QUANTITATIVE DETECTION OF TUMOR M2-PK: EVALUATION OF BLOOD AND STOOL SPECIMEN STABILITY

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Introduction

Pyruvate kinase plays a key role in the glycolytic pathway. Proliferating cells express M2-PK, a special isoenzyme of pyruvate kinase which can occur in a tetrameric and a dimeric form. In tumor cells the dimeric form is usually predominant and therefore termed Tumor M2-PK.

An ELISA with monoclonal antibodies to Tumor M2-PK has been developed (ScheBo® Biotech AG). Determination of this metabolic marker has been described in a variety of different tumor types. Interestingly, this assay can be performed with several types of specimen, e. g. EDTA-plasma, feces or tissue homogenates.

The vast majority of data have been obtained under study conditions, which may not represent the daily routine where considerable delay can occur between obtaining the sample and receipt at a hospital or private laboratory. To demonstrate the commercial ELISA's feasibility under everyday conditions the sample stability was evaluated.

Material and Methods

We measured Tumor M2-PK with a commercially available ELISA according to the manufacturer`s instructions.

1. Blood samples

Whole human blood, EDTA-, heparin and citrate-anticoagulated blood samples were collected from healthy volunteers. The samples were centrifuged at 2000 x g for 10 min., followed by removal of the serum or supernatant plasma. Then the specimens were treated as indicated in the results section.

2. Stool samples

Stool samples from patients with and without malignancies of the gastrointestinal tract were tested. To 100 mg of stool, 10 ml of extraction buffer was added. After vortexing, samples were diluted 1:10 in sample washing buffer and tested using the enzyme immunoassay after handling as described in the results section.

Results

1. Blood samples

Blood was drawn from ten healthy volunteers, and serum, EDTA-, heparin and citrate-plasma were handled as follows (at room temperature):

1. Centrifugation immediately after blood collection (0 hrs); 2. centrifugation 4 hrs after collection, either shaking the blood specimen during this period (4 hrs + shaking) or without shaking (4 hrs); 3. centrifugation 24 hrs after collection without shaking the samples (24 hrs). Figure 1 shows the reproducibility of Tumor M2-PK detection: EDTA-plasma and citrate-plasma was not affected by shaking the blood samples or exposure to room temperature for 24 hrs without prior centrifugation. In contrast, detection of Tumor M2-PK in serum or heparin-plasma was only reproducible when the specimens were centrifuged within 1 hr after collection (data not shown).

The long-term stability of Tumor M2-PK was tested by dividing EDTA-blood from each of seven volunteers into two aliquots: A) One aliquot was centrifuged and 1/3 of the supernatant EDTA-plasma was kept at 4°C, 1/3 at room temperature and 1/3 at 37°C. B) The second aliquot was not centrifuged and 1/3 of the total EDTA-blood was kept at 4°C, room temperature and 37°C respectively. Samples of all aliquots were collected on seven consecutive days. Figure 2 demonstrates the Tumor M2-PK concentrations on each day. Tumor M2-PK is stable at room temperature for one day in EDTA-plasma and EDTA-blood, and for seven days at 4°C in EDTA-plasma. Long-term storage of EDTA-blood leads to non-specific release of Tumor M2-PK from blood cells at all three temperatures.

2. Stool samples

The long-term stability of Tumor M2-PK in feces was investigated using stool specimens from ten patients, as follows: aliquots of stool from each patient were kept at room temperature or 4°C , respectively, and measured with the ELISA on days 1, 2, 3 and 7. In an additional investigation , the extraction buffer was added to the stool specimen and this suspension was stored and measured under the same conditions as described above. Figure 3 summarizes the results: when stool samples are kept at room temperature the assay should preferably be done within one day. Better stability could be ensured by storage at 4°C, when the ELISA could be performed after up to three days without significant loss of immunological detection of Tumor M2-PK. On the other hand, Tumor M2-PK should be measured on the same day as extraction from the stool specimen, because thereafter its detection declined, with a more rapid decrease at room temperature compared to storage at 4°C.

To demonstrate the ELISA´s reliability the intra- and inter-assay coefficient of variation were estimated. Fifteen stool samples ranging in concentration from 3 U/ml to 30 U/ml were each measured twenty times, resulting in an intra-assay CV of 4.48%. Measuring these samples on ten different days yielded an inter-assay CV of 6.08%.

Conclusions

1. Tumor M2-PK is stable at room temperature for one day in EDTA-plasma and in EDTA-blood, and for seven days at 4ºC in EDTA-plasma. Serum or heparin plasma is not recommended.
2. The assay can also be performed on feces. Stool samples can be kept at 4ºC for three days without loss of immunological detection of Tumor M2-PK. However, Tumor M2-PK should be assayed on the same day as extraction from the stool specimen, because thereafter its detection declined, especially at room temperature.
3. Quantitative determination of Tumor M2-PK by ELISA is practical in EDTA-plasma and fecal samples.
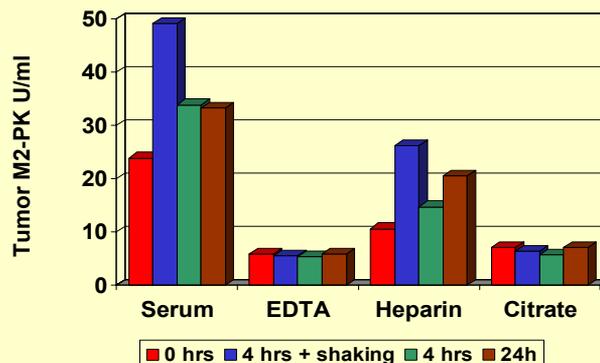

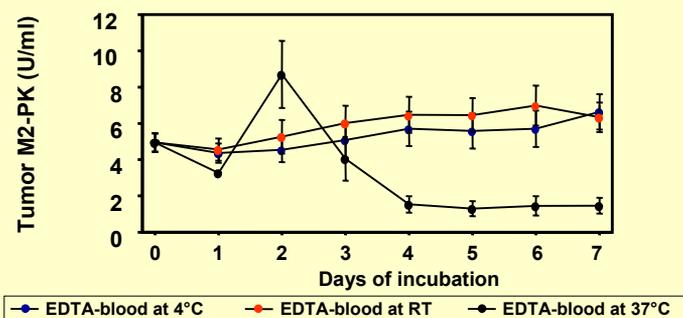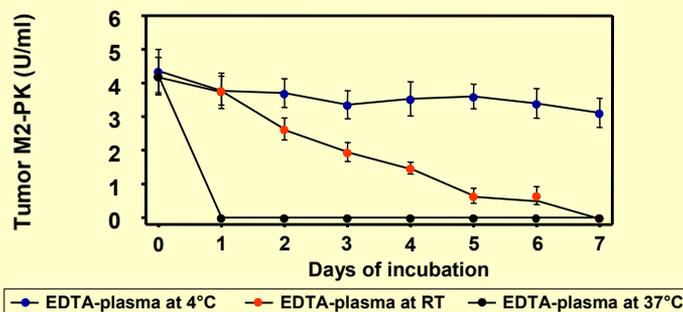Figure 1: Reproducibility of Tumor M2-PK detection

Figure 2: Tumor M2-PK concentrations on each day in EDTA-plasma and EDTA-blood (x̄ ± S.E.M.).
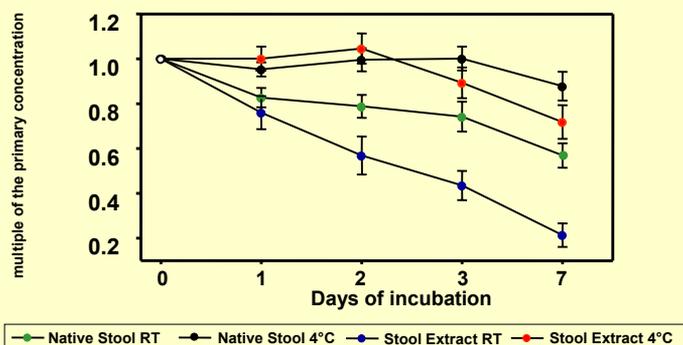

Figure 3: Stability of Tumor M2-PK in feces. The initial concentration of each fresh stool sample was normalised to 1. Subsequent results are expressed as a ratio of the original value (x̄ ± S.E.M.).