Application Note:
HbA1c Testing Using Capitainer®

Purpose

This document presents a comprehensive overview of both published and unpublished evidence, along with protocols for testing HbA1c using Capitainer sampling cards. It outlines how the testing process can be implemented across various standard clinical chemistry platforms and highlights key considerations and precautions that must be taken into account.

HbA1c, also referred to as Hemoglobin A1c or glycated hemoglobin, is a diagnostic blood test that reflects an individual's average blood glucose levels over the preceding two to three months. As glucose circulates in the bloodstream, it binds to hemoglobin within red blood cells. The HbA1c test quantifies the proportion of hemoglobin molecules that have glucose attached. Given that red blood cells have a lifespan of approximately three months, this test provides a reliable long-term indicator of blood sugar control.

HbA1c test is used for:

Diagnosis: Used to detect prediabetes, type 2 diabetes and one test in type 1 diagnosis.
Monitoring: Helps assess how well a person with diabetes is managing their condition.
Treatment Decisions: Guides adjustments in medication, diet, and lifestyle.

HbA1c can be reported in two different units, percentage glycated Hb (%) or as mmol/mol were 6.5% HbA1c ≈ 48 mmol/mol. The data in this application note is using mmol/mol.

Capitainer®B10 is a microfluidic card designed to enable at-home self-sampling that meters exactly 10 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.

HbA1c can be tested from both Capitainer®B10 and Capitainer®B50.

Rev. date 2026-01-27

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Overview of the Method

Detection Principles for HbA1c Testing in Central Laboratories
Currently, four main analytical principles are commonly used for HbA1c testing in central laboratory settings:

1. **Immunoturbidimetric Assays**
   In this application note represented by the Roche Tina-Quant method.

2. **Enzymatic Assays**
   Represented here by the Abbott Alinity platform.

3. **High-Performance Liquid Chromatography (HPLC)**
   This note highlights the Tosoh G8 HPLC Analyzer.

4. **Capillary Electrophoresis**
   Illustrated by the Capillarys 3 TERA system from Sebia.

Other detection technologies and point-of-care solutions fall outside the scope of this application note and are therefore not covered.

Assay and instrument considerations:

Hemoglobin is contained within red blood cells, which must be lysed to release hemoglobin for accurate HbA1c quantification. In liquid blood samples, this is typically achieved using a hemolyzing reagent that disrupts the cell membrane while simultaneously diluting the sample to a concentration suitable for analysis. As a result, most HbA1c assays include a pre-analytical step involving treatment with a specific hemolyzing buffer.

In dried blood samples—such as those collected using the Capitainer device—red blood cells undergo lysis during the drying process. However, the use of the recommended hemolyzing reagent remains essential, as it is a critical component of the assay protocol and may also support the measurement of additional analytes. Depending on the analytical platform, the hemolysis step may be performed onboard (automatically for liquid blood tubes) or externally.

For dried blood spot samples, external hemolysis during the reconstitution phase is preferred. This approach improves matrix compatibility and enhances overall assay performance.

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Laboratory Workflow for Capitainer® Samples

This section outlines the workflow followed when a Capitainer® sampling card is received in the laboratory. To enable analysis, the dried blood matrix must first be transferred into tubes or plate wells. It is then reconstituted into liquid form, as all current HbA1c detection systems are designed for liquid sample analysis.

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®B 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 like PH96 or our PA496 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 of dried samples:

In a study published by Rollborn et al. (2024) [1], various elution parameters were evaluated to determine the most effective protocol for releasing analytes from the paper filter disc in Capitainer®B10 cards. The investigation assessed different durations and modes of agitation, as well as the impact of various buffer solutions. Key findings from the study include:

1. **Passive elution without agitation results in poor recovery.**

2. **Agitation intensity (e.g., vortexing) has a greater impact on elution efficiency than extended incubation time.**

3. **The Roche Tina-Quant hemolysing reagent alone is suboptimal for reconstituting dried blood samples.**

4. **Optimal elution was achieved using a brief pre-incubation with PBS, followed by the addition of the Tina-Quant hemolysing reagent.**

Further testing demonstrated that efficient elution with PBS is critical for achieving high reproducibility. In one experiment, 30 EDTA whole blood samples with HbA1c values ranging from 28 to 109 mmol/mol were applied to Capitainer®B10 cards. After a 10-minute PBS elution step, the samples showed a coefficient of variation (CV%) of 3.56%, compared to 1.33% for liquid blood samples analyzed on the cobas® 6000 platform.

In a second experiment involving 39 samples with HbA1c values ranging from 25 to 108 mmol/mol, liquid blood samples showed a CV% of 1.51%, while Capitainer®B10 samples yielded a CV% of 1.58%. Extending the PBS pre-incubation from 10 to 30 minutes significantly improved result stability, producing values comparable to those from liquid samples.

The original Roche Tina-Quant protocol recommends adding 10 µL of whole blood to 990 µL of hemolysing reagent. In the adapted Capitainer®B10 protocol, which collects exactly 10 µL of blood, this was modified to 100 µL of PBS followed by 900 µL of hemolysing reagent—resulting in optimal performance.

When using the Capitainer®B50 card, a pre-elution step with 500 µL of PBS is recommended. From this eluate, 100 µL can be combined with 900 µL of hemolysing reagent to create a comparable sample matrix. This approach also preserves additional 400 µl of eluate for use in other assays if a panel including HbA1c is desired.

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Analysis

Turbidimetric assays

Roche cobas® TinaQuant

As described in the previous section, the protocol involving a 30-minute PBS incubation demonstrated excellent reproducibility between the two sample discs when Capitainer®B10 samples were analyzed one day after blood application. Furthermore, the correlation between results from liquid blood samples (processed using the standard Roche Tina-Quant protocol) and those from Capitainer®B10 samples—eluted using the optimized protocol—was very strong, with an R^2 value exceeding 0.99.

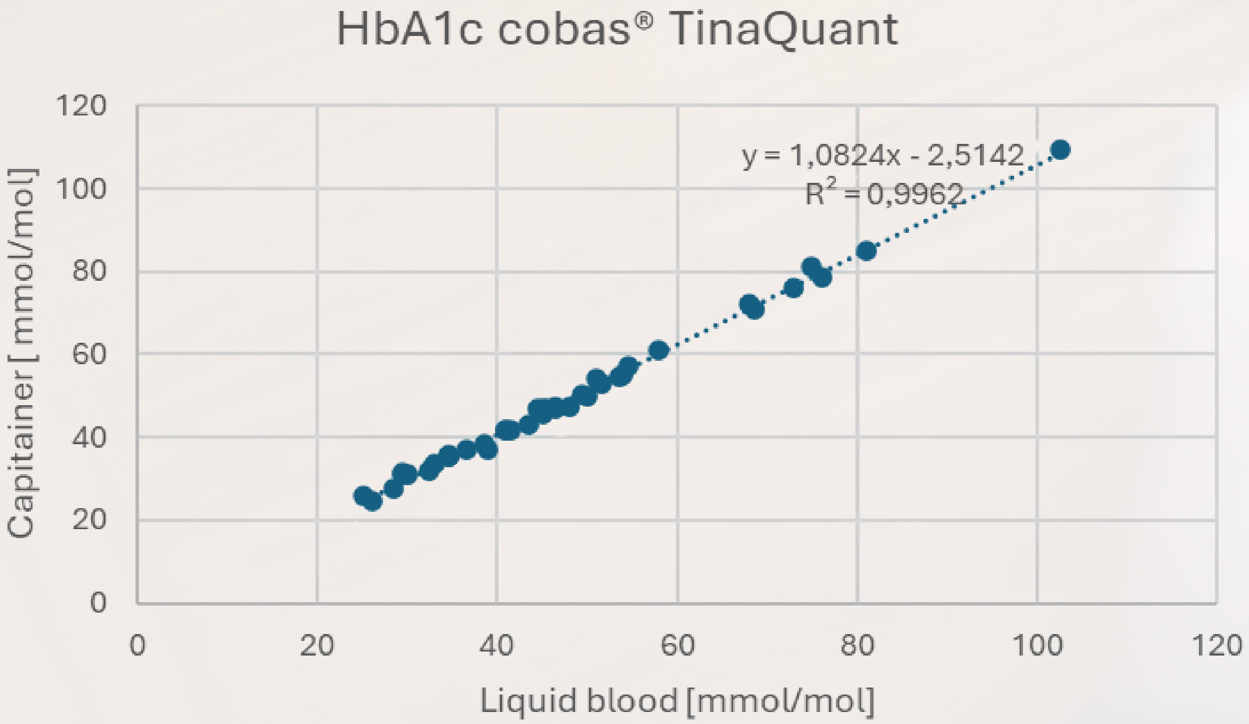

Figure 1: Correlation of XX venous blood samples analyzed from EDTA tubes versus as eluate one day post application to Capitainer®B10. The eluates were analyzed with the hemolysate application and the liquid blood with the whole blood application. 6.5% HbA1c ≈ 48 mmol/mol

In conclusion, the bias observed with this enzymatic protocol is comparable in magnitude to that seen with turbidimetric assays—but negative rather than positive. As discussed later, HbA1c levels tend to increase during storage in dried format. Therefore, a small negative bias observed on day 1 under controlled lab conditions may be offset in at-home sampling scenarios, where samples are shipped and analyzed after a longer delay.

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Enzymatic assays

Unlike cobas® and Indiko, the Abbott Alinity platform uses a different assay chemistry and blood-to-diluent ratio in its manual hemolysis protocol. The recommended procedure involves mixing 10 µL of whole blood with 222 µL of A1c diluent.

Replacing part of the A1c diluent with PBS was found to introduce a strong negative bias, with the bias increasing proportionally to the amount of PBS used. However, using only A1c diluent also failed to achieve effective elution.

An evaluation of 109 EDTA samples (HbA1c range: 27-109 mmol/mol) identified an optimal protocol for Capitainer®B10 samples: 50 µL PBS incubation for 30 minutes, followed by 182 µL of A1c diluent with another 30 minutes incubation. This approach yielded a CV% of 0.58% between the two sample discs, but introduced a negative bias of -1.59 mmol/mol. Increasing the PBS volume to 100 µL, followed by 132 µL of A1c diluent, resulted in a stronger negative bias of -2.40 mmol/mol.

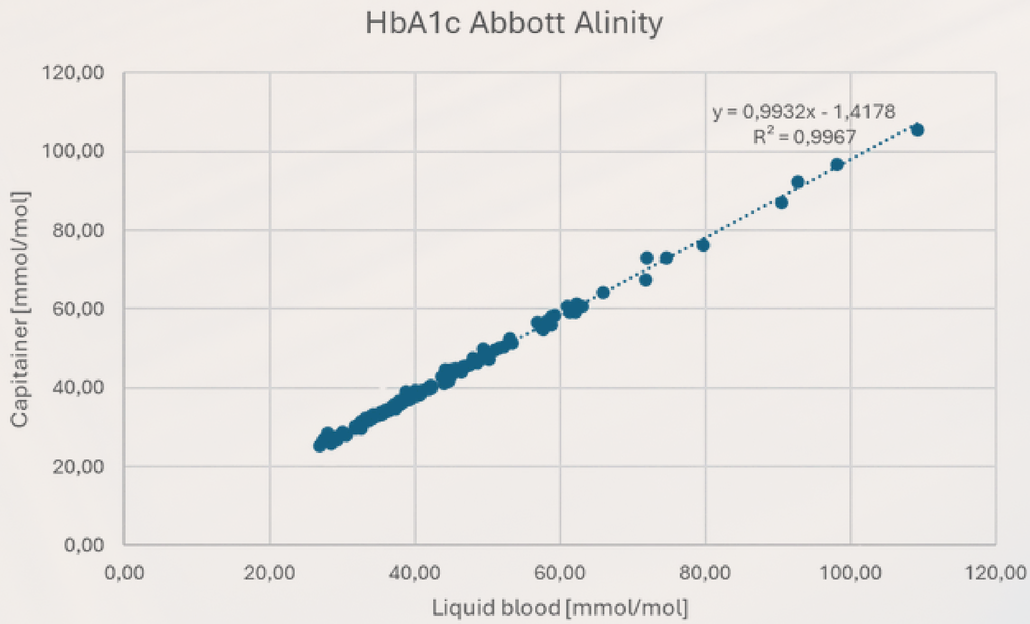

Figure 2: Correlation of 109 venous blood samples analyzed from EDTA tubes versus as eluate one day post application to Capitainer®B10. The eluates were analyzed with the hemolysate application and the liquid blood with the whole blood application. 6.5% HbA1c ≈ 48 mmol/mol

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HPLC & Capillary Electrophoresis methods

The Tosoh G11 platform delivers highly consistent results between fresh liquid samples and those collected using Capitainer®B10 cards eluted in 2 mL 1xPBS under agitation for 30 minutes. As indicated by the m-value in the graph below, a consistent positive bias is observed with this method.

A practical drawback, however, is that the instrument frequently generates alarms related to extra peaks—occurring in over 50% of measurements. While these alarms do not appear to affect analytical accuracy, they can present challenges in routine daily use by requiring additional review or manual intervention.

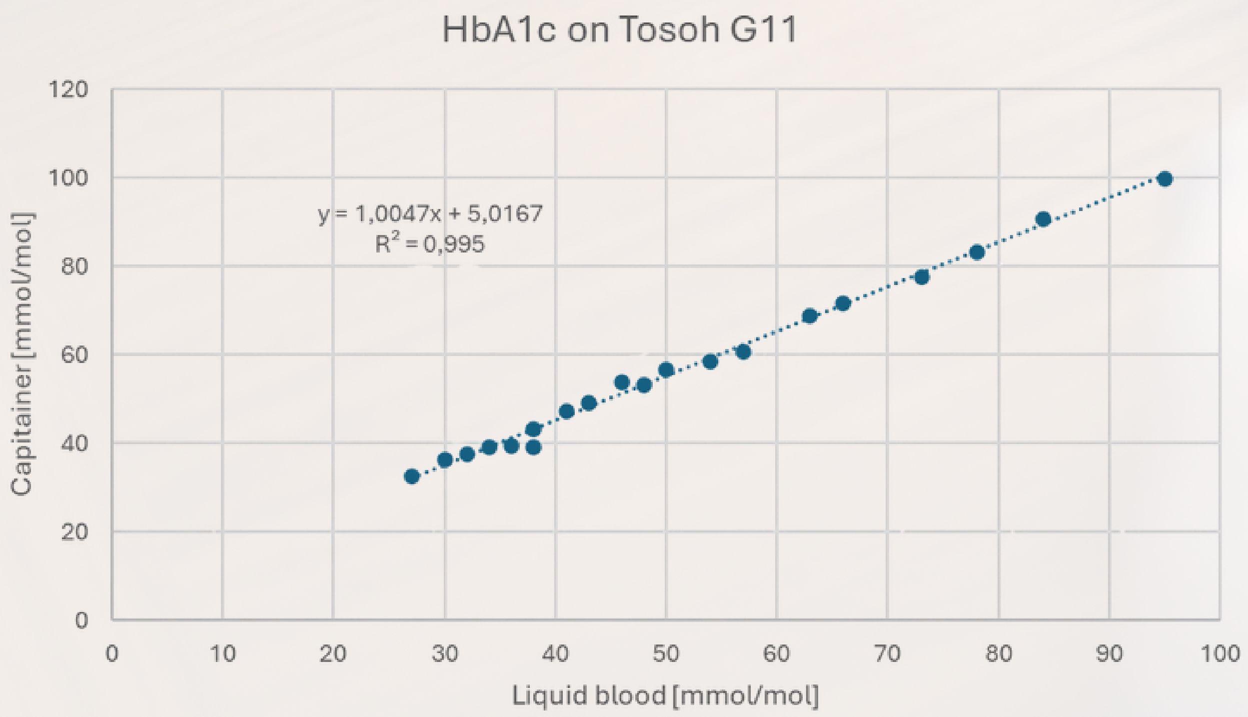

Figure 3: 20 EDTA samples were analyzed with standard protocol and as a eluate from Capitainer®B on Tosoh Automated Glycohemoglobin Analyzer. Samples were eluted 1 day post application to the Capitainer®B10 cards.
6.5% HbA1c ≈ 48 mmol/mol

Capillary electrophoresis has proven more challenging due to altered peak patterns, which primarily cause software-related calculation issues. These changes are likely similar to those that trigger alarms in HPLC systems, but in this case, they also compromise result consistency.

At present, instruments such as the Sebia Capillarys 3 cannot be recommended for HbA1c testing in combination with Capitainer®B10, due to these limitations.

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Usability & Analytical Stability

The Capitainer® system is designed for at-home blood sampling, with dried samples returned to the laboratory via standard mail at ambient temperature. To ensure reliable results, it is essential to evaluate how time and temperature affect analyte stability in dried blood.

In general, proteins are well preserved in dried format due to the absence of enzymatic activity found in liquid samples. However, stability varies between analytes, and each may degrade differently over time or under varying conditions. Note: Stability testing should be performed for each analyte to confirm robustness under expected transport and storage conditions.

An external validation study conducted by SKUP [2] assessed HbA1c testing with Capitainer®B10 using the Roche cobas® method. The evaluation focused on three key criteria:

1. **User-friendliness**
2. **Repeatability**
3. **Accuracy**

In total, 207 individuals in Swedish primary care self-sampled with Capitainer®B10 during their clinic visit for venous blood draw. Venous samples were transported via standard liquid sample logistics, while Capitainer®B10 cards were mailed in envelopes via regular postal service to the hospital laboratory in Östersund. The study was conducted in two separate rounds.

User-friendliness

SKUP assessed user-friendliness by having participants who used Capitainer®B10 for self-sampling complete a questionnaire. Two biomedical laboratory scientists also evaluated the process from a laboratory perspective. Respondents rated each category as Satisfactory, Intermediate, or Unsatisfactory, with an option for No opinion.

- Over 80% of lay users rated Satisfactory in 6 out of 7 usability categories.
- The Instructions for Use (IFU) were overwhelmingly rated as Satisfactory or No opinion, with only 2 out of 324 responses marked as Intermediate and none as Unsatisfactory.

For a detailed breakdown of the usability evaluation, we recommend consulting the full SKUP report [2].

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Repeatability

Repeatability was assessed by calculating the coefficient of variation (CV%) between the two sample discs on each Capitainer®B10 card. Since the card has two inlets, each disc technically represents a separate sample, though both originate from the same blood drop via a single incision.

Repeatability was summarized across three HbA1c concentration levels using the optimized protocol.

Mean HbA1c values (mmol/mol) were used as the basis for comparison characterized into three groups.

Mean value HbA1c (mmol/mol)	CV% (90% CI)	n*
36,2	1,3 (1,0-2,1)	11
46	0,8 (0,6-0,9)	39
69,6	1,0 (0,7-1,7)	8

Table 1: Repeatability results based on the cohort of lay persons performing self-sampling with Capitainer®B10

Accuracy and sample stability

In the first evaluation cohort, samples were analyzed up to 14 days after collection. A positive bias of 8.4 - 12.2% was observed when comparing Capitainer®B10 samples to liquid blood. When bias was broken down by time to analysis, a clear time-dependent trend emerged: bias remained low during days 1–5, but steadily increased from day 6 to 14.

To address this, a second cohort was included with the requirement that all samples be analyzed within 5 days of collection.

Using a quality goal of:

- ±3.0 mmol/mol for HbA1c < 35.3 mmol/mol
- ±8.5% for HbA1c ≥ 35.3 mmol/mol

74% of samples met the criteria when analyzed within 14 days. When the analysis window was reduced to 5 days, accuracy improved to 89%. Notably, applying a correction factor for bias would have resulted in >95% of samples meeting the quality goals.

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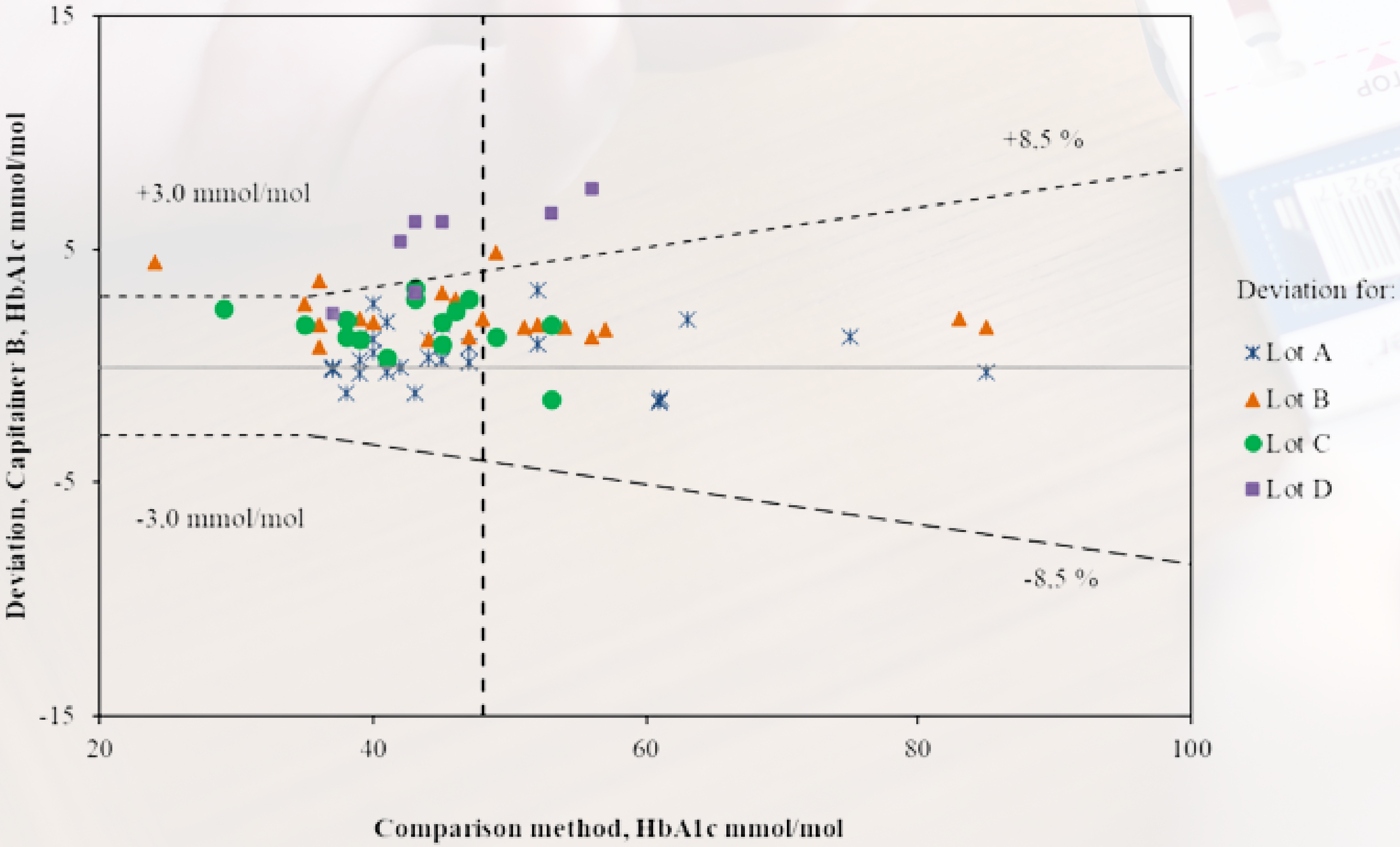

Figure 4: Accuracy of HbA1c results from Capitainer B samples when the samples were collected by lay persons in the second evaluation. The x-axis represents the HbA1c result of the comparison method. The y-axis represents the HbA1c deviation of the first Capitainer B sample measurement from the result of the corresponding sample of the comparison method. The vertical line at 48 mmol/mol HbA1c illustrates the diagnostic threshold value for diabetes. The different lots of Capitainer B are illustrated with the symbols ж (Lot a),▲ (Lot b), ● (Lot c) and ■ (Lot d). Stippled lines represent the allowable deviation limits according to the APS (within ±3,0 mmol/mol of the results of the comparison method for HbA1c concentrations <35,3 mmol/mol and within ±8,5 % for HbA1c concentrations ≥35,3 mmol/mol). Number of results (n) = 70.

The graph and the accompanying descriptive text are reprinted from the report by SKUP [2]. Published with permission.

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Conclusions

The methods and results presented support the use of Capitainer® for reliable HbA1c testing. However, certain factors must be considered to ensure accuracy:

1. **Shipping time and conditions**
2. **Choice of analytical platform**
3. **Management of bias in dried blood samples**

The observation of a positive bias on dried blood is not unique to Capitainer and has been published before. Studies using Bio-Rad D100 HPLC method has shown a positive bias and also changes in baseline levels [3] and for immunoturbidimetric methods this has been reported but also manageable with correction in the laboratory [4]. Further, there are indications that elevated temperatures during storage further can increase the positive bias. We conclude that a consistent bias can be handled by applying a correction factor in the laboratory.

A patient-centric sampling solution like Capitainer® opens new possibilities in diabetes care, including:

- Cost-effective and user-friendly screening for undiagnosed diabetes
- At-home sampling for long-term glucose monitoring, complementing continuous glucose meters
- Remote follow-up through telemedicine
- Support for decentralized care models

HbA1c is a key marker in diabetes diagnosis and monitoring, with standalone clinical value.

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Summary

This application note demonstrates that Capitainer®B10 offers a user-friendly and reliable alternative to venous blood draws for HbA1c testing. It enables enhanced diabetes monitoring and supports scalable screening initiatives.

Key considerations when analyzing HbA1c from dried blood samples include:

- **High concordance between venous and capillary blood results**
- **Excellent reproducibility between sample discs**
- **Consistent positive bias in dried blood vs. fresh blood—laboratories should apply a correction factor**
- **Timely analysis is critical; samples should ideally be processed within 5 days of collection**
- **Immunoturbidimetric and enzymatic assays show the best compatibility with Capitainer®B10**

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References

1.Rollborn, N., Larsson, A., & Kultima, K. (2024). Analysis of HbA1c using microfluidic card (Capitainer qDBS card) as a pre-step before determination of the HbA1c value with an immunological method. Scandinavian Journal of Clinical and Laboratory Investigation, 84(1), 11–15. https://doi.org/10.1080/00365513.2024.2303720

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