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Experience of using MAIIA columns for EPO testing at the 2012 Olympic and Paralympic Games

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Abstract

The MAIIA EPO purification kit (MAIIA Diagnostics, Sweden) has been evaluated as a tool to help simplify the original EPO sample preparation method, which requires several concentration and ultrafiltration steps to enhance the signal obtained from the very small concentrations (~10 ng/L) of EPO in urine. This work describes the experience and results obtained with the MAIIA EPO purification kit during the London 2012 Olympic and Paralympic Games. A total of 702 Olympic and 144 Paralympic urine samples for EPO analysis were immunopurified using the MAIIA columns prior to IEF-PAGE or SDS-PAGE analysis, for the first time in a Summer Olympic Games. Validation and evaluation of the new sample preparation method was completed by the end of 2011. The use of the columns showed a clear improvement of the IEF-PAGE results. MAIIA columns were considered a viable and reliable tool and, by then, a cost-effective method for the preparation of urine samples for EPO analysis. During the Games, 1-2% of the columns were defective and needed replacement and approximately 5% of the samples clogged the columns causing difficulties collecting the eluates. Considering the use of over a thousand anti-EPO columns, the overall experience was good. Hence, if the costs continue reducing and some alterations are made to the anti-EPO columns to help solve the existing problems, the establishment of these columns as a pre-step in EPO doping analysis is assured.

Introduction

Erythropoietin (EPO) is present at very low concentrations in urine, typically 10 ng/L. The sample preparation method preceding the urinary EPO doping test is based, in its original form, on several concentrations and ultrafiltrations steps [1]. In order to minimise band deformation or smearing, new sample preparation methods relying on affinity purification (anti-EPO antibody-coated ELISA wells [2], monolithic discs [3] or beads [4]) have been recently proposed. The MAIIA EPO purification kit comprises disposable Anti-EPO columns containing a thin monolith with immobilised monoclonal anti-EPO antibody 3F6, which is claimed to capture very specifically both endogenous and recombinant human EPO from the many other abundant proteins in the urine matrix. Here we describe our use during the London 2012 Olympic and Paralympic Games.

Experimental

The MAIIA EPO purification kit was obtained from MAIIA Diagnostics (Uppsala, Sweden). Of the total 4,872 Olympic urine and blood samples, 702 urine samples were tested for EPO. Of the total 1,256 Paralympic urine and blood samples, 144 urine samples were tested for EPO (Fig 1). The immunopurification of the samples was performed according to the manufacturer's directions for use, with minor changes in the case of the clogged columns (see below). Validation and evaluation of the new sample preparation method was completed by the end of 2011 and subsequently used routinely in our laboratory to immunopurify all urine samples prior to isoelectric focusing (IEF) or SDS-PAGE analysis. In brief, twelve blank urine samples from different subjects, an authentic positive urine sample and urine samples spiked with EPO Biological Reference Preparation (BRP) and NESP were prepared using both ultrafiltration (UF) and immunopurification (IP) techniques. Plasma and serum blanks together with CERA spiked samples were also immunopurified and analysed.

The problem of clogged columns was usually solved by the addition of 1 mL extra MAIIA *Urine Precipitate Dissolution buffer* to the samples, or by increasing the vacuum or length of the centrifugation step or, ultimately, as a last resort, using a second column.

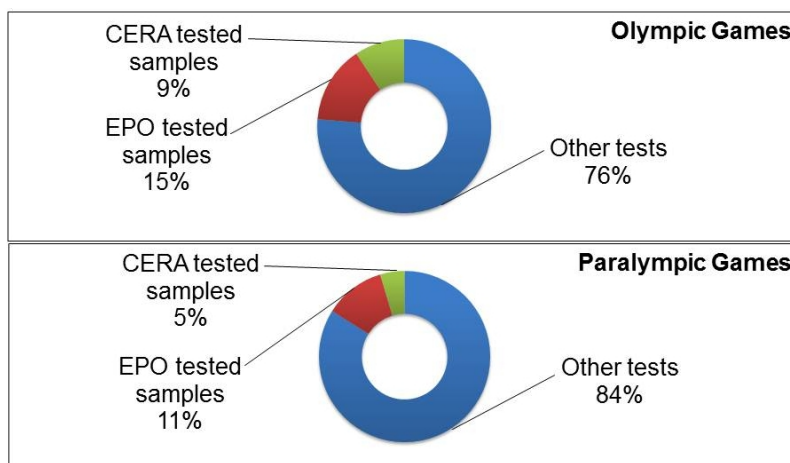


Figure 1: EPO testing during the 2012 Olympic & Paralympic Games.

Results and Discussion

The use of the disposable Anti-EPO columns showed a clear improvement of the IEF results when compared to the classical ultrafiltration method (Fig 2) and is also a simple and less time consuming procedure. The eluates obtained (55 μ L) load onto the gels (20 μ L) consistently and results are easier to interpret. Approximately 8% of the Olympic samples needed further work as they appeared to be active urines, effort urines (due to the immediate collection of samples after strenuous physical effort), or had atypical IEF-profiles.

Having 35 μ L of the MAIIA eluates left, together with the benefits of the SDS-PAGE technique, avoided having to use more urine and saved time. Figure 3A shows a urine sample with an atypical profile shifted to the basic area of the IEF gel. This urine produced a band with lower apparent molar mass in SDS-PAGE indicating a possible degradation of the uEPO [5]. Results of 'effort urine' samples were also observed. The IEF-profiles were slightly more basic but only one band corresponding to hEPO was observed on SDS-PAGE (data not shown). Only one AAF, which was a control, was reported during the Games (Fig 3B). Samples from the CERA ELISA screen greater than the cut-off decision limit (100 pg/mL) were immunopurified and analysed by IEF (eight samples). Results of these samples clearly indicated that the samples were negative as they did not meet the WADA identification criteria [6]. During the Olympics, 1-2% of the columns were defective (monolith not properly placed inside the column, broken monolith, or monolith with little holes) and needed replacement (Fig 4). Another unfortunate and frequent problem (~5% of the samples), was clogging of the columns requiring further work or replacement of the column.

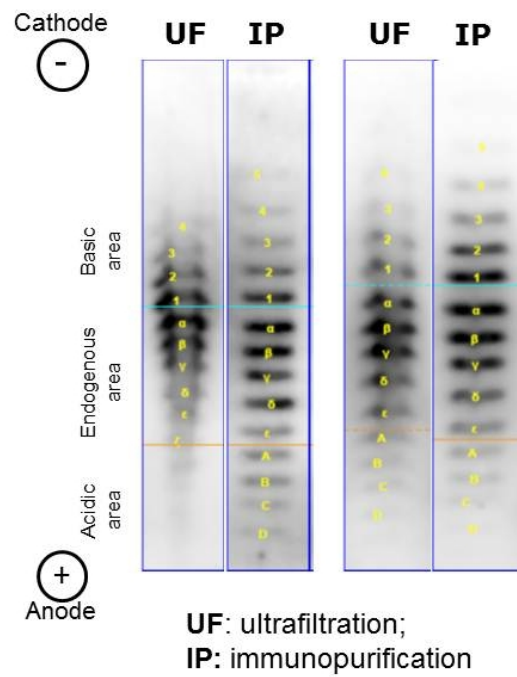


Figure 2: IEF results of retentates & eluates from blank urine samples. 20 µL of retentate & eluate loaded.

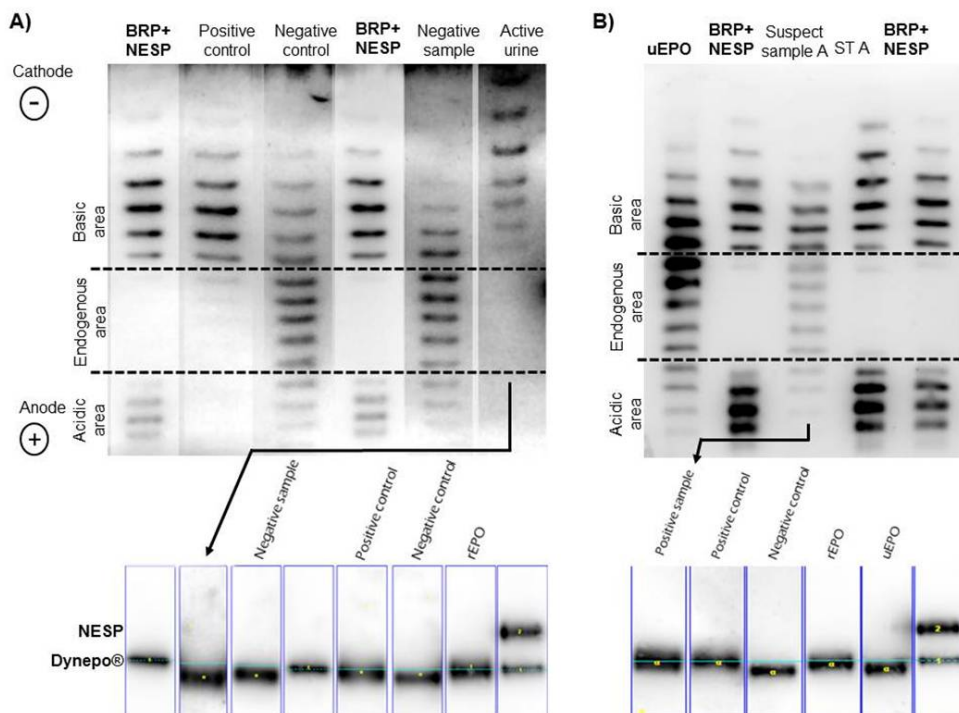


Figure 3: IEF- and SDS-PAGE results of A) an active urine, the IEF-profile was shifted towards the basic area of the gel. The SDS-PAGE result showed a band with lower apparent molecular mass compared to hEPO, B) Sample A met the identification criteria for rEPO in the IEF- and SDS-PAGE results. The stability test result of sample A (ST A) demonstrated the stability of this sample.



Figure 4: Defective monoliths in the MAIIA columns (monolith not properly placed inside the column, broken monolith, or monolith with little holes).

Conclusions

This was the first Summer Olympic Games where MAIIA columns were used and, perhaps, the largest use by a WADA accredited laboratory over such a short time period. After validation and evaluation of the new sample preparation method, MAIIA columns were considered a viable, reliable and cost-effective tool for the preparation of the urine samples prior to EPO analysis.

More than 1,000 anti-EPO columns were used during the Games, for routine testing and training temporary analysts and performed well. The columns should be cost-effective for routine EPO analysis provided that the product consistency is improved and the problem of clogging is solved.

References

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