to 8.83 nmol/L (3.89 µg/L) – was insufficient to account for the decreased results obtained with the restandardized Folate III assay [4,5]. A later study also found a −8.3 % negative bias compared to the WHO International Standard 03/178 (assigned value: 12.1 nmol/L), despite the manufacturer's claim of traceability to this standard [6].

The Folate III assay is used on Cobas e 801 instrument modules at both our university hospital laboratories – serving populations of approximately 2.5 million in Stockholm County and 0.4 million in Uppsala County. In October 2023, Roche Diagnostics introduced a new version of the Folate III assay with enhanced biotin tolerance, designed to reduce interference from biotin present in some patient samples. Many immunoassays rely on the strong binding affinity between biotin and streptavidin as part of their analytical design. However, if a patient's blood contains excess free biotin – due to supplements or high-dose therapy – it can interfere by competing with the assay's biotinylated reagents, potentially leading to erroneous results. Manufacturers increase biotin tolerance of their immunoassays to ensure result reliability and maintain clinical confidence in their diagnostic products [7]. Prior to implementation, we evaluated the updated reagent using surplus patient samples across the measurement range to assess any bias relative to the existing version. While no significant bias was detected in Stockholm, the Uppsala laboratory observed a slight to moderate negative bias averaging −9 %. Other clinical laboratories in Sweden have also reported similar findings during verification of the updated assay. The decline was also evident in the Swedish EQA scheme for folate (Equalis.se). Roche has been informed about these findings.

Implementation of the new Folate III version occurred at slightly different times: March–April 2024 in Stockholm and October 2024 in Uppsala. Shortly after transitioning to the new version, both laboratories began receiving inquiries from clinicians about unexpectedly low patient results, with an apparent increase in values falling below the lower reference limit (LRL). This prompted us to investigate trends

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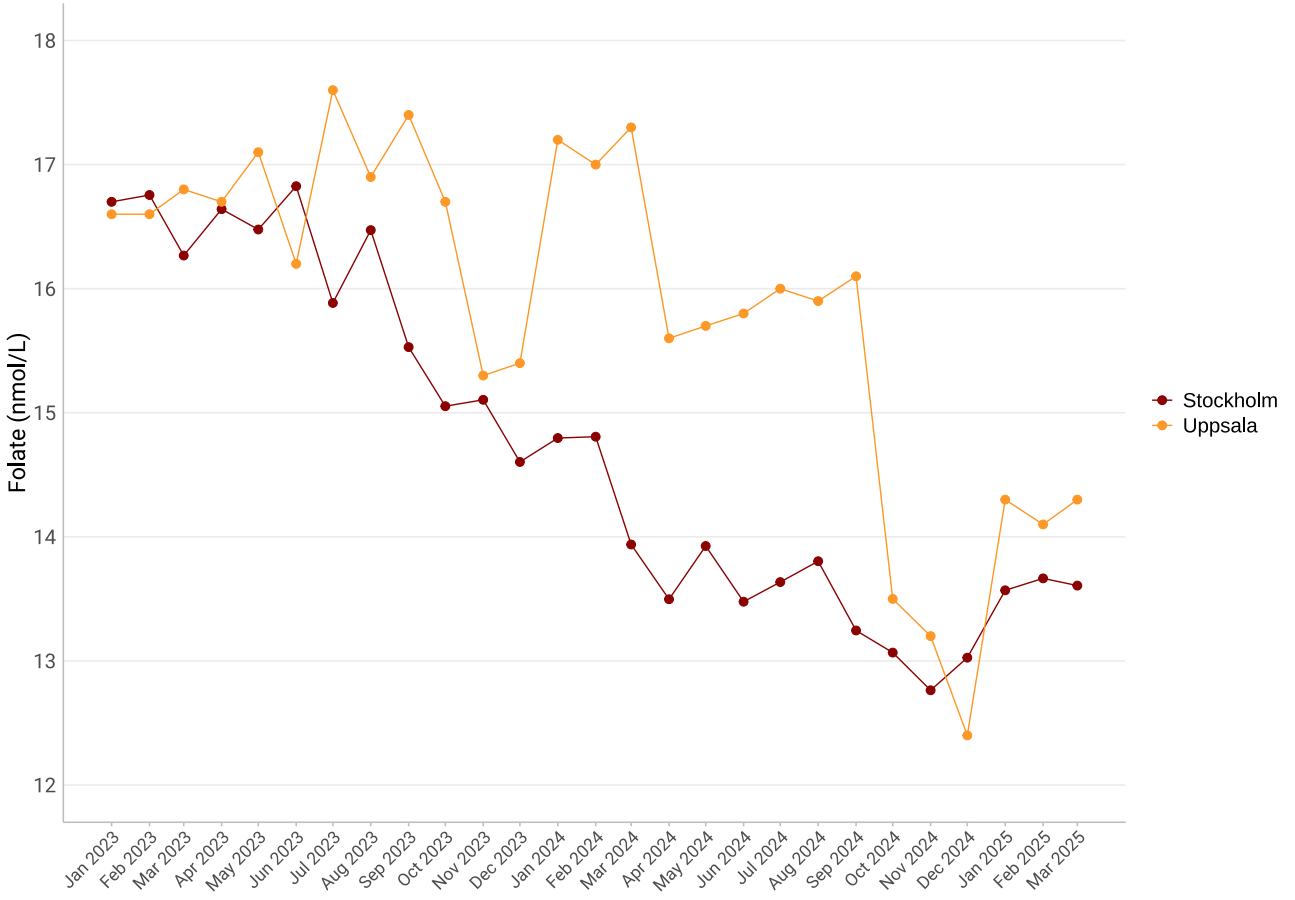

Figure 1: Monthly median patient folate values for Region Stockholm and Region Uppsala from January 2023 to March 2025.

in patient median folate levels. Extracting patient medians from the Laboratory Information System (LIS) provides a robust tool for monitoring laboratory methods and offers a clinically meaningful complement to conventional quality control (QC) procedures. Unlike synthetic QC materials, patient medians reflect real-world sample matrices and biological variability [8]. Medians are preferred over means as they are less affected by outliers and skewed distributions.

At the start of 2023, both laboratories observed similar patient median folate values of approximately 16.6 nmol/L (Figure 1). From late 2023, a gradual decline in median values was observed in Stockholm, while Uppsala showed a more marked decrease beginning in late 2024 (Figure 1), possibly reflecting the later assay transition. By October 2024, both laboratories reported similar medians of approximately 13.5 nmol/L. Compared to early 2023, this represents a 19 % decline in patient median folate values – exceeding the –9 % negative bias observed during our verification of the updated assay. This suggests that, in addition to increased biotin tolerance, a further downward shift in assay calibration may have occurred.

Over the two-year observation period, the proportion of patient results falling below the current LRL of 7.0 nmol/L rose significantly – from approximately 4 % to nearly 15 %. If Roche's suggested LRL of 8.8 nmol/L had been used, the increase would have been from about 11 % to roughly 25 %. This trend should not be interpreted as an actual increase in folate deficiency prevalence; rather, it highlights how sensitive prevalence estimates are to both the chosen LRL and calibration shifts.

A discrepancy between the Folate III assay calibration and the manufacturer's LRL was previously noted after the 2016 restandardization [4]. Our current data indicate that this discrepancy has grown more pronounced, leading to an increasing number of patients being classified as folate deficient. The manufacturer should either recalibrate the assay to improve recovery or adjust the LRL downward. It is important that the method is calibrated according to the WHO International Standard. In the absence of any such measures, laboratories may themselves need to consider adjusting either their own test results or their reference intervals.

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