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Fast screening for the detection of HES and dextran

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INTRODUCTION

Hydroxyethyl starch (HES) and dextran are the most frequently used plasma volume expanders (PVE) in the medical field due to their limited effects. Because of its various properties, HES is used by some athletes to increase body fluid amounts to prevent a decrease in exercise performance due to dehydratation. The misuse of HES in high performance endurance sports was officially mentioned in 1998 and, since January 2000, the Medical Commision of the International Olympic Committee (IOC) prohibited the use of any plasma expander. These substances are still included in the WADA list of prohibited substances and methods [1]. Currently, there are different methods to screen for the presence of HES and dextran in urine, some based on a microtiter colorimetric assay [2-4].

Here we present a protocol based on a colorimetric assay for the detection of polysaccharide-based plasma volume expanders (PVEs) through glucose detection, in human urine, using commercially available reactive strips for clinical diagnosis.

EXPERIMENTAL SECTION

Sample preparation and colorimetric detection

To 50 µL of urine, 0.6 mL of hydrochloric acid (3 M), were added and incubated for 1 hour at 100 ºC. After hydrolysis the sample was evaporated to dryness. The residue was reconstituted in 50 µL of phosphate buffer (pH 7.4).

The detection was done using reactive strips based on two enzymatic reactions, one catalyzing the oxidation of glucose into gluconic acid and hydrogen peroxide, instead the second enzyme catalyzes the reaction between hydrogen peroxide and orthotolidine (Diastix)

or potassium iodide (Clinstix). The colour of the final solution range from pink to bleu (Clinstix) or from green to brown (Diastix) (see Figures 1).

Figure 1: reactive strips: Clinstix (A), Diastix (B)

RESULTS

The approach proposed for the plasma expander screening shows a good specificity and colour repeatability; limits of detection are around 1 mg/mL for HES and 500 µg/mL for dextran. Figure 2 shows a representative example of the specificity of this colorimetric test: ten samples without glucose or glucose-containing polymer were tested, using the two different reactive strips, before and after chemical hydrolysis. No significant interferences were found thus excluding the possibility of false positive results.

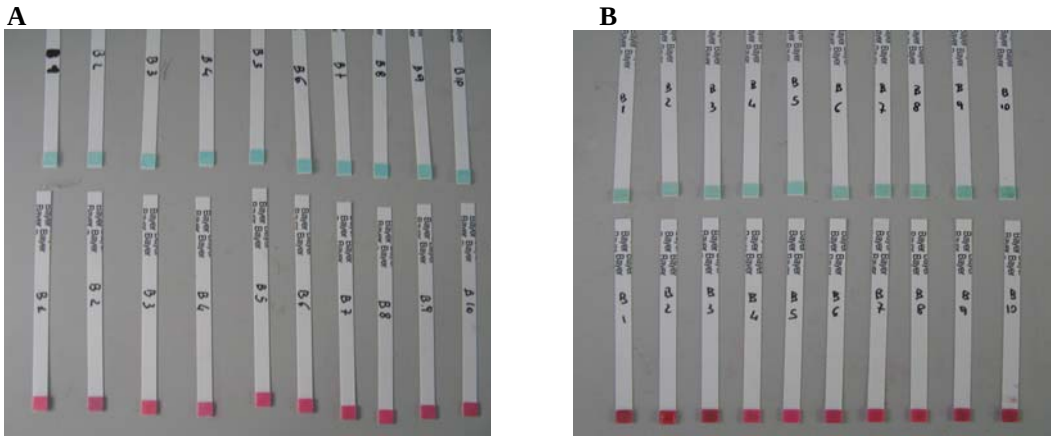

Figure 2: Specificity test: 30 blank urines are tested before (A) and after (B) hydrolysis

Figure 3 shows the results after acid hydrolysis of a blank urine compared with spiked samples a three different concentration (HES: 500 µg/mL, 1 mg/mL and 2 mg/mL; dextran: 250 µg/mL, 500 µg/mL and 1 mg/mL), to establish the limit of detection (LOD) for both HES and dextran.

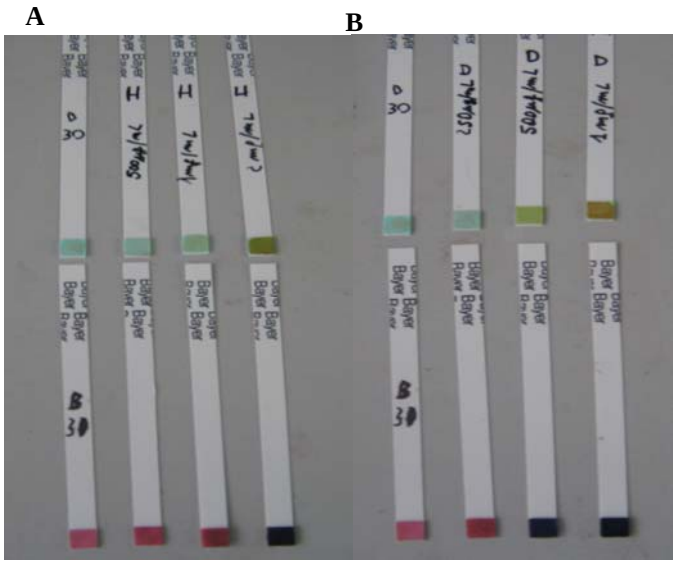

Figure 3: Limit of detection for HES (A) and dextran (B)

More specifically, the suitability of the developed method for routine analysis was checked by analysing real samples positive for the presence of dextran (Figure 4).

Figure 4: Dextran excretion study

CONCLUSION

The approach proposed as a screening method shows limit of detection values according with the glucose levels encountered in urine after HES or dextran administration.

In conclusion, we believe that the method is suitable for routine use in anti-doping laboratories, being particularly advantageous for those laboratories analyzing a considerable number of samples and/or with limited instrumental resources in terms of the total available

number of GC/MS stations; for indeed, the colorimetric test is simple and quick, taking less than one minutes, and the analysis costs (especially in terms of solvents and supplies) are lower than traditional GC/MS methods. These features are extremely significant whenever the time constraint is critical, as it is in the case of major International sport events, like Olympic Games, when two concurrent situations increase the specific workload for the laboratory: (i) the expected reporting time must be shorter than 24/48 h (for negative or positive samples, respectively), and (ii) the daily number of samples received by the laboratory increases.

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REFERENCES

1. World Anti-Doping Code. The 2008 Prohibited List International Standard. Montreal (2008) http://www.wada-ama.org/rtecontent/document/2008_List_En.pdf (access date 25.04.2008).
2. Avois L, Lund HS, Hemmersbach P, Saugy M. (2004) Rapid screening of HES in urine with Colorimetric Detection In: Schänzer W, Geyer H, Gotzmann A, Mareck U (eds.) *Recent advances in doping analysis*, (12), Köln, pp 371-376 .
3. Gutièrrez Gallego R, Such-Sanmartin G, Sgura J. (2005) A microtiter assay to detect the presence of glucose-based plasma volume expanders in urine. In: Schänzer W, Geyer H, Gotzmann A, Mareck U (eds.) *Recent advances in doping analysis*, (13), Köln, pp 383-386.
4. Sook Yi Mun, Normaliza AM, Manaf A, Latiff AA. (2006) Rapid screening of plasma volume expanders using Benedict's solution. In: Schänzer W, Geyer H, Gotzmann A, Mareck U (eds.) *Recent advances in doping analysis*, (14), Köln, pp 427-430.