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ORIGINAL RESEARCH ARTICLE

The Urine Marker Test: An Alternative Approach to Supervised Urine Collection for Doping Control

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Abstract

Background Urine sample collection for doping control tests is a key component of the World Anti-Doping Agency's fight against doping in sport. However, a substantial number of athletes experience difficulty when having to urinate under supervision. Furthermore, it cannot always be ensured that athletes are actually delivering their own urine. A method that can be used to alleviate the negative impact of a supervised urination procedure and which can also identify urine as coming from a specific athlete is the urine marker test. Monodisperse low molecular weight polyethylene glycols (PEGs) are given orally prior to urination. Urine samples can be traced to the donor by analysis of the PEGs previously given.

Objective The objective of this study was to investigate the use of the urine marker during urine doping control testing.

Methods Two studies investigated athletes' acceptance of this new method via two questionnaires ($n = 253$). Furthermore, a third study ($n = 91$) investigated whether ingestion of the marker can identify the urine as coming from a specific person and whether the marker interferes with the detection of prohibited substances.

Results and conclusions The results indicate that this new method finds wide acceptance both from athletes who have only heard about the procedure and those who have actually tested the new method. Furthermore, the marker, which can identify urine as coming from a specific person, does not interfere with the detection of prohibited substances.

Key Points

The urine marker can identify urine as coming from a specific athlete.

The urine marker does not interfere with the detection of prohibited substances routinely monitored by a doping control laboratory.

The majority of surveyed athletes feel that the urine marker method is a good alternative to supervised urine collection and that it would help eliminate the problems some athletes experience with the standard doping control procedure.

1 Introduction

Urine sample collection is a key component of the World Anti-Doping Agency's (WADA's) fight against doping, and elite athletes are obliged to provide doping control samples at any time and any place without prior notice. The most common procedure for detecting the consumption of illegal substances is by testing urine [1], although there are discussions about further increasing the number of blood

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tests [1] and expanding the biological passport program. Furthermore, since 2014, the athlete's individual steroid profile parameters for each urine sample have to be reported through the WADA platform Anti-Doping Administration and Management System (ADAMS) after each doping control. The advantage of urine doping controls is that many substances can be more easily identified in urine than in blood, and the collection of urine is also less physiologically invasive and less harmful than collection of a blood sample [2].

The collection of urine samples has to follow a strict, standardized procedure following the World Anti-Doping Code "International Standard for Testing and Investigations" [3]. This means that prior to the doping control, the athlete either has to wash his/her hands thoroughly with water only or he/she has to wear suitable (e.g., latex) gloves during the provision of the sample. Afterwards, the doping control officer (DCO) instructs the athlete to remove and/or adjust any clothing that might restrict the DCO's clear and unobstructed view of the athlete providing the urine sample. The athlete needs to provide at least 90 mL of urine, and the DCO will verify that a suitable volume of urine has been provided while in full view of the athlete. This means that the DCO will directly observe the urine sample leaving the athlete's body.

Just recently, the media were filled with the story about FC Barcelona's star player Gerard Pique who missed his plane after a game because he could not urinate during a doping control [4]. This case is not unique; several recent studies have shown that a fairly large percentage of athletes experience difficulty regarding providing a urine sample. This pertains to difficulties urinating under supervision [5, 6], feelings of one's freedom being infringed because of being watched during the urination process [6, 7], impaired recovery following a stressful doping control [8], which can also negatively impact future performance [8], or an unpleasant urine control triggering the clinical disorder of paruresis [7, 8]. The reasons for the inability to urinate during a doping control are manifold and are of both a physiological and a psychological nature. For example, physiological causes are that the athlete has just been to the toilet or is too dehydrated from sport, whereas psychological causes refer to reasons like the athlete not being able to urinate while others are watching or while others can hear him or her. Studies conducted with both DCOs [5] as well as athletes [9] on questions related to psychogenic urine retention during doping controls indicate that factors like gender, age, number of previous sample collections and whether the collection is being performed at home, at training, or after a competition have no influence on the phenomenon.

Athletes mention problems in connection with doping controls not only in general but also with the collection of urine samples in particular. In a study conducted by Elbe and Overbye [6], one-third of 400 surveyed Danish athletes reported that they experienced stress because they had difficulty urinating; about one out of seven felt their personal integrity was violated when someone was watching them urinate; and slightly fewer sometimes felt under suspicion during doping tests.

Negative experiences during a doping control may lead to several other consequences for an athlete. The most immediate effect of negative experiences during a control concerns the athlete's recovery level. Recovery entails psychological, social, and physiological processes [10]. A doping control that lasts much longer than anticipated due to an athlete's inability to urinate can lead to an imbalance of an athlete's stress and recovery levels. Some athletes report delays in urinating of up to 3 h or more [5]. This difficulty in urinating at doping controls caused by psychological factors negatively impacts athlete recovery [8]. Furthermore, Elbe et al. [8] showed that athletes who experience psychologically induced urination delays during doping controls are more likely to express a lack of confidence about their athletic competence.

Another negative aspect of urine doping controls that can have a strong psychological impact on athletes' well-being is the possibility that a problematic doping control triggers paruresis. Paruresis, also known as shy bladder syndrome, is the clinical diagnosis of a general state of psychogenic urine retention involving the inability to urinate when other people are around [11]. Triggers for paruretic behavior are (a) the presence of other people, (b) a perceived threat to privacy, and (c) the experience of intense emotions such as anxiety or anger [12]. Soifer et al. [12] pointed out that paruretics often report the onset of their problem as being caused by one unpleasant event while trying to urinate either in a public restroom or during a drug or medical test.

The most successful intervention for psychogenic urination difficulties during doping controls is the use of a urine marker. The marker, used in this study, consists of a mixture of monodisperse low molecular weight polyethylene glycols (PEGs) that is given orally prior to urination. The urine sample can be traced to the donor by analysis of the marker composition. A urine marker can reduce the negative impact of providing a urine sample for drug testing [13, 14] in relation to feelings of embarrassment, infringement of privacy, and stress associated with the collection process. Urine markers are widely used in the drug testing of pilots and convicts. As there are more than 500 possible marker combinations, individual markers can be easily discriminated from each other. Thirty minutes after having swallowed the marker, athletes are allowed to urinate without supervision.

Another advantage of the urine marker, in addition to the fact that it can alleviate psychogenic urine retention during doping controls, is that it can identify urine as coming from a particular person. Urine doping tests in the past were often discussed with relation to the opportunities they offer for cheating because they can tempt athletes to deliver someone else's urine, e.g., by injection into the bladder or insertion of a urine sack into the vagina. One of the most prominent cases was that of the Hungarian Olympic athletes Adrian Annus and Robert Fazekas, who delivered someone else's urine by means of a hose fastened to the back of their penises. Furthermore, in the UK DCOs were warned about the "Whizzinator", a fake penis that comes complete with drug-free urine, and a heater pack to ensure it is maintained at body temperature [15]. Other famous cases of manipulation are those of the German sprinters Katrin Krabbe, Grit Breuer, and Silke Moeller, who all delivered identical urine samples during a training camp in South Africa [16]. By introducing the monitoring of the individual steroid profile in 2014, the manipulation of urine samples can now be discovered more easily. However, by marking the urine with one of the 500 possible versions of the marker, cheating during doping controls can be deterred even further and can also easily be identified. The marker ensures that the urine actually originates from that particular athlete.

The following research questions guide this study:

1. What attitudes do athletes have towards swallowing a urine marker prior to a urine doping control?
2. What are the experiences of athletes who actually swallow a urine marker prior to a urine doping control?
3. Can the urine marker identify urine as coming from a particular person?
4. Does the urine marker interfere with the detection of doping agents by a doping control laboratory?

2 Methods

Three separate studies were conducted to answer the four research questions.

2.1 Study 1

Study 1 was a combination of an online and a paper/pencil survey of athletes with previous experience in urine doping testing. The online study was programmed with SurveyXact. A total of 143 athletes viewed the first page of the online version of the study. Athletes were approached through personal contacts (e.g., sport psychology consultants, coaches, athletes) and Facebook groups, and the link was sent out to sport federations and associations worldwide. In addition, a sample of 69 athletes was recruited through the National Institute of Sport, Expertise and Performance (INSEP), the national elite sport federation of France. Another sample of four athletes was recruited in Greece through the University of Thessaly. The questionnaire focused on biographical information (e.g., age, gender, competitive level, previous experience with doping testing) and contained open and closed questions about the athletes' attitudes towards the use of a urine marker during a urine doping control.

A total of 162 athletes (mean age 26.07 $\pm$ 6.5 years, 61 female) from the overall sample of 212 surveyed athletes indicated previous participation in at least one doping control and were therefore included in the further analysis. The majority of the athletes were French (37 %), Danish (36.4 %), or Belgian (9.3 %). In total, athletes from 13 different countries participated in the study (missing data from one athlete). The competitive level of the athletes was distributed as follows: international level (72.2 %), national level (21.6 %), and regional level (6.2 %). Mean years of sport career were 13.6 $\pm$ 6.1 (range 1–36 years), and the mean number of completed doping controls was 7.2 $\pm$ 11.3 (range 1–100 doping controls). 52.2 % of athletes indicated that they had previously experienced problems urinating during at least one doping control. The athletes participated in the following sports, categorized according to Alaranta et al. [17]: speed and power sports (e.g., sprinters, 44.4 %), team sports (e.g., volleyball, 25.3 %), motor skill-demanding sports (e.g., gymnastics, 17.9 %), and endurance sports (e.g., cycling, 11.7 %) (missing data from one athlete). All participants provided informed consent and were ensured of the confidentiality of their responses.

2.2 Study 2

Study 2 involved the actual testing of the urine marker on athletes and a survey about their experiences with this method. After the athletes had given written consent, they swallowed the marker (PEGs) and delivered their urine after a waiting period of at least 30 min. After they delivered their urine, they filled in the questionnaire about their experiences with the procedure.

Prior to conducting the study, we looked at several urine samples from people who had not ingested the PEG marker and found in the US that all the samples contained baseline PEG concentrations. It was decided that a pilot study was needed to determine the concentration of PEGs to administer so that the baseline PEGs would not interfere with the ability to determine, based on the PEGs, that the urine came from a particular athlete.

A total of 91 athletes participated in either the pilot or main study (mean age 21.3 $\pm$ 5.4 years), with 50 being

female (54.9 %). All athletes were living in Germany during data collection; 39.6 % of athletes ($n = 36$) had previous experience with doping control testing, with a mean number of completed doping controls of 4.6 ± 6.1 (range 1–30 doping controls). For the other 55 athletes (60.4 %), this study was their first experience with a doping control test. The athletes competed in the following sports: athletics ($n = 68$), combat sports ($n = 19$), and ice hockey ($n = 4$).

There were two different studies testing the marker. In a pilot study, athletes received different concentrations of the marker in order to determine the optimal marker concentration. To determine the optimal amount of PEGs for oral intake, 36 of the 91 athletes (described above) received different concentrations of the marker. Two participants received 125 mg each of PEG 8, 9, 10, and 11. Five participants received 125 mg each of PEG 8 and 9. Five participants received 100 mg each of PEG 8, 9, 10, and 11. Three athletes received 100 mg each of PEG 8 and 9. Seven athletes received 50 mg each of PEG 8, 9, 10, and 11. Seven athletes received 25 mg each of PEG 8, 9, 10, and 11. Seven athletes received 5 mg each of PEG 8, 9, 10, and 11. The results from this pilot study indicated that 50 mg of each PEG is sufficient for unambiguous assignment of the applied marker in urine samples.

In the main study, 51 athletes received PEG 8 and 9 each at a concentration of 50 mg, and 28 athletes received PEG 10 and 11 each at a concentration of 50 mg. A total of five athletes received all four PEGs (8, 9, 10, and 11), each at a concentration of 50 mg.

2.3 Study 3

Study 3 involved the analysis of urine samples collected in the second study. A Shimazu Prominence LC-20AD liquid chromatography (LC) system with an SIL-20AC HT autosampler coupled to an AB Sciex 5500 QTRAP tandem mass spectrometer (MS/MS) with an electrospray ionization interface was used for this study. Low molecular weight monodisperse PEGs were purchased from CS, Am Parir 27, 52379 Langerwehe, Germany. All chemicals were of chromatography grade. An LC–MS/MS method was developed to monitor two *m*/*z* transitions for PEGs with 8, 9, 10, and 11 repeating units and molecular weights of 370.4, 414.5, 458.6, and 502.6.

Urine samples were diluted in deionized water and 5 μL of diluted sample were injected onto the LC column. No sample cleanup was performed. PEGs were separated by a Phenomenex Kinetex C18 50- × 2.1-mm LC column with a 2.6-micron particle size and 100-Å pore diameter at 40 °C. Solvent A was 5 mM ammonium acetate in water (pH 3.47), and solvent B was acetonitrile. A gradient of 10–60 % solvent B over a 5-min period at a flow rate of 0.3 mL/min was used for separation. Parameters for the electrospray ionizations source and mass spectrometer were optimized for each PEG by direct infusion of each PEG standard (PEG 8, PEG 9, PEG 10, and PEG 11). Optimal signal-to-noise ratio was obtained with a spray voltage of 5500 volts and a gas temperature of 450 °C. The mass spectrometer was operated in multiple reaction monitoring (MRM) mode. Identification of PEGs in urine was done by monitoring retention times and two MRM transitions. The following transitions (precursor/product pairs) were monitored: PEG 8 371.2 → 89, 371.2 → 133; PEG 9 415.2 → 89, 415.2 → 133; PEG 10 459.2 → 89, 459.2 → 133; and PEG 11 503.2 → 89, 503.2 → 133. MRM transitions are unique for each PEG, and each PEG elutes off the column at a different time. The linearity of the method was determined by analysis of known concentrations of each PEG. Replicate samples were analyzed by LC–MS/MS, and the method was shown to be linear from 0.4 to 100 ng/mL based on R^2 values greater than 0.99.

Quantitation of each PEG was achieved by comparing the peak area against a calibration curve of known standards. Standards were prepared gravimetrically and each pure monodispersed PEG was re-suspended in water prior to analysis. The majority of urine samples were diluted with water prior to analysis to obtain peak areas that were within the linearity range of the method.

Urine samples from athletes (30 min post administration) in the pilot study that had received 100 mg of each PEG were subjected to a liquid–liquid sample cleanup procedure, and the extracts were evaluated by an LC–MS/MS method that is routinely used by a doping control laboratory to detect anabolic steroids, anti-estrogenic agents, and glucocorticoids, as described in Langman and Snozek [18].

2.4 Ethics

All participants gave informed consent prior to participation in the studies. The use of PEGs as a marker substance was approved by health authorities in Europe (European Medicines Agency) and the USA [Food and Drug Administration (FDA)]. All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

3 Results

3.1 Study 1

The results indicate that 61.9 % of the athletes would be willing to swallow a capsule containing a urine marker during a doping control test. 33.1 % of athletes feel that

this alternative method could alleviate the problems some athletes experience during a supervised urine collection. There were no significant differences between athletes who had experienced previous problems urinating during a doping control test and those who had not with regard to these questions. Overall 47.6 % of the athletes would prefer taking the marker instead of a regular supervised urine collection. However, of the athletes that had problems with doping control tests in the past, 59.0 % preferred taking the marker versus 32.3 % that did not have problems. A Chi square test revealed that there was a significant difference between responses from athletes with problems in the past and the athletes with no previous problems ($p < 0.01$).

Furthermore, athletes were asked to respond to several open questions. With regard to the question of why the urine marker would alleviate urination problems during a doping control test, a total of 43 athletes gave an explanation. The main reasons were more privacy because of less supervision (15; 34.8 %), less stress and pressure (13; 30 %), the control is faster (4; 9 %), more freedom (3; 6.9 %), it is easier to urinate if no one is watching (3; 6.9 %), the procedure is less embarrassing (3; 6.9 %), and it is easier to swallow a pill than to urinate in front of someone (2; 4.6 %). With regard to anticipated negative impacts of swallowing the urine marker prior to a training session, 11 athletes mentioned possible impacts. The most frequently mentioned negative impact was that athletes were unsure about the exact content of the pill and that these worries could negatively impact their training (10; 90.9 %). One athlete was concerned about throwing up and "losing" the marker (1; 9.1 %).

Furthermore, 17 athletes used the possibility to add comments about the study. They voiced full support of the new method (6; 35.3 %), that they would like more detailed information about the content of the capsule and possible side effects (3; 17.6 %), they would never swallow a capsule given to them (2; 11.8 %), they would prefer to give blood over swallowing a capsule (2; 11.8 %), they would never swallow a capsule given to them outside of their home country (1; 5.9 %), they had concern that this procedure is more expensive (1; 5.9 %), they see no necessity in a new procedure because the old one works fine for them (1; 5.9 %), and they are concerned about possibly not being able to deliver 90 mL of urine after having swallowed the capsule (1; 5.9 %).

3.2 Study 2

In study 2, the athletes' experiences with taking the marker prior to a doping control were investigated. On a scale from 1 (unpleasant) to 7 (pleasant), athletes on average rated placing the capsules in their mouth as 5.05 (SD = 1.4) and swallowing the capsule as 5.08 (SD = 1.5). The fact that athletes had to wait for 30 min before they were permitted to urinate was rated on a scale from 1 (very burdensome) to 7 (no burden at all) as 4.81 (SD = 1.5). 95.5 % of the athletes in study 2 indicated that they would be willing to take the urine marker during a doping control test. Furthermore, 85.3 % of the athletes who had previous experience with the standard urine doping control procedure reported that they preferred to take the urine marker instead of having to provide a supervised urine sample.

In the open questions addressing which negative impact a doping control test with the urine marker prior to a training session could have on their training, a total of five athletes mentioned potential impacts. These were that they felt having to urinate during their training would be an interruption (2; 40 %), they were worried that the marker might have an adverse effect like an allergic reaction (2; 40 %), and one athlete was concerned about throwing up due to strenuous training and then losing the marker (1; 20 %). A total of 27 general comments were given about the study. The majority of athletes expressed their support of the study and expressed how positive they were towards this new type of doping control test (13; 48 %); they mentioned that they find the new procedure much less intrusive and also faster (6; 22.2 %); they voiced their concern that additional substances could be added to the urine since no one is supervising the urination process (2; 7.4 %) and that the delay of 30 min before urinating was too long (2; 7.4 %); they wanted even more detailed information about the content of the urine marker pill before they could comment (2; 7.4 %); they voiced concern about how to really ensure that athletes actually swallow the marker (1; 3.7 %); and they commented that he/she would never swallow a capsule given by anyone (1; 3.7 %).

3.3 Study 3

The lower level of quantification and the lower level of detection for LC–MS/MS analysis were determined to be 1 and 0.3 ng/mL, respectively, for each low molecular weight PEG tested. Linearity was observed from 0.4 to 100 ng/mL. As shown in Fig. 1, the different low molecular weight polymers of monodispersed PEGs can easily be separated without interference from unwanted compounds. The retention time and the precursor product pairs (transitions) can then be used to correctly identify and quantify the concentration of each PEG in urine samples.

In many of the urine samples tested and also in the preliminary study in the USA, we observed background signals for polydisperse low molecular weight PEGs. In the samples from athletes that consumed at least 50 mg of each monodispersed PEG orally (e.g., four markers for a total concentration of 200 mg or two markers for a total

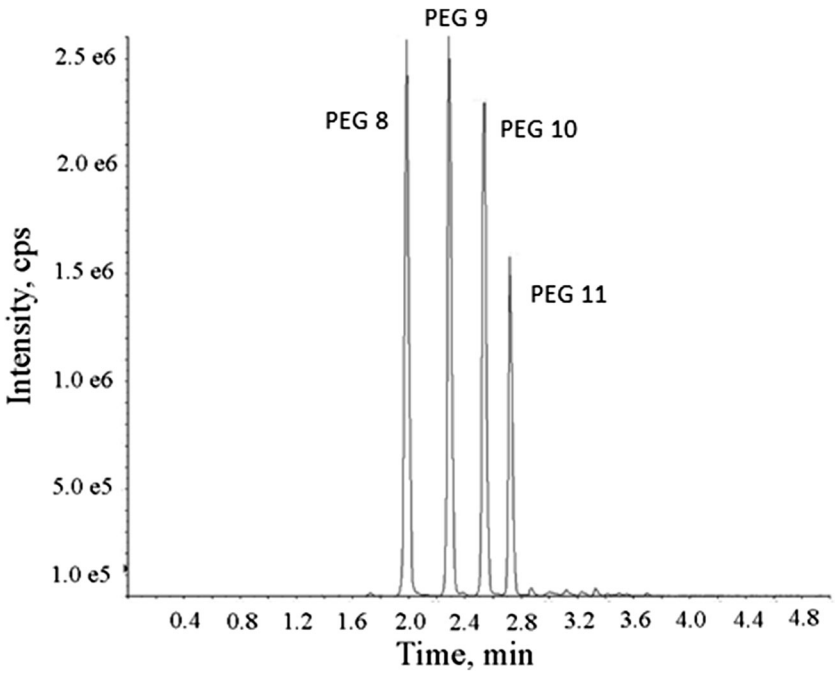

Fig. 1 LC–MS/MS chromatograph of four different low molecular weight polyethylene glycols in a urine sample. The urine sample was spiked with PEG 8, PEG 9, PEG 10, and PEG 11 each at a concentration of 50 ng/mL. *CPS* counts per second, *LC–MS/MS* liquid chromatography tandem mass spectrometry

concentration of 100 mg), the marker could clearly be identified from background signals. It was therefore concluded that the marker should be given in a total concentration of 50 mg of each monodispersed PEG (e.g., 200 mg in total for four different PEGs in a concentration of 50 mg each). The four different types of PEGs in the urine samples of participants who had consumed 100 mg of each monodisperse PEG orally ($n = 8$) did not produce chromatographic peaks for any of the transitions that were routinely monitored for a wide range of prohibited substances. This indicates that the monodispersed PEGs in a concentration of up to 400 mg in total do not interfere with the ability to detect the presence of prohibited substances in urine samples routinely analyzed by doping control laboratories.

4 Discussion

The results of the two questionnaires indicate that athletes in general seem to be positive towards the marker. Athletes, however, who actually tested the alternative method seem to be even more positive and for a large part prefer this procedure to the standard doping control procedure. It needs to be mentioned, though, that the samples of the two studies were not identical. In study 1, all athletes had previous experiences with a doping control. This was not the case with athletes in study 2, where only around 40 % of the athletes had previously experienced a doping test. Also, the geographic distribution of athletes was more diverse in study 1. Athletes felt that swallowing the marker was more pleasant than unpleasant and the delay of 30 min before urinating was not too burdensome. It could have been expected that more athletes find swallowing a "capsule" for anti-doping purposes burdensome as it goes against the idea of clean sport and capsules are a symbol of "unclean sport." It was surprising that only one athlete commented on the fact that he would never swallow a pill that was given to him by anyone. There were a small number of concerns and questions voiced in both studies. Prior to implementation, athletes would need even more information about the exact contents of the markers and potential negative side effects. Also, questions like what to do if one has to throw up or is unable to provide 90 mL of urine need to be explained to the athletes. For some athletes, it was also of concern that additional substances could be added to the urine after urination since no one supervises the urine collection procedure. However, additional substances added to the urine after urination are commonly identified with the sample check test, the Trinder reaction, and/or a general solid phase test to uncover falsifications, which are very sensitive towards adulteration. These tests can identify common procedures used to adulterate a urine sample. A concern that was not mentioned by the athletes but which needs further consideration is the fact that according to the WADA "International Standard for Testing and Investigations" "… any urine Sample provided by the Athlete to the Sample Collection Personnel should be the first urine passed by the Athlete subsequent to notification, i.e., he/she should not pass urine in the shower or otherwise prior to providing a Sample to the Sample Collection Personnel" [3].

Therefore, supervision of the athletes during the 30 min needs to be ensured.

Analysis of urine samples indicated that different polydisperse low molecular weight PEGs are already present in athletes' urine without consumption of the marker. PEGs, for example, are on the FDA and many other health authorities' "list of inactive ingredients." PEGs can easily be mixed with water or many of the most common organic solvents and are an inexpensive, potent solvent. PEGs are used to a large extent by the pharma and food industry and therefore can be commonly found in many urine samples. With the high degree of security that is particularly needed with regard to urine doping control, the results of this study suggest that the monodisperse PEG peak or signal should be significantly higher than what is obtained for background polydisperse PEGs. Therefore, the recommendation is to orally ingest 200 mg of monodisperse PEG in order to ensure that the PEG contained in the urine marker can be easily distinguished from background PEGs. Even a concentration this high does not interfere with the analyses that are commonly conducted in a doping control laboratory.

And lastly, it needs to be addressed that this alternative method will only help athletes who have difficulties with providing urine under supervision. This method will not help athletes who, in general, have urination difficulties because they fear doping controls and/or fear a positive test result because, for example, they have taken forbidden substances by mistake [6].

5 Study Limitations and Future Research

There are some limitations to our study that need to be addressed. First of all, the samples in study 1 and 2 showed differences with regard to previous experiences with doping testing and geographic distribution which could have an effect on the results. Also in study 2, participants were informed that the doping control was performed for study purposes and therefore was not a "real" doping test. The fact that it was not an actual doping control could have impacted the results. With regard to study 3, it needs to be stated that only four different markers were tested although there are up to nine different markers currently available, allowing for over 500 different combinations. Future studies therefore could include the use of more markers. Another important future research question is whether the application of preparations or formulations that contain polydispersed PEGs in varying amounts (e.g., orally given laxatives) interfere with the urine marker method.

It would also be interesting to investigate the detection of PEGs in urine together with the presence of doping agents. This would require a study in which urine samples were spiked with doping agents.

Last, it would be relevant to look for the expulsion rate of the PEG solution, expressed as units per min, to get information about the kidney excretion rate of PEGs (e.g., from 30 min to 2 h). For this, an additional study would need to be conducted in which all urine is collected at various time periods after giving PEGs.

6 Conclusion

This study is the first to investigate athletes' acceptance of the urine marker in a questionnaire study as well as in its practical application. Furthermore, it is the first study to test whether the urine marker can identify urine as coming from a particular person and whether it interferes with the detection of prohibited substances by a doping control laboratory. The results of the three studies indicate that this new procedure finds acceptance from athletes and can make the doping control procedure less invasive and eliminates the possibility of delivering someone else's urine during the doping control procedure. The results suggest that the urine marker should be given in a concentration of 200 mg in order to ensure that it can clearly be distinguished from other PEGs the athlete has ingested through food and drink. Several limitations of the studies were addressed and future research perspectives were given.

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Compliance with Ethical Standards

Conflict of interest Ruprecht Keller, Anne-Marie Elbe, and Anthony W. Butch received a grant from Partnership for Clean Competition entitled "A new labelling procedure which allows to identify urine as coming from a particular person."
Stine Nylansted Jensen, Peter Elsborg, Monika Wetzke, Getachew A. Woldemariam, and Bernd Huppertz declare that they have no conflict of interest.

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