

# Colloidal gold based electrochemical immunoassays for the diagnosis of acute myocardial infarction



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The assays performed are directed to the determination of protein markers for **Acute Myocardial Infarction (AMI)**. The choice of this application has been done since cardiovascular diseases are the most lethal diseases in western world and the interest in developing fast and sensitive tests based on specific markers is very high.

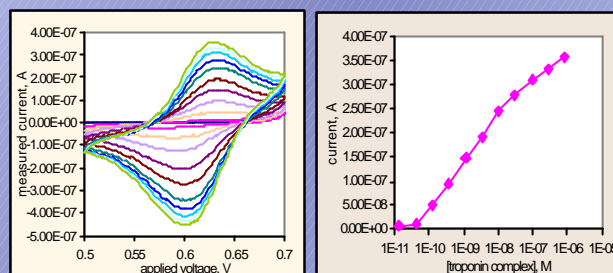
**Myoglobin** is expressed in cardiac and skeletal muscle so it is **not cardiac-specific**, but it appears in patients blood 1-3 hours after the onset of symptoms (peak within 8-12h) so it is the earliest marker and its predictive value in case of negative result is 100%.

**Troponin** is more cardiac specific and is present in **binary and ternary complexes** of its isoforms (**cTnI-TnC** and **cTnI-TnC-cTnT**).

They can be detected 16-30h after onset and last for 5-8 days (useful for late diagnosis)

**Creatin kinase** is an enzyme present in three isoforms **MM, BB, and MB**, the last one more heart specific. The presence of this enzymatic activity in blood is indicative of muscle damage but the immunoassay permits to discriminate the tissue specific isoforms [1]

The electrochemical immunoassay for cardiac troponin complex employing the antitroponin-gold conjugated antibody is shown below. The cyclic voltammograms recorded for each troponin concentration are shown on the left, while the calibration curve on the right is obtained by plotting the oxidation peak current vs troponin complex concentration.



The electrochemical detection of immunoassays is not very widely used but it is gaining growing interest because it has the potential of being applied to low cost miniaturisable electrodes. The transducer material is very cheap and by the screen printing technology a large scale production of graphite and other conductive ink electrodes is possible. Different designs can be adopted in order to perform the measurements in low volumes.

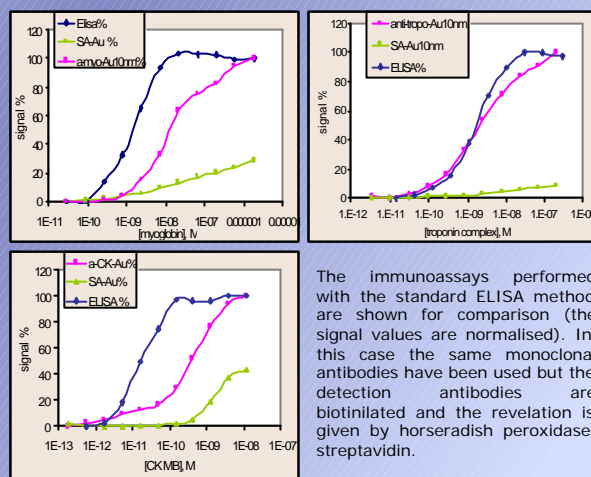
There are different electrochemical techniques adoptable for the revelation of immunoassays, depending on the labelling principle chosen. Colloidal gold is used as a label especially for histological samples in optical as well as in electron microscopy but it has also very interesting electrochemical properties. It can in fact be detected quantitatively with various voltammetric as well as potentiometric methods [2]. One of the simplest methods which can be used is the cyclic voltammetry whose operating principle consists in the application of a forward and reversed potential scan (triangular wave) to an electrochemical cell.

The electrochemical immunoassay is performed in microtiter plates and the last step is the oxidative solubilisation of gold with a solution containing HBr and Br<sub>2</sub>. The measurement is performed in a 50µl drop.

The measurement set up is shown in the photo: the working electrode is a screen printed graphite one, the counter electrode is a platinum wire and a standard reference electrode Ag/AgCl just touches the sample drop.

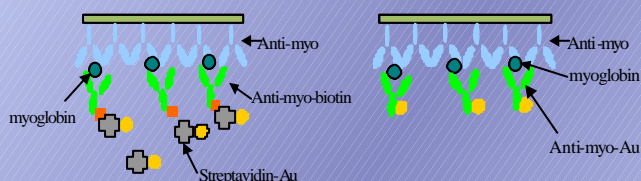


The assay performed with the specific colloidal gold labelled antibody and with the gold streptavidin are compared for each of the three cardiac markers analysed:



The immunoassays performed with the standard ELISA method are shown for comparison (the signal values are normalised). In this case the same monoclonal antibodies have been used but the detection antibodies are biotinylated and the revelation is given by horseradish peroxidase-streptavidin.

The schemes of immunoassay adopted here is shown below:



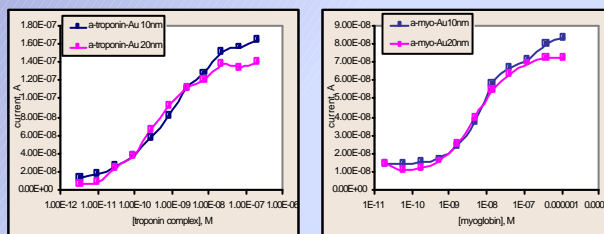
While the gold labelled streptavidin is commercially available, the conjugation with colloidal gold particles has been performed in the lab for the antibodies specific for the three cardiac markers analysed.



The interaction between the antibody molecule and the colloidal gold particle is based on electrostatic, hydrophobic and dative bonds with the SH- groups. The conjugation stabilises the colloidal particles against high ionic strength. Since disruption of the particles due to high salt concentration results in a change of color from red to grey, it is possible to visualise the conjugation and to perform a titration of the particle surface.

The use of the gold-antibodies results in a lower sensitivity level if compared to ELISA, both for myoglobin and for CK MB. On the other hand the assay based on gold-streptavidin, which is much more similar to ELISA is even less efficient so it offers no advantage over the use of directly labelled antibodies.

In the case of the troponin complex assay however the difference between the ELISA and the electrochemical assay based on the directly labelled antibody is less pronounced, maybe because in this case polyclonal antibodies have been used and this could possibly reduce steric hindrance factors.



The influence of the colloidal particle size on the signal has been investigated. It is reasonable to think that larger particles would yield higher signals, but the higher radius of the particles can pose some steric limitations to the assay. The use of 20 nm particles labelled antibodies gives no significant advantage over 10 nm particles. Probably steric hindrance overruns the effect of the larger Au quantity per particle. The use of lower diameter particle will be considered.

## Acknowledgements:

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## References:

- [1] J. McCord, R. M. Nowak, M. P. Hudson, P. A. McCullough, M. C., Tomlanovich, G. Jacobsen, G. Tokarski, N. Khoury and W. Douglas Weaver, *Annals of Emergency Medicine*, 42-2, (2003), 343-350.
- [2] Dequaire, M. Degrand, Ch. and Limones, B. *Anal. Chem.* (2000), 72, 5521-8.