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ORIGINAL ARTICLE

Challenges of *In Vitro* Glycation when Producing Blood Materials for Hemoglobin A_{1C} Immunoassays

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SUMMARY

Background: **Blood materials are essential for quality control and assurance of hemoglobin A_{1C} (HbA_{1C}) measurements. This study presents an optimal condition for *in vitro* glycation to prepare blood materials for HbA_{1C} with desired high HbA_{1C} content and commutable with two immunoassays.**

Methods: **Washed erythrocytes were adjusted to a hematocrit (Hct) of 50 - 55% and glycated *in vitro* at 37°C for up to 120 hours with various concentrations of D-glucose in phosphate buffer saline to prepare blood materials for HbA_{1C}. After glycation in each condition, glycation of blood material was inhibited and HbA_{1C} level was monitored. The HbA_{1c} in blood materials from *in vitro* glycation was compared in terms of stability and commutability with blood materials from other preparation methods.**

Results: **Incubation of erythrocytes with 400 mM D-glucose for 15 hours at 37°C resulted in a significant increase ($p < 0.001$) of HbA_{1c} in blood materials by at least 40% with a remaining Hct between 38% to 42%. Hemoglobin A_{1C} in blood materials was stable at 3.8 ± 0.8°C for 70 days and during transport for 3 days (temperature ranges from 8.1 to 23.5°C), after inhibition by glucose concentration solution. Hemoglobin A_{1C} values in blood materials from *in vitro* glycation were commutable between enzymatic and turbidimetric immunoassay.**

Conclusions: **An optimal condition for *in vitro* glycation by incubation of erythrocytes with 400 mM D-glucose for 15 hours at 37 °C was able to generate HbA_{1C} material with intact erythrocytes that is sufficiently stable and commutable between enzymatic and turbidimetric immunoassay. Therefore, this condition is suitable for the preparation of blood material for HbA_{1C} immunoassays.**

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Supplementary Data

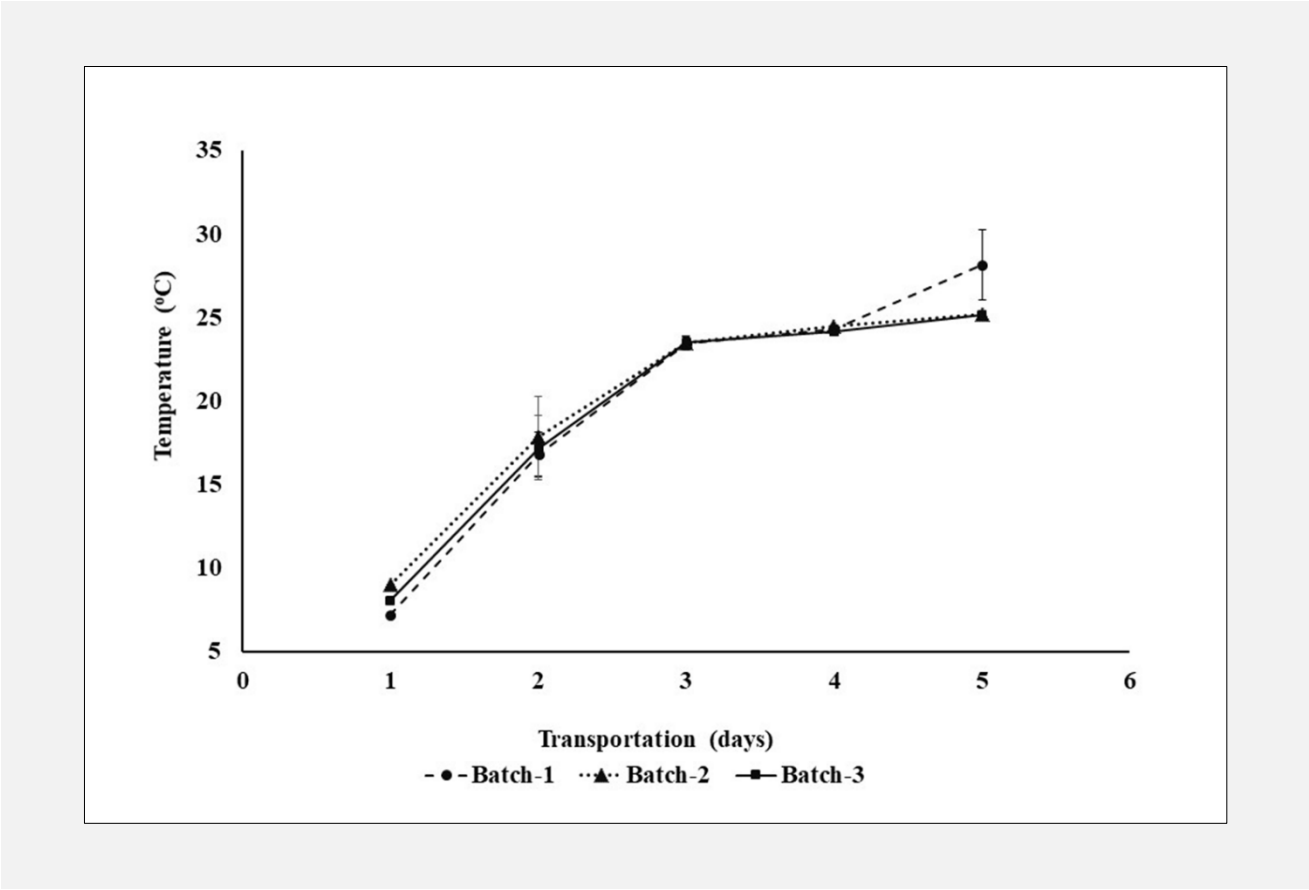

Figure S1. Temperature during three days of transportation.