Application Note:
TSH Analysis Using Capitainer®B50

Purpose

This document provides a protocol for laboratories to implement thyroid-stimulating hormone (TSH) analysis from capillary blood collected via the Capitainer®B50 microsampling card. The application is based on a clinical validation that is currently under scientific review for publication.

TSH is the primary biomarker used to diagnose and monitor hypothyroidism. Elevated TSH levels indicate underactive thyroid function, while suppressed levels suggest hyperthyroidism.

The method described enables decentralized, home-based sampling with high analytical precision and strong agreement with traditional venous blood testing through the use of Capitainer®B50 samples for the test.

Capitainer®B50 is a microfluidic card designed to enable at-home self-sampling that meters exactly 50 µL of whole blood from a simple finger prick.

The sample is dried and mailed to a certified lab for analysis — no refrigeration, no clinic visit, no hassle.

Advantages

- Home self-sampling enabled
- Room temperature stability
- Postal logistics in ambient temperature
- Analysis on standard
- High correlation with venous samples ($R^2 \approx 0.97$)

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Overview of the Method

Analyte: Thyroid-Stimulating Hormone (TSH)

Sample Type: Capillary whole blood (50 µL) via microsampling card Capitainer®B50

Analyzer Platforms: Roche cobas® e411 or e801 immunoassay systems

Required equipment: PBS buffer, Hitachi cups and orbital shaker table

Laboratory Analysis Protocol

This section describes the workflow when a Capitainer®B50 cards is received in the laboratory. In order to analyse the samples, the dried matrix needs first to be transferred into tubes or plate wells and thereafter reconstituted back into liquid form again.

Sample Card Handling:

To begin the reconstitution process, transfer the Capitainer® sample disc—containing the volumetric dried blood sample—into a suitable tube or plate well.

Accessing the Sample Disc

- Remove the protective tabs on the back of the Capitainer®B50 card to expose the sample discs.
- Only one of the two sample discs on the card is required for analysis.

Transfer Methods

- Manual Transfer: Use tweezers or the MH1t manual card handler for precise handling.
- Automated Transfer: For high-throughput workflows, consider automated solutions and 96-well plate formats to streamline processing.

Proper handling of the sample disc is essential to ensure accurate reconstitution and downstream analysis.

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Reconstitution:

The following protocol and considerations were taken when eluting the dried samples back into liquid form:

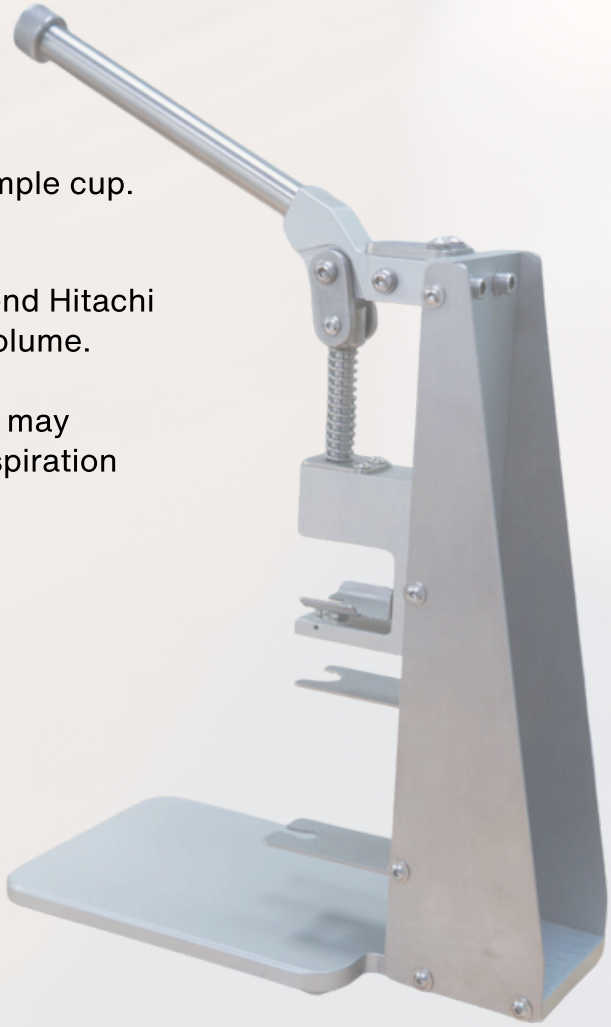
1. Buffer Addition & Incubation
- Add 300 µL of PBS buffer to each tube containing a 6 mm sample disc.
- Place the tubes on an orbital shaker and shake vigorously for 60 minutes at room temperature.
- This incubation step is essential for consistent and reproducible elution.

2. Tube Format Recommendations
- Use a tube or well wider than the sample disc to ensure proper mixing of the disc and buffer.
- Narrow wells, such as those in plate formats, may require more vigorous shaking to achieve efficient elution.

3. Post-Incubation Handling
- After incubation, either:
- Remove the sample disc from the tube, or
- Aspirate the eluate and transfer it to a new sample cup.

4. Instrument Compatibility
- For use with cobas® instruments, we recommend Hitachi cups from Roche, which help minimize dead volume.

Important: If the sample disc remains in the cup, it may obstruct the sample needle, potentially causing aspiration errors.



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Analysis:

After reconstitution, analyze the eluate using the standard cobas® e411 or e801 application for TSH. Extract the results directly from the analyzer.

Note: TSH values obtained from Capitainer®B50 eluates will be significantly lower than those from corresponding venous serum samples from the same individual. This difference is expected and can be explained by the following factors:

Key Factors Affecting TSH Values

1

Measurement Basis

- The unit reflects TSH per volume of Capitainer®B50 eluate, not per volume of serum.

2

Dilution Effect

- The reconstitution involves eluting 50 µL of dried blood in 300 µL of PBS, resulting in a 1:5 dilution.
- Approximately 50 µL of PBS rehydrates the dried blood, while the remaining 250 µL contributes to dilution.

3

Serum vs. Whole Blood

- TSH is traditionally measured in serum, which is only a fraction of whole blood.
- The haematocrit level (cellular portion) varies between individuals but typically accounts for just under 50% of total blood volume.

4

Elution Efficiency

- The recovery of TSH from the paper matrix is not 100%, but the process is highly consistent across sample discs and runs.

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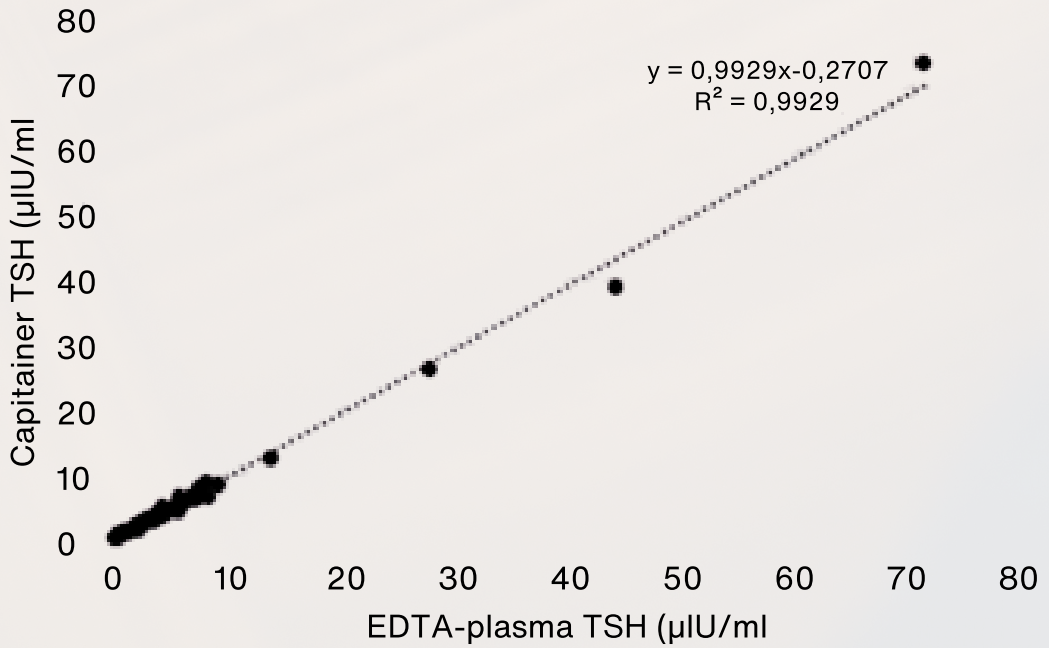
Method validation and Sample correction:

The described method utilizes a pre-defined cobas® serum assay, which reports results based on a calibration curve established for serum samples. The cobas® system does not support alternative calibration curves or adjustments for different sample matrices.

Clinical reference ranges for TSH (Thyroid-Stimulating Hormone) are defined in terms of concentration per volume of serum (mIE/L). To ensure medical relevance, results obtained from Capitainer®B50 eluates must be converted to equivalent serum concentrations.

In this study, a correction factor of 21.65 was determined using a separate dataset and applied to 73 validation samples. This factor adjusts the TSH values obtained from Capitainer®B50 eluates to align with serum-based reference ranges.
Important: The correction factor must be established individually for each laboratory and analytical platform.

While following the described protocol may yield similar correction factors, the exact value may vary depending on instrumentation and workflow.



In the analytical validation an excellent correlation (R2=0.99) was seen in 69 venous patient samples when analyzed from tubes versus put on Capitainer®B50 cards. The Total imprecision for 100 duplicates in the full measure range was 7.27%.

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Analytical Performance with capillary samples

The described method was evaluated in a clinical setting under conditions similar to those used during method validation.

Study Design

A total of 73 patients from primary care centers in Sweden participated in the study. At the time of their hospital visit, each patient provided:

- A Capitainer®B50 sample via fingerprick
- A venepuncture sample
- Both samples were collected simultaneously to ensure comparability.

Sample Processing

- All samples were analyzed in duplicate.
- The coefficient of variation (CV%) between duplicates was low, with a median CV% of 2.2% and an interquartile range (IQR) of 0–4.4%.
- No individual sample showed a CV% exceeding 20%.

Performance Observations

- Lower TSH concentrations exhibited slightly higher variability compared to higher concentrations.
- The method demonstrated strong performance within the 2–10 mIE/L serum range, with consistently low CV%.
- For TSH values below 2 mIE/L, interpretation should be made with caution due to elevated assay variability.

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Sample stability

The Capitainer® solution is designed to support at-home blood sampling, with samples shipped via standard mail at ambient temperature back to the laboratory.

To ensure reliable results, it is important to assess how time and temperature may affect the stability of the target analyte in dried blood samples.

In general, proteins are well preserved in dried blood due to the absence of enzymatic activity that would otherwise degrade them in liquid form. However, stability can vary between different analytes, and each molecule may exhibit unique degradation patterns over time or under varying temperature conditions.

Note: Stability testing should always be performed for each analyte to confirm robustness under expected transport and storage conditions.
In this study two stability studies were performed:

- Room temperature stability up to 10 days
- Summer/winter scenario according to U.S. Food and Drug Administration (FDA) templates.

Winter profile

Temperature	Cycle Period	Cycle Period Hours	Total Time Hours
-10°C	1	8	8
-10°C	2	4	12
-10°C	3	2	14
-10°C	4	36	50
-10°C	5	6	56

Summer profile

Temperature	Cycle Period	Cycle Period Hours	Total Time Hours
40°C	1	8	8
22°C	2	4	12
40°C	3	2	14
30°C	4	36	50
40°C	5	6	56

No significant degradation of TSH was observed after 56 hours under simulated cold and hot conditions.

In long-term storage tests, a slight decrease in signal (up to 10%) was noted between day 0 and day 6. After day 6, the signal remained stable, with no further decline observed up to day 10.

These findings indicate that non-temperature-controlled shipment conditions—such as standard postal services—are suitable for TSH testing using Capitainer® samples. A 10-day window covers most postal delivery lead times, supporting the robustness of the method under typical ambient transport conditions.

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Clinical Applications

The above-described methods and results from a clinical validation supports that this method is ideal for at-home sampling offerings for TSH measurements and could facilitate:

- Long-term monitoring of hypothyroidism
- Elderly or immobile patients
- Population screening programs for undiagnosed hypothyroidism
- Telemedicine solutions to enable a TSH value with remote consultations
- Decentralized care models

Limitation: Based on the increased CV in lower levels and the reduced dynamic detection range in lower levels, the method is not suitable for diagnosing or monitoring hyperthyroidism.

Summary

A simple pre-analytical protocol was applied

- Add 300µL PBS to one Capitainer®B50 sample disc
- Incubate for one hour under strong agitation on orbital shaker
- Use eluate on cobas TSH assay
- Adjust results according to established correction factor

Excellent agreement between whole blood hemolysate and venous serum TSH measurements with a strong correlation.

Correction of raw results is needed to transform values back to corresponding serum levels and laboratory and instrument dependent correction factors should be established in each laboratory.

The diluted hemolysate has reduced sensitivity and higher lowest limit of detection compared to venous serum, thereby not suitable for hyperthyroidism diagnosis but excellent for all applications within the area of hypothyroidism.