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Technical note

Standardisation of metalloimmunoassay protocols for assessment of silver nanoparticle antibody conjugates

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ABSTRACT

The application of silver nanoparticles to electrochemical metalloimmunoassays has previously been reported (Szymanski et al., 2009) and is now used in the electrochemical Argento immunoassay platform via UK Patent No. 2458420. The development of an immunoassay in this format requires the optimisation of the antibody to silver nanoparticle conjugation process. Issues such as pH, antibody concentration and other factors can affect the assay performance. In order to determine the effect of these variables it is necessary to understand and control the effects of other factors that may affect the assay signal.

In this study a number of conditions which affect the assay signal were identified and methods developed to minimise variability in the assay signal, resulting in a standardised method allowing easy comparisons of silver nanoparticle conjugates prepared and assayed at different times.

1. Introduction

The advent of highly specific biomarker tests has created an environment in which the very definition of a disease can be based upon detection of the biomarker, rather than the biomarker indicating the disease. One example of this is acute myocardial infarction, for which elevated levels of biomarkers, particularly troponin, is included in two of the four criteria that define this condition (Alpert et al., 2008; Schreiber and Miller, 2009). The UK National Health Service also state that troponin (T and I) is the preferred marker of myocardial damage (Burtonwood and Halloran, 2006). For this reason a standard reference assay for troponin is being developed (Panteghini et al., 2008).

Multiplexed cardiac marker assays including troponin, myoglobin and cytokines have been developed, but it has been shown that troponin levels alone match or better the clinical performance of these multiplexed assays. (Eggers et al., 2005; Morrow and Antman, 2009).

A number of laboratory and point-of-care systems are available for the detection of troponin by immunoassay. . The point-of-care tests are sensitive enough to at least match the performance of laboratory analysers, although there are also disadvantages to the currently available systems (von Lode, 2005; Burtonwood and Halloran, 2006).

Electrochemical immunoassays are well suited to point-of-care diagnostic devices since the electronics can be incorporated into hand-held instruments and the measurement can be made in complex matrices with small sample volumes, giving high sensitivity and specificity. Metalloimmunoassays based on gold, silver and other metals are increasingly being used for these applications, a number of examples are reviewed by (Campbell and Compton, 2010). The Argento system, based on methods described previously (Porter et al., 2009; Szymanski et al., 2009) is one such platform, which utilises silver nanoparticles functionalised with antibodies. Other metalloimmunoassays require strongly acidic hazardous reagents to break up the metal nanoparticles e.g. Dequaire et al. (2000)). In contrast the Argento assay is followed by reaction with a non-corrosive reagent, ammonium thiocyanate, which is more suited to use at the point of care. Electroactive silver ions are formed under

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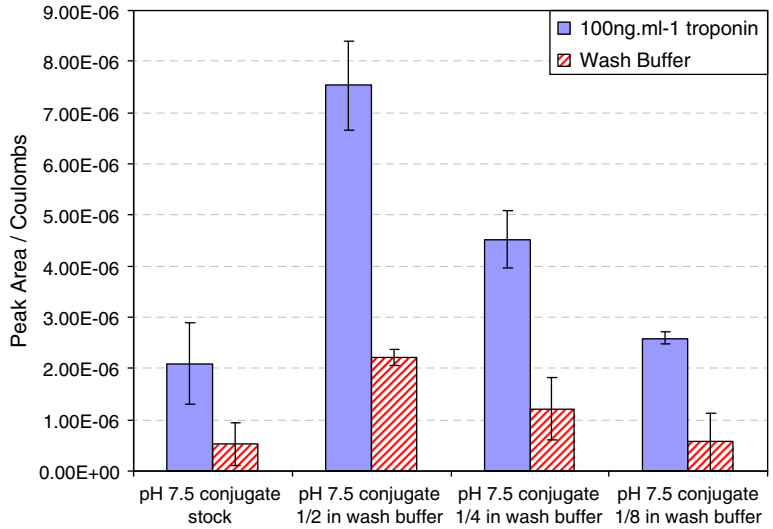

Fig. 1. Effect of dilution of silver nanoparticle conjugate in wash buffer. Error bars represent 1 standard deviation from triplicates.

electrochemical oxidation at the electrode and allow measurement of the assay signal by stripping voltammetry.

The initial validation step for silver antibody conjugates used in these immunoassays is based on the method described by Szymanski et al. (2009). Developing assays in this format requires optimisation of the silver-antibody conjugation process, which in turn requires a method to compare different silver conjugates under the same conditions. During optimisation of the assay for troponin, it was found that a number of factors affected the assay signal independently of the performance of the conjugate, potentially making comparison of conjugates prepared and assayed at different times difficult. The authors believe the results reported here to be generally applicable to metalloimmunoassays of this type.

2. Materials and methods

2.1. Silver nanoparticle conjugation

1 ml aliquots of 40 nm diameter silver nanoparticles (British Biocell International (BBI), Cardiff, UK or NanoComposix, San Diego, Cam USA) were centrifuged at 16,100× *g* for 10 min to form a soft pellet. The supernatant was discarded and the pellet re-suspended in 1 ml of 10 $\mu g.ml^{-1}$ antibody (in house anti-troponin monoclonal) in 0.1 M boric acid adjusted to pH 7.5 with sodium hydroxide (borate buffer). The silver-antibody solution was incubated for 2 h at 20 °C on a rolling mixer. The solution was then centrifuged at 16,100× *g* for 10 min and the pellet re-suspended in 1 ml of borate buffer containing 0.1% w/v BSA (storage buffer). After a 5 minute incubation at ambient the solution was centrifuged for 10 min at 16,100× *g*, the supernatant removed and discarded and the pellet re-suspended in 250 μl of storage buffer.

2.2. Microplate electrochemical immunoassay

96-well ELISA plates (Corning 9011, Amsterdam, The Netherlands) were coated with 50 μl per well of a mixture of 2.5 $\mu g.ml^{-1}$ HyTest MF4 anti-troponin and 2.5 $\mu g.ml^{-1}$ HyTest 560 anti-troponin (HyTest, Turku, Finland) in PBS at 4 °C overnight. The plate was then washed three times with 300 μl wash buffer (0.02 M TRIS, 0.15 M NaCl, 0.05% v/v TWEEN 20 pH7.5), and blocked with 150 μl SuperBlock (Thermo-Fisher, PN37536) for 1 h at 20 °C. The plate was then tapped dry and sealed, then stored at 4 °C until used. The stability of the plate was at least 1 month.

For each assay, 50 μl of either wash buffer or 100 $ng.ml^{-1}$ HyTest troponin I standard in wash buffer was added to each well and incubated for 1 h on a 3D orbital shaker. The wells were then washed three times with 300 μl wash buffer, and a total volume of 50 μl of silver nanoparticle conjugate (neat or diluted in wash buffer) was added. The plate was incubated at room temperature for 1 h, then the wells washed three times with 300 μl wash buffer. 50 μl of ammonium thiocyanate (Sigma-Aldrich, Poole, UK) (NH_4SCN) of appropriate concentration (1 M unless otherwise stated) in MilliQ water was then added to each well and incubated for up to 2 h on a 3D orbital shaker (longer incubations were continued static at ambient temperature). The ammonium thiocyanate reacts with the silver nanoparticles to remove antibody from the surface and give the particles a negative charge to enable electrochemical detection as described by Szymanski et al. (2009). The signal was then read by anodic stripping voltammetry, based on an initial hold at +0.6 V for 10 s, a second hold at −1.6 V for 5 s, a hold at −1.2 V for 120 s and then a sweep from −1.2 to +0.2 V at 1 V/s. The sweep produces a characteristic peak for silver, and area under this peak was calculated to give a signal in Coloumbs.

3. Results and discussion

3.1. Sample matrix

The effect of sample matrix was assessed by serial dilution of the silver nanoparticle conjugate in wash buffer. Fig. 1 shows that diluting the conjugate 1 in 2 in wash buffer gave a 4-fold increase