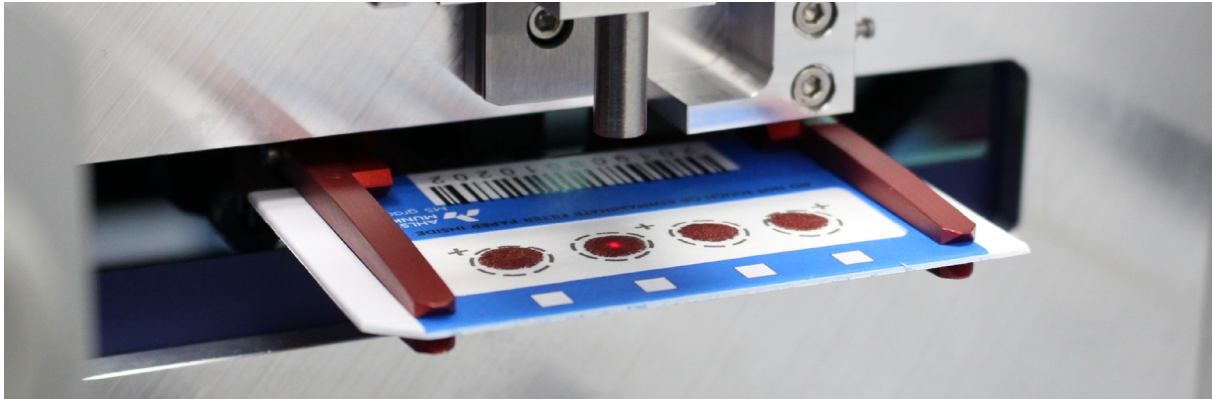


Fully automated Hematocrit correction for Dried Blood Spots



Keywords

Hematocrit correction, Hematocrit Normalization, Truly quantitative DBS Analytics, Volumetric DBS sampling, Anti-Doping

Introduction

To address hematocrit (HCT) related dried blood spot properties, CAMAG developed a fully automated HCT detection module that allows rapid and simple DBS HCT assessment and normalization.

The working principle is based on non-destructive, reflectance spectroscopy and has been fully validated. Being embedded within the automated CAMAG DBS-MS 500 HCT, the setup permits the high-throughput analysis of the DBS' reflectance at a specific wavelength, which correlates with the HCT of the DBS. The novel workflow makes the necessity of volumetric sampling for quantitative DBS analysis obsolete: By using a fixed extraction area that is completely saturated with blood, eluting from the center of each DBS, and correcting for the HCT of the target analyte. The HCT correction compensates for the blood volume within the fixed sampling area, as the spreading area of blood on the filter paper is HCT dependent. Thus, knowledge of the exact sample volume is not necessary.

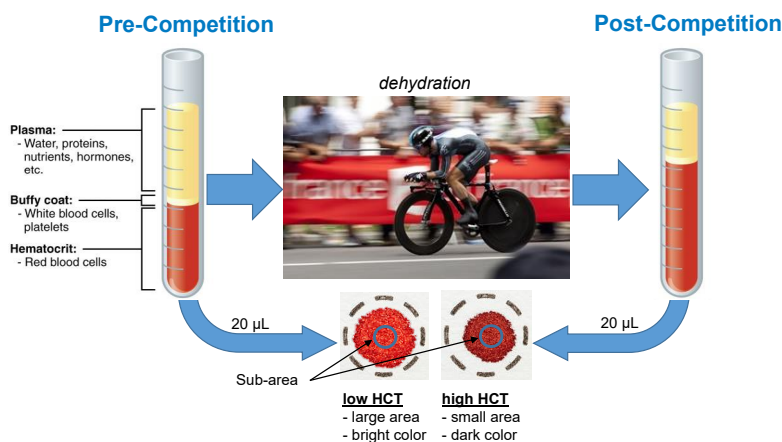


Figure 1: Example for a hematocrit change during dehydration and its effect on DBS

NOTE: The presented results are to be regarded as examples only!

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Scope

The HCT scanner allows normalizing for any known HCT related DBS effect such as the HCT area bias, the HCT recovery bias, or HCT related analyte properties. Furthermore, it is possible to normalize the analytical result to a selected HCT value. The system is ideally suited for the reliable, quantitative analysis of non-volumetric DBS samples.

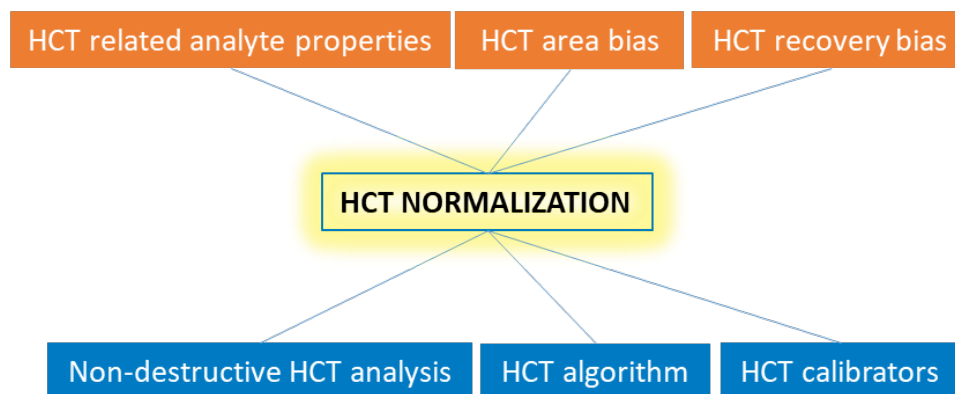


Figure 2: DBS related HCT issues depicted in orange, and the CAMAG solution depicted in yellow

Recommended devices

- CAMAG DBS-MS 500 HCT
- LC-MS/MS system for fully automated dried blood spot analysis (e.g. Shimadzu 8050 or higher)
- HCT centrifuge or hematology analyzer to prepare HCT calibration samples

Optional:

- CTC PAL robot for offline sample collection in vials or microtiter plates (e.g. 96-well plate)

Samples

Authentic DBS samples can be directly referenced to a calibration series of choice. Thereby, the user is free to build its own calibration curve, with filter paper material, anticoagulant, and calibration window of choice.

The HCT measurement based on reflectance has been validated to be independent of storage conditions, age, and temperature.

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HCT Calibrator Preparation

Hematocrit calibrator samples can be prepared using two different approaches:

Approach 1 is based on freshly collected blood that is collected and centrifuged at ambient temperature for 10 min at 1600 RCF to separate red blood cells (RBC) and plasma. Afterward, the two fractions are collected in individual falcon tubes and subsequently mixed in individual Eppendorf tubes using a manual positive displacement pipette to result in different HCT concentrations (e.g. 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7). Subsequently, DBS from each HCT and the native blood are generated on Ahlstrom TFN cards and dried for at least 3 h at room temperature.

Approach 2 relies on either an addition or a subtraction of plasma. Thereby, freshly collected blood of a known HCT is first centrifuged in individual tubes at 1300 RCF for 10 minutes, to ultimately result in different HCT concentrations (e.g. 0.2, 0.3, 0.4, 0.5, 0.6, and 0.7). After the centrifugation, plasma is either removed or added to each tube to reach the desired HCT value. Subsequently, DBS from each HCT and the native blood are generated on Ahlstrom TFN cards and dried for at least 3 h at room temperature.

To accurately reference the prepared HCT calibration samples, the liquid blood of each calibrator must be analyzed either using a hematology analyzer (e.g. KX-21N from Sysmex, Kobe, Japan) or an HCT centrifuge (e.g. hematokrit 200 from Hettich, Tuttlingen, Germany) on the same day of collection.

HCT analysis process

For each DBS sample, the reflectance of the blank filter paper and the reflectance at the center of the spot is measured. To create a calibration, the measured signal intensities are directly linked to DBS samples with known HCT values. Based on a linear regression analysis, an HCT calibration algorithm is developed. Based on the algorithm, the HCT of authentic DBS samples can be determined.

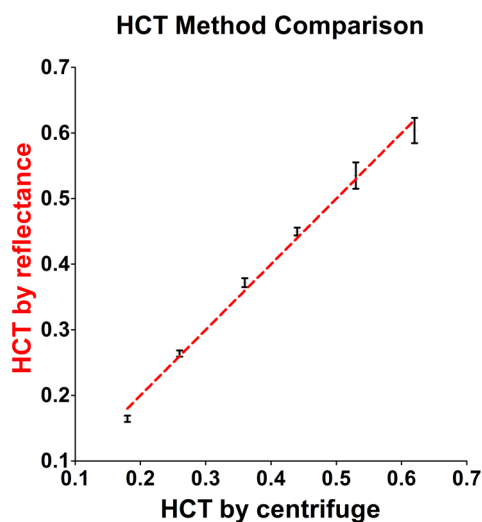


Figure 3: Comparison between reflectance-based HCT analysis and the analysis of liquid blood samples by centrifuge. An R square fit of 0.975 and a slope of 5.77 was observed

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Results

When the analyte related DBS HCT dependency is known, a normalization algorithm can be applied. Similar to the normalization of urine samples for their creatinine content, the normalization of HCT dependent analytes permits referencing for an HCT value of choice. This way, concentrations from male and female subjects, which generally have different HCT ranges, can be compared at the same HCT. This might be generally interesting for any assessment where threshold concentrations were applied indifferently to the specimen's HCT value.

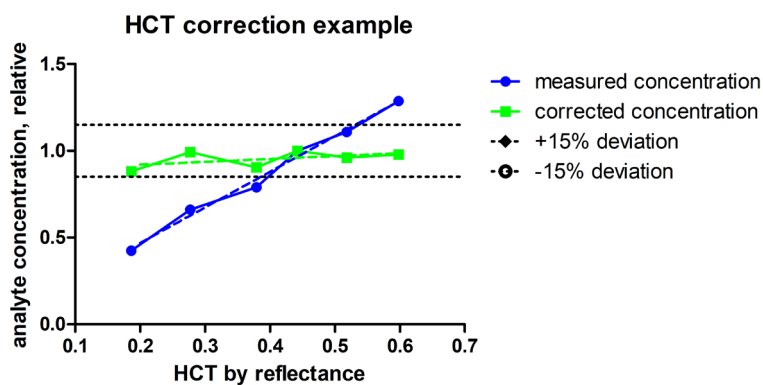


Figure 4: Example for the correction of the direct alcohol marker Phosphatidylethanol 16:0/18:1

Literature

Luginbühl M, Fischer Y, Gaugler S. Fully Automated Optical Hematocrit Measurement from Dried Blood Spots, *Journal of Analytical Toxicology*. 2020. doi:10.1093/jat/bkaa189

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