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Technical improvement of the screening method for the detection of erythropoietins in urine

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Introduction

Erythropoietin (EPO) is a glycoproteic hormone which is involved in several functions, the most important being the regulation of erythropoiesis. Since an increase of erythrocytes enhances athletic performance in endurance sports, its use is prohibited according to the rules of World Anti-Doping Agency (WADA). The current method for the detection of erythropoietin in urine, starting from sample preparation to final analysis, requires almost 3 days to obtain the gel image for evaluation. In order to perform antidoping analyses in shorter times - which is more and more important specially when particular time constraints have to be achieved (as it is the case of major sport events) - we focused on reducing the time required for the screening method.

The method we are presenting is based on the one proposed by Lasne *et al.* [1-2] and accredited by WADA, except for the use of the SNAP i.d. system (Figure 1) from Millipore for performing the double blotting steps.

Figure 1. The SNAP i.d. system

Experimental

The SNAP i.d. system is used after the DTT incubation and the first blotting; it consists of two small (7,9 x 8,8 cm) single-use blot holders; vacuum is applied to actively drive reagents through the blotting membrane so that the incubation time for the blocking step and the washing time can be drastically reduced with respect to the original procedure; more specifically, the blocking step and the three washing steps require 20 seconds each (compared to 1 h and 10 min compared to the original procedure, respectively). The increased speed of all these steps allows to obtain the results in less than 12 hours from the beginning of the isoelectric focusing to the EPO-detection by chemiluminesce. There are three possible modes of operation as described in Figure 2 - depending on the way chosen for incubating the membrane with the primary and with the secondary antibody. One limitation of this system is that for each small membrane only ten to eleven samples can be applied, so at the same time a maximum number of twenty to twenty two samples can be processed since the system consists of only two single well blot holders.

Results and Conclusions

More than 100 negative samples, which were already evaluated by the original method, were analysed in the three different ways. Consistent results were obtained with no false positives. The LODs of the original method were also confirmed. The reproducibility of this procedure was evaluated using blank urine samples spiked with epoetin α, epoetin β, darbepoetin alfa, or epoetin δ. The use of this system reduces the time for the complete analysis by four to five hours. The results obtained with the three different modes of operation are shown in Figure 3.

Acknowledgement

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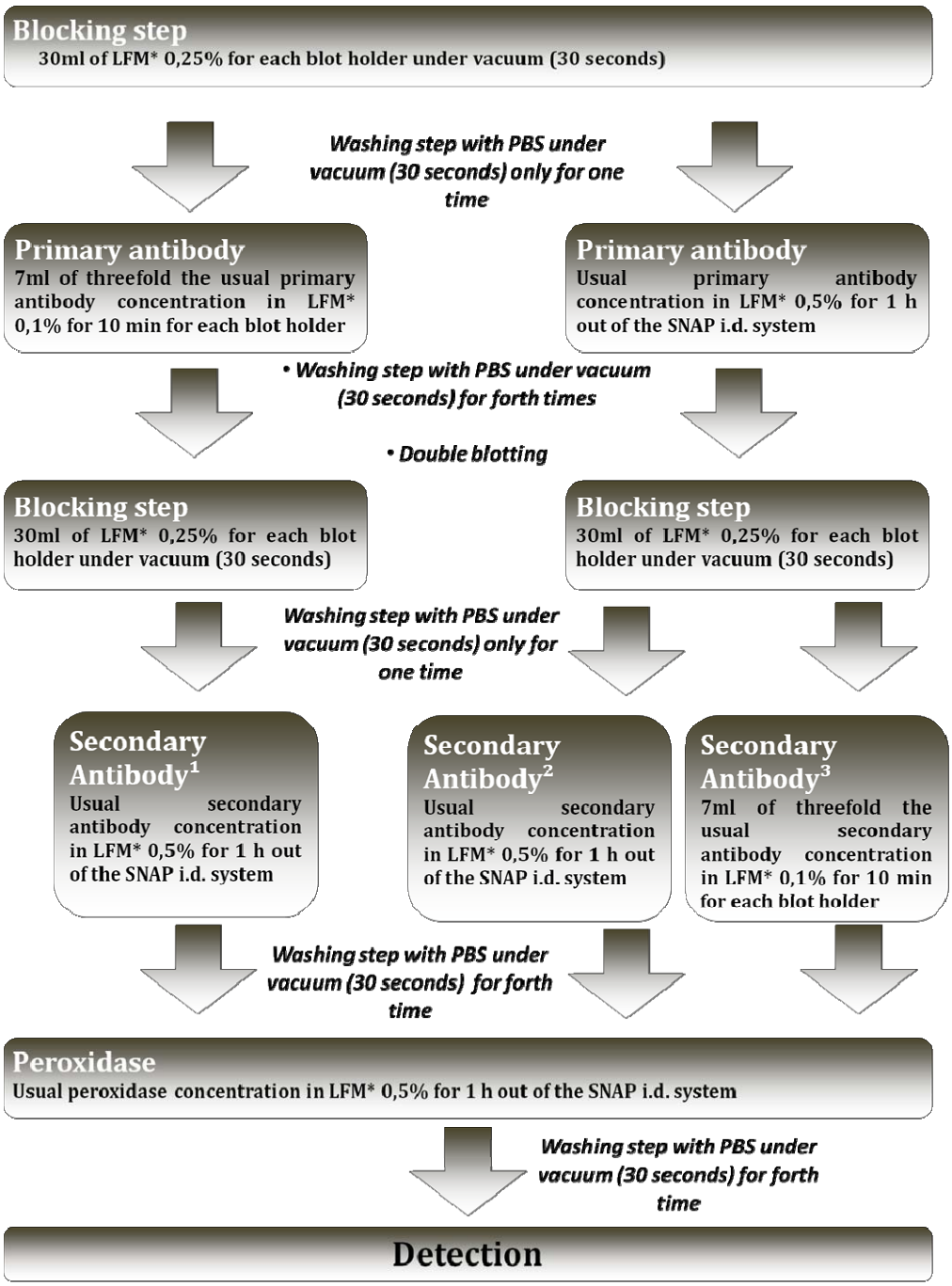

*) it is critical that the solution is completely solubilised to maintain a constant flow through the membrane and to avoid obtaining a high background

Figure 2. Schematic representation of the workflow as modified for the use with the SNAP system

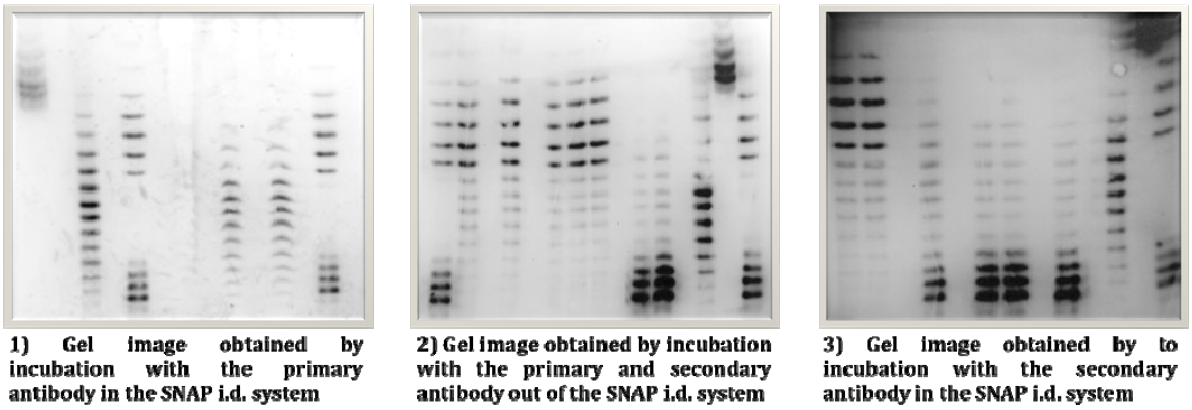

Figure 3. Chemiluminescence images of the gels obtained by the three different experimental protocols based on the SNAP system

References

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[2] Lasne F. (2003) Double-blotting: a solution to the problem of non-specific binding of secondary antibodies in immunoblotting procedures. *J Immunol Methods* **276**, 223-226.