SCREENING METHOD FOR THE MEASUREMENT OF CREATINE KINASE IN PORCINE WHOLE BLOOD SAMPLES

Mika Tuomola, Jani Vainio, Eeva-Riitta Wirta* and Timo Lövgren

Department of Biotechnology, University of Turku, FIN-20520 Turku, Finland
*LSO Foods Oy, FIN-20520 Turku, Finland

Background

Physical activity and muscle damage change the permeability of mammalian muscle tissue, and increase the levels of certain enzymes, *e.g.* creatine phosphokinase (CK-MM, EC 2.7.3.2), in the blood (1). In the animal production, these enzymes can be used as markers of stress conditions which are linked with animal welfare and final product quality. A rapid one-step immunoassay was developed in this study for porcine CK-MM using whole blood samples. The assay is based on time-resolved fluorescence and includes a stable lanthanide chelate (2) that enables also the measurement of fluorescence directly from the solid phase.

Materials and Methods

Six commercially available antibodies to CK-MM were biotinylated and labeled with an intrinsically fluorescent europium chelate (Fig. 1).
The measurements were performed in streptavidin coated microtitration wells to which biotinylated capture antibody had already been attached (Fig 2). The assay was started by adding standard or diluted whole blood sample (5 µL) and the Eu-labeled tracer antibody (25 µL) into the well. The wells were incubated for 15 min at 37 °C and washed four times. LANFIA enhancement solution (Wallac Oy, Turku, Finland) was added to each well (200 µL), and the strips were shaken for three minutes. The time-resolved fluorescence was measured with DELFIA 1234 Fluorometer (Wallac Oy, Turku, Finland).

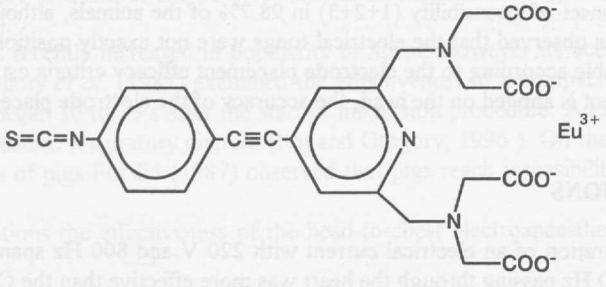

Figure 1. {2,2'2''2'''-{{4-[(4-isothiocyanatophenyl)ethynyl]pyridine-2,6-diyl}bis(methylenenitrilo)}tetrakis(acetato)} europium(III).

Selection of the antibodies was based on the specific signal obtained, linearity of the immunofluorometric assay and kinetics of the bioaffinity reaction. The best antibody combination was chosen and the assay was optimized and characterized. The results from porcine whole blood samples were compared with colorimetric CK activity reference method (Sigma UV-47). The distribution of CK-MM levels in normal Finnish slaughter pig population was also investigated by the analysis 300 samples obtained from the local abattoir.

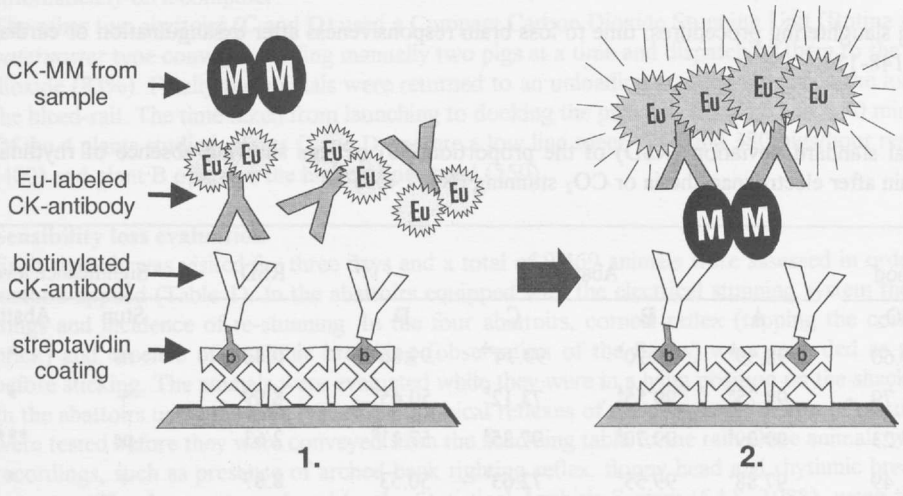

Figure 2. Immunofluorometric assay (IFMA) of creatine kinase.

Results

The method optimization resulted in a rapid assay that reached the equilibrium in less than 15 min. The release of the fluorescent component from the solid phase into solution was also fast, less than 3 min. This dissociation step could also have been omitted, because the label structure allowed also fluorescence measurement directly from the solid phase. This is due to the chelate structure that in normal assay conditions strongly binds the lanthanide ion and the excitation energy is efficiently absorbed by the energy-mediating moiety of the molecule.

The detection limit for CK-MM in whole blood samples was 470 ng/mL and the measurement was linear up to 5000 ng/mL. Analytical recovery was 90-95% and the immunoassay correlated well with the reference method (r=0.982, n=17, P<0.001) (Fig 3). The within-run and between-run imprecision (CV%) of the assay was 4.0-13.1% and 4.6-16.6%, respectively.

A total of 300 samples from normal Finnish slaughter pigs were measured with the assay. The results indicate that only 5% of pigs had elevated CK-MM concentrations (>5000 ng/mL) in their blood (Fig. 4).

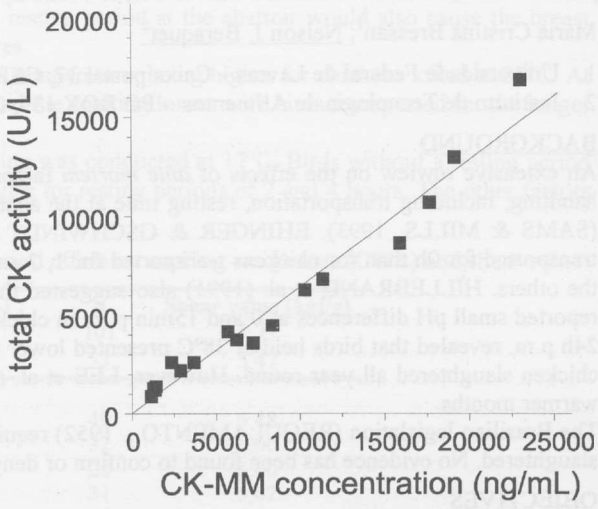

Figure 3. Correlation between one-step immunoassay and spectrophotometric CK-activity method

Conclusions

The simple and rapid time-resolved immunofluorometric assay of CK-MM in porcine blood is well suited for the screening of large number of samples. The technology as such makes it possible to carry out rapid and quantitative assays in whole blood samples.

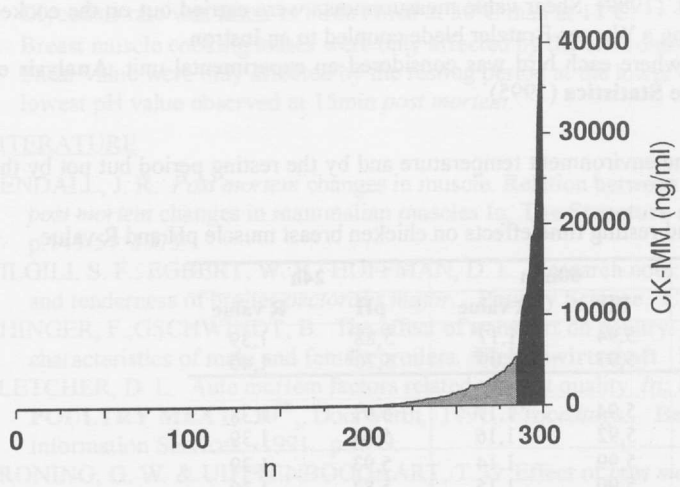

Figure 4. The distribution of CK-MM levels in normal slaughter pigs

References

1. Warnants N, Eeckhout W, Boucqué Ch V. **(1993)** *J Anim Breed Genet, 110*, 357-362
2. Meriö L, Pettersson K Lövgren T. **(1996)** *Clin Chem, 42*, 1513-1517