

## Letter to the Editor

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# Feasibility of an EQAS for HbA<sub>1c</sub> in Italy using fresh blood samples

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To the Editor,

The External Quality Assessment Schemes (EQAS) are an essential component in the quality management of clinical laboratories. Their scope is to monitor that results obtained on patient's samples comply with the required quality for patient care by evaluating either the results provided by any participant, but also by assessing the performance of the different measurement procedures [1, 2].

In the case of glycosylated hemoglobin (HbA<sub>1c</sub>), several EQAS programs are available in the world, but it is difficult to compare the state-of-the-art of the different methods nowadays available to measure such component, since they essentially differ in design, type of materials used and methods for assigning the target values [3, 4]. Particularly, with regard to the materials used, fresh whole blood samples are probably the best material to be used, with the main limitation that they require a fast transport system in order to avoid degradation. Indeed fresh pooled blood samples have been used with success in various proficiency testing and EQAS in the USA [5], Asia [6], Sweden [7], the Netherlands [8] and Norway [9]. In Italy there are a few EQAS organizations, but they all use lyophilized control materials of unknown commutability, so it would be advisable, at least once a year, to run an exercise with fresh blood samples. A nationwide study performed many years ago [10] did indeed show inter-laboratory CVs between 15% and 16%, and this information needs to be updated as soon as possible.

In order to run an EQAS study in Italy with fresh blood samples, and to limit the costs, the main issue to be investigated is the method of shipping samples to the participants. To this end, we have performed a preliminary investigation which is reported here.

One fresh blood sample in EDTA, collected in the second week of December 2013 from a donor with informed consent, was divided in 13 aliquots. Ten of them (200 µL per aliquot) were shipped (from the laboratory of CW in The Netherlands) in parallel by courier (FedEx, "courier en emballage") and by priority mail to nine laboratories, selected in order to cover either North, Center, and South of Italy, including Sardinia. The three remaining aliquots were stored at room temperature (between 20 and 23°C) and HbA<sub>1c</sub> was assayed in triplicate by three different IFCC calibrated methods (Menarini 8180 V, Trinity Premier 9210, Sebia Capillarys Flex2 Piercing) on the day of collection, and then after 4 and 7 days, respectively.

The results of the measurements performed on the aliquots stored at room temperature in the laboratory of one of the authors (CW) are reported in Table 1. As can be seen, HbA<sub>1c</sub> is stable at room temperature up to 4 days. After that time, HbA<sub>1c</sub> slowly decreases and some unusual peaks appearing between HbA<sub>1c</sub> and HbA<sub>0</sub>, probably related to degradation products as previously described [11], were detectable by the HPLC analysis. This finding may integrate the information on sample stability published by Szymezak et al. [11] and, later on by Rohlfing et al. [12]. Both groups did show that fresh whole blood is stable from 3 to 14 days at room temperature, depending on the method used to measure HbA<sub>1c</sub>. Particularly, the shorter stability was with the Bio-Rad Variant II and the longest one with the Siemens DCA 2000+.

In the present communication we provide further data concerning sample stability at room temperature, evaluated by another HPLC system (Menarini 8180 V), another affinity chromatography method (Trinity Premier 9210) and by a capillary electrophoresis equipment (Sebia Capillarys Flex2 TM). For all these systems, the stability of whole blood was limited to 4 days at room temperature.

**Table 1** Stability of HbA<sub>1c</sub> (mmol/mol) during storage at room temperature.

Method	Basal	After 4 days	After 7 days
Menarini 8180 V	44.3±0.5	43.7±0.7	41.9±0.9 <sup>a</sup>
Trinity Premier 9210	45.0±0.7	44.2±0.8	42.0±0.6
Sebia CapillaryS	45.5±0.8	44.7±0.7	42.5±0.8 <sup>a</sup>
Mean±SD	44.9±0.6	44.2±0.5	42.1±0.3 <sup>b</sup>

<sup>a</sup>Abnormal peaks appearing in the chromatograms and in the electropherograms; <sup>b</sup>p=0.002 respect to basal (t-test).

With regard to the shipment of the aliquots to the participants, the shipment by regular mail did not work reliably. Only two laboratories received the samples within 4 days, four laboratories received them from 6 to 13 days after shipment, and three laboratories did not receive them even after 13 days.

On the contrary, the shipment by courier did work well, and all the participants received the samples within 4 days after the time of sampling. The results of the measurements performed by the participants on these last aliquots were the following: HbA<sub>1c</sub>, Tosoh G8 (n=5), 46.2±1.3 mmol/mol; Bio-Rad Variant II, dual kit (n=3), 44.3±1.5 mmol/mol; Roche Tina-quant Hemoglobin A1c Gen.2 (n=2), 44 and 41 mmol/mol (this last value was obtained 7 days after the shipment by a participant who received the samples within 4 days but, for technical reasons, was not able to perform the measurement on the same day). None of the laboratories performing the HPLC methods detected any abnormality in the chromatograms.

In conclusion, we have proven that fresh blood samples can be shipped to Italy by courier only, in order to be analyzed within 4 days from the time of sampling. After that time results cannot be accepted because of sample degradation. The experiment we have performed was

during the cold season, in which external temperatures are usually much <20°C. A shipment in the period from spring to autumn, unless under controlled temperature, should therefore be avoided.

We hope that these findings may be helpful to integrate the existing EQAS programs using lyophilized control materials with fresh pooled blood samples, in order to have a more realistic estimate on performance of laboratories in the measurement of HbA<sub>1c</sub> in Italy.

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#### Conflict of interest statement

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