Review

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Anti-doping testing at the 2008 European football championship

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Big sports events like the 2008 European Football Championship are a challenge for anti-doping activities, particularly when the sports event is hosted by two different countries and there are two laboratories accredited by the World Anti-Doping Agency. This challenges the logistics of sample collection as well as the chemical analyses, which must be carried out timeously. The following paper discusses the handling of whereabouts information for each athlete and the therapeutic use exemption system, experiences in sample collection and transportation of blood and urine samples, and the results of the chemical analysis in two different accredited laboratories. An overview of the analytical results of blood profiling and growth hormone testing in comparison with the distribution of the normal population is also presented.

Keywords: blood profiling; football championship; growth hormone; sports doping

Introduction

Looking back into history, the first European Football Championship was held in 1960. The early competitions involved four European teams qualifying for the final tournament. The number was subsequently increased to eight teams and then to 16 teams for the four most recent competitions. The 2008 European Championship final tournament, hosted by Switzerland and Austria, took place from 7 June to 29 June. It was the thirteenth competition and involved the following 16 teams:

Group A: Switzerland, Czech Republic, Portugal, Turkey.
Group B: Austria, Croatia, Germany, Poland.
Group C: Netherlands, Italy, Romania, France.
Group D: Greece, Sweden, Spain, Russia.

Each squad contained 23 players, making a total of 368 players. The intention of the Union of European Football Associations (UEFA) for the 2008 tournament was not only to go further than the previous competitions in its anti-doping testing programme but also to use all World Anti-Doping Agency (WADA) approved analytical methods/tools available at the time in order to create the best possible testing programme.

Following the recommendation of the UEFA Anti-Doping Panel, the UEFA Executive Committee decided that blood should be collected in addition to urine every time a player was tested, in or out of competition. Compared to previous major international sports events like Olympic Games[1,2,3] this was the first time that blood and urine were collected from each tested player. In total, 286 players were tested. Analysis of the blood samples covered not only blood parameter determination and testing for homologous blood transfusions using whole blood as testing matrix, but also testing for human growth hormone (hGH) doping as well as for artificial haemoglobin (HBOC), both requiring serum as testing material. The hGH method, which had been recently improved, was approved by WADA prior to the final tournament.[4] The same method was subsequently used at the 2008 Olympic Games in Beijing. In addition, UEFA decided to conduct erythropoietin (EPO) analysis on every sample collected in competition (IC) as well as out of competition (OOC).[5] Another innovation was the systematic IRMS analysis of all in-competition samples to identify synthetic testosterone or testosterone precursor substances[6] for the first time during a sports tournament.

This article focuses on the logistics and the testing schedule and selected final results of the Anti-Doping programme of the European football championship in 2008.

Testing Schedule, Sample Collection and Transportation Logistics

On 5 March 2008, UEFA informed the participating teams that there would be OOC testing in the run-up to the tournament and that every match during the final tournament would be tested. The EURO 2008 anti-doping programme was presented at a press conference attended by a WADA representative as well as the UEFA president. A UEFA anti-doping charter accepting the implementation of UEFA's anti-doping programme was signed by all presidents of the participating teams. The next day, a meeting took place with the team doctors of all qualified teams focussing on the IC and OOC doping-control procedure, including the blood testing procedure and equipment used and the requirement to submit the players' declaration of consent on the

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A CZECH REPUBLIC
Bad Kleinkirchheim, AUT, 700 km, 21.05.08

B TURKEY
Marienfeld, GER, 1000 km, 21.05.08

C POLAND
Donaueschingen, GER, 1600 km, 22.05.08

D PORTUGAL
Viseu, POR, 3700 km, 23.05.08

E ROMANIA
Bucharest, ROM, 2000 km, 25.05.08

F RUSSIA
Rottach, GER, 950 km, 26.05.08

G ITALY
Florence, ITA, 800 km, 27.05.08

H NETHERLANDS
Apeldoorn, NED, 2100 km, 28.05.08

I GREECE
Neu-Isenburg, GER, 2500 km, 28.05.08

J SWITZERLAND
St Gallen, SUI, 1400 km, 29.05.08

K AUSTRIA
Graz, AUT, 500 km, 29.05.08

L SPAIN
Las Rozas, ESP, 3000 km, 29.05.08

M SWEDEN
Stockholm, SWE, 2600 km, 30.05.08

N GERMANY
Kamen, GER, 900 km, 30.05.08

O FRANCE
Clairefontaine, FRA, 2000 km, 02.06.08

P CROATIA
Bad Tatzmannsdorf, AUT, 450 km, 03.06.08

A AUSTRIAN RESEARCH CENTERS
Seibersdorf, AUT

B SWISS LABORATORY FOR DOPING ANALYSIS
Lausanne, SUI

Figure 1. Location of the training camps (light grey balloons) of all the teams participating in the EURO 2008 final tournament as well as the location of the testing laboratories (A and B). The distances describe the approximate length of the total journey each BCO took from leaving home to the venue of sample collection and back to the laboratory.

in-competition and out-of-competition programme implemented at this final tournament (in particular blood testing). In addition, UEFA explained to the team doctors the whereabouts information that needed to be received and the therapeutic use exemption (TUE) process. At the end of the meeting all 16 team doctors also signed the charter.

Two countries with two WADA–accredited laboratories hosted the tournament. Since the UEFA Doping Control Officers (DCOs) were based in Lausanne, near UEFA's headquarters, for the duration of the tournament, it made sense to use the Lausanne laboratory for all in-competition sample analysis as the DCOs could deliver the samples when they returned to their base camp. It was also the guarantee that all samples collected on Swiss territory could be delivered to Lausanne within a few hours after the end of the match and that the analysis could start without delay upon receipt. As a consequence, Seibersdorf was appointed to analyse all OOC samples.

Teams had to provide UEFA with weekly reports of their players' whereabouts from the beginning of May. As the teams only arrived at their tournament base camps in Austria and Switzerland at the beginning of June, most of the out-of-competition tests were carried out at pre-tournament training camps elsewhere in Europe. Figure 1 presents the training locations of the participating teams, the day of OOC sample collection as well as the distance to the testing laboratory (A) for OOC-samples. Ten players from each team were tested in the pre-tournament period.

The UEFA had its own panel of doping control officers (DCOs) and appointed them directly. All were qualified doctors and experienced DCOs. Most of them had been conducting out-of-competition testing (OOCT) programme in the UEFA Champions League since the 2005/2006 season. Eleven blood-collection officers (BCOs) were selected according to their professional experience in blood collection and they underwent special preparation and training for EURO 2008. Six of them were retained for in-competition testing during the final tournament. All BCOs received selected blood equipment. BCOs trained twice daily with this material from January 2008.

Clear instructions were drafted by UEFA for the BCOs on how to use the blood equipment by issuing a step-by-step procedure for actual blood sampling.

A preparation workshop for the 12 collection officers selected for in-competition testing (six DCOs plus six BCOs) was held at the anti-doping base camp in Lausanne the day before the opening match. The aim was to provide the UEFA DCOs with the feedback of the out-of-competition controls and to provide them with instructions for the in-competition testing in order to have a harmonized approach.

The testing conducted by UEFA DCOs follows the UEFA Anti-Doping Regulations,[7] which comply with the WADA International Standard for Testing[8] and FIFA AD Regulations.[9]

Four separate tubes were needed for whole blood (A- and B-sample) and for serum (A-and B-sample) in order to conduct the blood-test scheme. The WADA-approved blood-analysis method for hGH and HBOCs analysed serum only, while the analytical methods for blood transfusion and blood parameters needed whole blood.

The blood samples were intended to be analysed shortly after collection and had to be kept at a stable temperature range (between 4–12 °C). It was therefore necessary to develop a cooling box having enough capacity to keep the blood close to this temperature range for at least 48 hours. A temperature-monitoring device was used to monitor the temperature during transportation. Deviations were recorded by an alarm system. Butterfly needles were used for venipuncture as this equipment had already been used successfully at the 2002 FIFA World Cup in Japan and Korea. It was found to be less intrusive for the player. Special doping-control forms were created for blood collection.

Whereabouts System

All 16 participating teams had to collect whereabouts information according to the World Anti-Doping Code[10] for all their players and provide UEFA with weekly reports/schedules for the following week every Friday before 5 pm using a whereabouts form specifically developed for the EURO 2008. Whereabouts information comprised details of all training camps/sessions, friendly matches of the national team, the address of the training ground or match venue and the address of the hotels where the team were staying between 1 May and 6 June, at the latest the first time the team gathered together.

Therapeutic Use Exemption Management

Therapeutic use exemptions had to be submitted to UEFA in agreement with the WADA International Standard for Therapeutic Use Exemptions,[11] 21 days before the start of the tournament, as per the WADA requirements. Including the preparation weeks before the start of the tournament, 30 TUEs were dealt with by UEFA (22 abbreviated, eight standard) for 28 different players. Of the 22 abbreviated TUEs, 20 were delivered by the UEFA Therapeutic Use Committee and two were recognitions of certificates delivered by national anti-doping organizations. Two cases concerned asthma and the remaining cases concerned local use of glucocorticosteroids. In addition eight standard TUEs were submitted to the UEFA TUE Committee, which accepted four and refused four.

Out-Of-Competition Testing

UEFA decided to test the 16 teams during the preparation weeks prior to the start of the final tournament. Consequently 160 players were tested OOC. There were no positive tests, no whereabouts failures (filing failures/missed tests) and the co-operation of the teams was excellent.

This was regarded as a major improvement in testing capacity and density compared to previous tournaments. At the EURO 2000 in Belgium and Holland, four teams out of 16 were tested OOC (four players from each team). At the EURO 2004 in Portugal, all 16 teams were tested OOC (four players per team). In total 64 players were tested OOC at the EURO 2004 compared to 160 at EURO 2008. For the first time at a UEFA competition, each player tested had to provide a blood sample in addition to the standard urine sample so that new prohibited substances and methods could be detected. All available WADA-approved methods were used to analyse the blood samples, including human growth hormone (hGH), blood transfusion and the haemoglobin-based oxygen carriers (HBOCs).

As mentioned previously, the target was to deliver all collected samples to the WADA-accredited laboratory of Seibersdorf/Austria in time (within about 12 hours from collection) and with the samples of adequate quality for testing. Although some of the DCOs experienced transport problems due to airline restrictions for liquid transportation within the cabin, all urine and blood sample exhibited sufficient quality for further analysis.

In-Competition Testing

Two players plus two reserves were drawn from each team to undergo testing. The reserves were called upon if a player drawn suffered serious injury which required hospital treatment. Any of the 23 squad players could be drawn for testing, regardless of whether they actually played in the match or were sitting on the bench. In 2000, 2004 and 2008 all 31 matches of the tournament were tested. However in 2004, EPO was not analysed systematically in all samples; in 2000, the EPO analysis method was not approved at that time.

In addition, all IC samples collected in 2008 were analysed with isotope ratio mass spectrometry (IRMS) and for hGH.

All test results of the samples collected IC (126 players' blood and urine samples) were negative too.

The national anti-doping organizations (NADOs) of both hosting countries collaborated with UEFA on the 2008 final tournament. Their DCOs acted as chaperones. Four chaperones were present at each of the eight venues. In 2004, the chaperones were not part of the Portuguese NADO but were recruited by the Portuguese NADO according to UEFA's requirements and then trained jointly by UEFA and the Portuguese NADO.

At the 2000 and 2004 tournaments, all IC samples were analysed within 24 hours and 48 hours for EPO. However, in 2008, the analysis for both standard, EPO and in addition IRMS was done within 24 hours. The Lausanne laboratory worked 24 hours a day during the period of the tournament. The results were therefore available before the relevant team's next match.

For the final tournament of the EURO 2008, six teams of two doctors were appointed to carry out in-competition testing. Each pair comprised a BCO in charge of blood collection and DCO in charge of urine collection. The 12 doctors were based in Lausanne, close to the WADA-accredited laboratory.

The laboratory is based in Epalinges near Lausanne (Switzerland), so deliveries of samples after testing carried out after

matches in Switzerland were relatively quick, whereas the samples collected in Austria were delivered on the next day. Despite some transportation problems with one airline the quality of samples was satisfactory at delivery and could be analysed in time.

Urine Collection

The same urine collection procedure was used IC and OOC, except that a suitable doping-control station (DCS) had to be planned and organized by the teams for the OOC controls, either at the training venue or at the hotel where the team was staying. For IC collection, the DCS of each venue had already been designed and organized by UEFA in the course of 2007. Each DCS had a urine sampling area with toilets adjoining, a blood sampling area and a waiting room. Standard urine collection kits were used.

Blood Collection

For in-competition tests, the cooling box was prepared by the Lausanne laboratory and collected by the BCO prior to departure. For OOC tests, the BCO prepared the cooling box himself based on a protocol and procedure defined by UEFA and both anti-doping laboratories.

The following equipment was used for the blood control of each player:

One bag containing:

- 2 × 3 ml whole-blood tubes with EDTA as stabilizer
- 2 × 5 ml serum tubes with spray coated silica as clot activator

One bag containing:

- 1 butterfly needle
- 1 tube (to attach to the butterfly needle)
- 1 pair of sterile gloves
- 1 disinfectant towel
- 1 plaster

Two blood transportation kits with different numbers for whole blood and serum tubes were used. Each small blood kit contained:

- 2 small hard plastic bottles (A+B)
- 1 absorbent pad
- 8 stickers with sample code number and bar code (for lab)
- instructions for use (small)

In addition one doctor's case contained spare blood sampling equipment (butterfly needle, tubes, etc.), one cooling box (including the refrigerated elements), a temperature-monitoring system and a seal for customs purposes.

In order to compare profiles, two players were target tested in competition in addition to the scheduled testing scheme.

It took an average of about 12 minutes to conduct blood sampling for one player. It took an average of about 50 minutes for four players during in-competition testing and an average of two hours for 10 players during OOC testing.

Transport to the Laboratory

Out-of-competition samples

Out-of-competition testing started on 21 May 2008, close to the beginning of the tournament. The reason for starting to test teams close to the first matches is that most teams did not gather for training before this time because many players were still playing with their clubs in national-level competitions.

Ten players were tested each time; each collection consisted of 20 urine samples, 20 whole blood samples and 20 serum samples (both A and B). Although the temperature monitoring device alarmed for some collections (see Table 1), none of the whole blood samples showed any sign of degradation or haemolysis. All data from blood profile analysis were valid for further conclusions.

Table 1. Overview of the delivery conditions of the OOC samples (ten blood and ten urine samples per mission) to the Seibersdorf laboratory; travel times reflect the approximate mean duration of sample transfer. Temperature information (Q-Tag system) of both deliveries is presented

Collection date	End of collection	Delivery date	Travel time (h)	Q-Tag information	Max. temp. (°C)
21.05.2008	15.20	21.05.2008	7	OK	
21.05.2008	15.50	21.05.2008	7	OK	
22.05.2008	15.35	22.05.2008	9	Alarm	15.8
23.05.2008	22.11	24.05.2008	15	OK	
25.05.2008	16.25	25.05.2008	6	OK	
26.05.2008	23.05	27.05.2008	10	Alarm	11.8
27.05.2008	22.15	28.05.2008	15	Alarm	14.2
28.05.2008	15.10	28.05.2008	8	Alarm	18.6
28.05.2008	15.10	28.05.2008	8	Alarm	22.8
29.05.2008	15.25	29.05.2008	5	Alarm	14.9
29.05.2008	21.10	30.05.2008	11	No recording	
29.05.2008	17.30	30.05.2008	19	Alarm	14.8
30.05.2008	15.55	30.05.2008	8	OK	
30.05.2008	21.42	31.05.2008	11	OK	
02.06.2008	12.30	02.06.2008	10	OK	
03.06.2008	17.10	03.06.2008	3	Alarm	13.6

In-competition samples

In-competition testing started on 7 June 2008, with the opening matches. All IC samples collected were transported to the WADA-accredited laboratory in Lausanne (by road for samples collected in Switzerland or air for the ones collected in Austria).

Two players from each playing team were tested each evening. After each match, collection consisted of four urine samples, four whole blood samples and four serum samples. In Table 2, the travel times are given for each competition event. Samples from both matches in Austria arrived at the day after the match, while samples from Switzerland arrived at the night of the same day. This meant that a maximum of eight urine samples as well as 8 serum and 8 whole blood samples arrived in parallel for screening analyses. Within a maximum reporting delay of 24 hours negative results could be reported before the next match of the tested team.

Results and Discussion

Sample collection logistics

As a result of the experiences in transporting the blood samples from OOC testing, the instructions for cooling the samples have been adjusted. The BCOs were asked to put the cooling box in the hotel fridge or, if it was too small, at least all the sample bottles and the cooling elements.

Table 2. Overview of the delivery conditions of the IC samples to the Lausanne laboratory (four blood as well as four urine samples per match); only the longest duration of two deliveries per match day is given. Temperature information (Q-Tag system) of both deliveries is presented

Collection date	End of collection	Delivery date	Travel time (h)	Q-Tag information	Max. temp. (°C)
07.06.2008	23.35	08.06.2008	3	OK/OK	
08.06.2008	23.40	09.06.2008	11	Alarm/Alarm	14.2/17.4
09.06.2008	23.20	10.06.2008	4	OK/Alarm	–/15.5
10.06.2008	22.45	11.06.2008	13	OK/Alarm	–/21.2
11.06.2008	23.20	12.06.2008	4	OK/OK	
12.06.2008	23.35	13.06.2008	12	Alarm/Alarm	14.5/18.5
13.06.2008	23.05	14.06.2008	3	OK/OK	
14.06.2008	23.20	15.06.2008	15	Alarm/Alarm	14.0/19.6
15.06.2008	23.20	16.05.2008	4	OK/OK	
16.06.2008	23.25	17.05.2008	12	OK/Alarm	–/12.4
17.06.2008	23.50	18.06.2008	3	OK/OK	
18.06.2008	23.15	19.06.2008	13	OK/Alarm	–/17.6
19.06.2008	23.25	20.06.2008	4	OK	
21.06.2008	0.15	21.06.2008	11	Alarm	15.4
21.06.2008	24.00	22.06.2008	4	OK	
23.06.2008	0.15	23.06.2008	11	Alarm	17.7
25.06.2008	23.20	26.06.2008	4	OK	
26.06.2008	23.25	27.06.2008	11	Alarm	17.3
29.06.2008	23.45	30.06.2008	11	Alarm	19.2

Table 3. The pH and specific gravity of the urine samples collected during IC and OOC testing

	pH		Specific gravity	
	OOC samples	*IC samples*	*OOC samples*	*IC samples*
No. of samples	160	126	160	126
Mean	6.09	5.7	1.019	1.016
Median	6.1	5.5	1.021	1.017
Min.	4.7	4.8	1.004	1.003
Max.	7.6	7.6	1.039	1.032
SD	0.58	0.57	0.008	0.007
CV	10%	10%	1%	0.7%

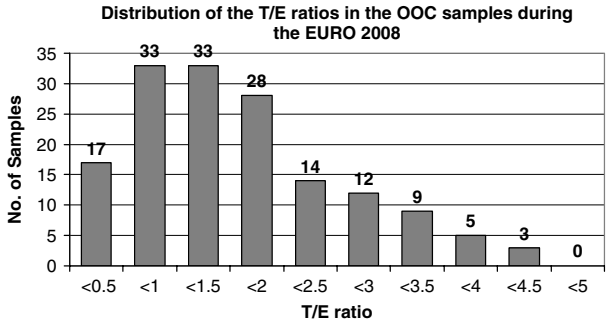

Figure 2. Distribution of the testosterone to epitestosterone (T/E) ratios in the out-of-competition samples during the EURO 2008.

Sample analysis

Due to WADA accreditation of both laboratories, which requires constant external quality control of the laboratories, the analysis of the samples was of equal quality in both laboratories, objectified by equal results of WADA - proficiency tests performed prior to the tournament. Significant differences in sample treatment and results evaluation could be excluded by the close co-operation of both laboratories prior to the tournament. Analysts of each laboratory were trained in the other laboratory for method harmonization, especially the detection of rEPO, hGH and homologous blood doping.

Harmonization in case of blood parameter determination was achieved by the fact that both laboratories participated in external quality control programme[12] for blood samples.

Pre-analytics

Table 3 summarizes the pH and specific gravity of the urine samples (A-samples) collected during IC and OOC testing.

Urine testing

Out-of-competition urine samples were tested for substances prohibited out-of-competition, which comprised substances in the following groups mentioned in the 2008 Prohibited List[13]

S1: Anabolic Agents
S2: Hormones and Related Substances; 1. Erythropoietin, 3. Gonadotrophins
S3: Beta-2-Agonists
S4: Hormone Antagonists and Modulators
S5: Diuretics and other Masking Agents

IC urine samples were further screened for:

S6: Stimulants
S7: Narcotics
S8: Cannabinoids
S9: Glucocorticoids

Each OOC sample showing a testosterone to epitestosterone ratio significantly above 4 was further analysed for synthetic testosterone or testosterone precursors using gas chromatography combustion isotope ratio mass spectrometry (GC/C/IRMS). Each IC sample was analysed by GC/C/IRMS, regardless of the TE ratio.

To meet the strict time requirements of the event organizer for the competition phase (reporting within 24 h) a fast clean up of the samples was done for IRMS analysis. To render clear negative results within this timeframe, samples were cleaned with SPE[14] and analysed using IRMS. Endogenous reference substance concentrations were measured from the IRMS extract in parallel.

Only clear negative results were given in 24 hours. Samples with increased T/E or any results leading to a need for confirmation would have needed longer for a final result (Figure 2 and 3).

The presence of recombinant erythropoietin was tested using the method described by Lasne *et al*. comprising ultrafiltration, isoelectric focussing (IEF), double blotting and chemiluminescence detection.[15] No suspicious profiles were detected. For some samples a clear negative result was not achieved immediately after the first screen; these samples passed an activity test and a second analysis yielding clear negative results. The percentage of samples with undetectable erythropoietin using this method was approximately 6% for the OOC samples and approximately 13% for the IC samples. When compared with data from the literature, these can be regarded as a rather low percentages.[16,17]

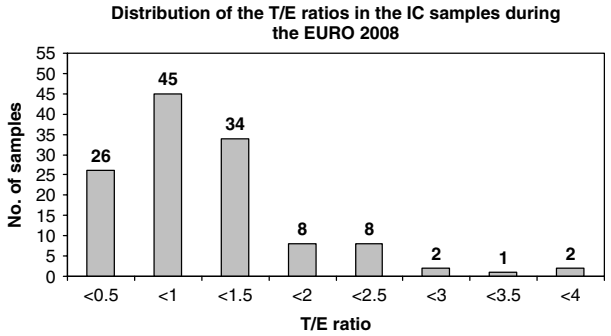

Figure 3. Distribution of the testosterone to epitestosterone (T/E) ratios in the in-competition samples during the EURO 2008.

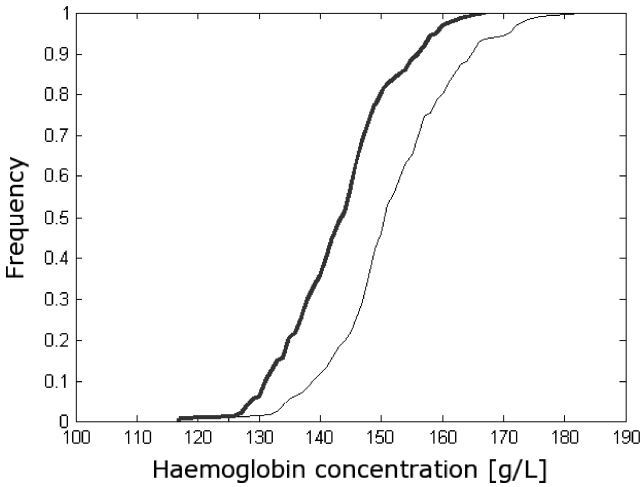

Figure 4. Cumulative frequency distribution of the haemoglobin concentration [g/L] in the in-competition (thin line) and out-of-competition (thick line) samples during the EURO 2008.

A correlation between the samples with undetectable erythropoietin and the specific gravity showed that the distribution of the specific gravity of these samples is bimodal with ranges between 1.004 – 1.008 and 1.024 – 1.032. This is in agreement with previously published data.[12]

Blood parameter distribution

A full blood count was obtained for all blood samples and the stimulation index (OFF-score)[18] and Abnormal Blood Profile Score (ABPS)[19] were calculated. Figure 4 shows the cumulative distribution functions for IC and OOC samples for haemoglobin level. Comparison is made between IC and OOC samples and not toward a 'normal population' due to potential differences in pre-analytical protocols.[20,21]

In Figure 4., a shift toward the right hand-side of the IC sample curve (thin line) compared to OOC sample curve. This shift can be due to dehydration of athletes after competition, plasma volume expansion from the OOC sample or pre-analytical differences (time of collection, delay of resting, feeding status, laboratory specificities, temperature). The OOC samples were predicted to have higher plasma volumes due to the long competition season and training.[22]

In Figure 5., the IC and OOC sample distributions are not significantly different. The trend toward a lower percentage of

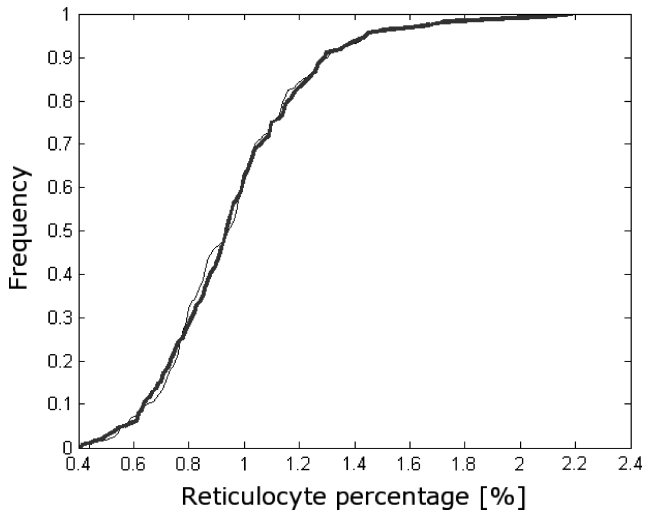

Figure 5. Cumulative frequency distribution of reticulocyte percentage [%] in the in-competition (thin line) and out-of-competition (thick line) samples during the EURO 2008.

reticulocytes is not significant when the uncertainty resulting from sampling combined with analytical and pre-analytical variations is taken into account.

Reticulocytes are not dependent on plasma expansions as long as they are expressed as a percentage. Then, a small variation in the plasma volume would not be expressed by the percentage of reticulocytes in Figure 5, but it would appear in Figure 4 as haemoglobin is linked to plasma volume. To avoid bias due to pre-analytical differences, collection and transport protocols must be strictly followed. Laboratories must work with the same protocol and the same quality controls to be able to check that all results can be interpreted on the same basis. Without this guidance, there is no guarantee of proper use of blood parameters for anti-doping purposes.[2326]

In Figure 6, variations are present between IC and OOC samples, but they only reflect variations of haemoglobin concentration observed in Figure 4.

In Figure 7, ABPS values from both IC and OOC samples suggest a close to zero prevalence of blood doping in the population. ABPS values inferior to −3 suggest that some athletes present a pathological condition such as anaemia. For OOC samples, the departure from normal population out-of-competition explainable by a plasma volume expansion due to intense training or fatigue induced by a long competition season.

Growth hormone testing

Human growth hormone was tested in serum and plasma. Out-of-competition testing was performed on 160 athletes, resulting in 320 hGH test results, and 127 athletes were tested IC, resulting in 254 hGH test results. All hGH tests were carried out using the method described by Bidlingmaier *et al.*[1] comprising two different chemiluminescence immuno-assay kits ('screening' and 'confirmation') and each result was expressed by two measured concentrations : the concentration given by the 'REC' assay (corresponding roughly to the recombinant hGH) and the concentration given by the 'PIT' assay (corresponding to all hGH forms circulating in the blood) . The ratio of both concentrations is the basis for further decisions about the occurrence of recombinant growth hormone in the en-

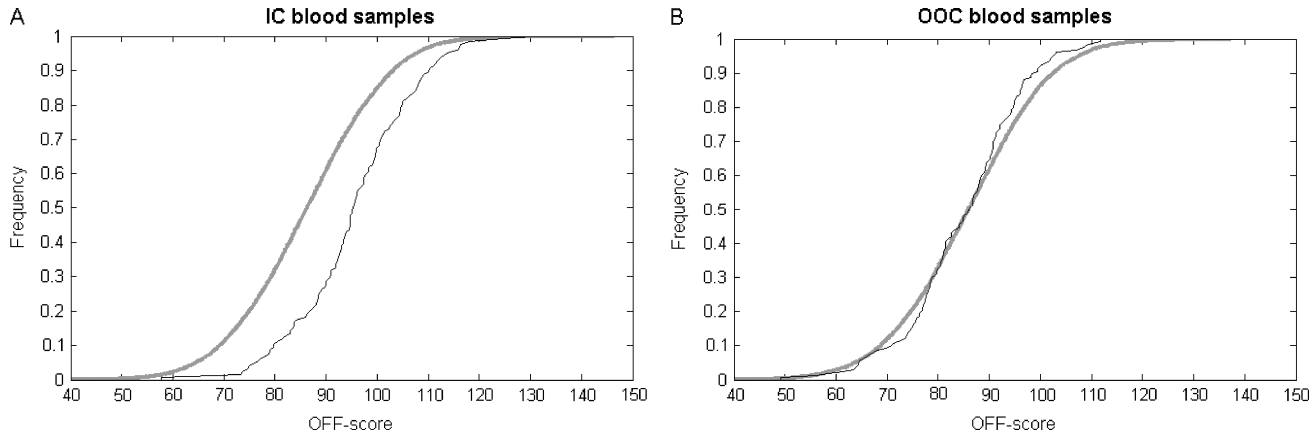

Figure 6. Distribution of OFF-score in the in-competition (A) and out-of-competition (B) samples during the EURO 2008. Part A shows IC results and part B shows OOC results. The light grey thick line represents the distribution expected from a normal population.

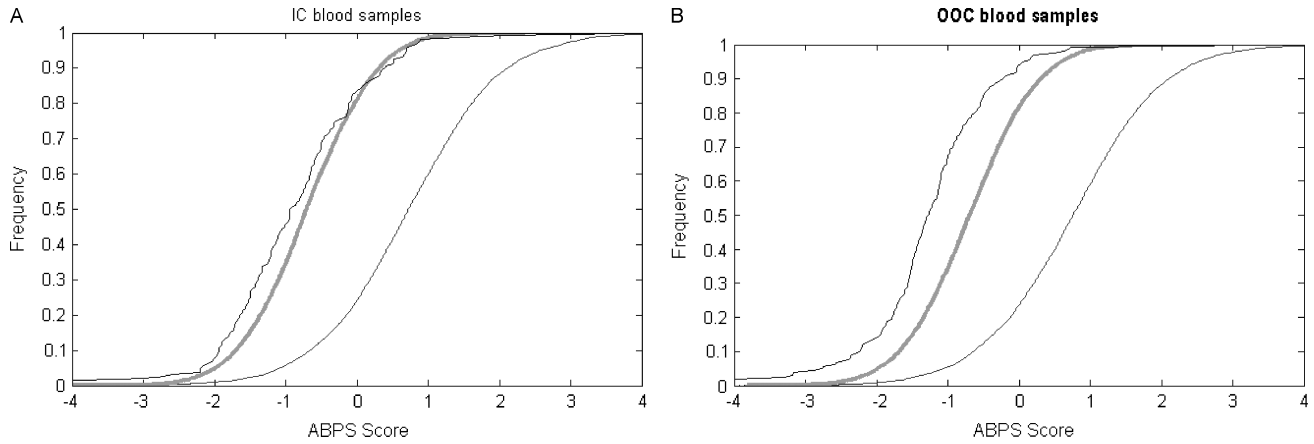

Figure 7. Distribution of ABPS Score in the in-competition (A) and out-of-competition (B) samples during the EURO 2008. The light grey thick line represents the distribution expected from a normal population. Thin light grey line represents the distribution obtained from a population who has abused from recombinant erythropoietin and/or blood transfusion.

tire sample. Interpretation of result are based on numerous publications.[27–35]

From the 320 GH results of the OOC samples, less than 40% had hGH concentrations above the limit of quantification (LOQ). More than 70% of the IC samples had hGH concentration above the limit of quantification for each assay (Table 4). This difference is most probably linked to the time of collection of the blood samples. Most OOC samples were collected during the afternoon while IC samples were collected in the night. The hGH concentration is linked to a circadian cycle and is known to have a minimum for males during the afternoon and to have a peak during night.[36]

Based on results given in Table 4., all samples were below the WADA cut-off including the measurement uncertainty (MU); no indication of the use of recombinant hGH was found. Only results from serum are considered as this matrix is the reference one.

The ratio distribution is given in Figures 8 and 9 for the screening and confirmation kit results respectively.

Due to the low number of samples, a comparison between IC and OOC samples is difficult, especially if the aim is to show any differences. Figure 8 appears to show that no difference is present between IC and OOC samples. Figure 9 appears to show that the population variance is bigger in OOC samples than IC samples. There could be a small shift toward a bigger mean of OOC ratios, but no significance can be given with classical statistics. This shift could also be explained as resulting from the fact that more data were below the quantification limits for OOC samples and as such biased the distribution. In any case, no ratio approaches the limit given WADA cut-off and therefore only negative samples were observed.

Conclusion

The EURO 2008 anti-doping programme included as a major new advantage the simultaneous collection of blood (whole blood and serum) and urine from every athlete, providing the ability for the analysing laboratory to gain a complete picture for doping testing. All possible analytical WADA-accredited methods were implemented in both testing labs and applied to all the samples, including GH testing as well as testing for homologous blood transfusion and the determination of blood parameters.

As a consequence blood profiles were recorded and stored, as well as endogenous steroid parameters as a starting point for the concept of the biological passport. Differences between IC and OOC samples for haemoglobin led to further improvements in blood testing.

Table 4. The hGH results for blood samples collected during IC and OOC testing

	All OOC blood samples		All IC blood samples	
	Screening kit	*Confirmation kit*	*Screening kit*	*Confirmation kit*
No. of samples	320	320	254	254
No. of Samples above the LOQ	106	120	198	180
Percent below the LOQ	63%	67%	22%	29%
	OOC samples above LOQ		**IC samples above LOQ**	
	Screening kit	*Confirmation kit*	*Screening kit*	*Confirmation kit*
No. of ratios	56	60	98	86
Mean of ratios	0.527	1.008	0.569	0.892
Median of ratios	0.515	1.005	0.544	0.824
SD	0.24	0.40	0.23	0.33
Mean CV of ratios	11%	11%	11%	9%
Minima	0.05	0.23	0.17	0.40
Maxima	1.04	1.73	1.32	1.95
WADA cut-off (incl. MU)	2.04	2.55	2.04	2.55

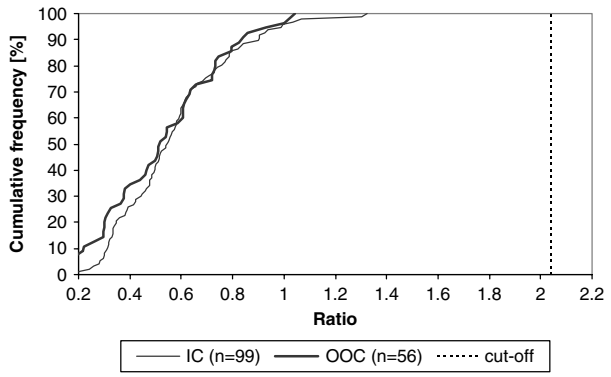

Figure 8. Cumulative frequency distribution of the screening hGH "REC" to "PIT" ratios in the in- and out-of-competition samples during the EURO 2008.

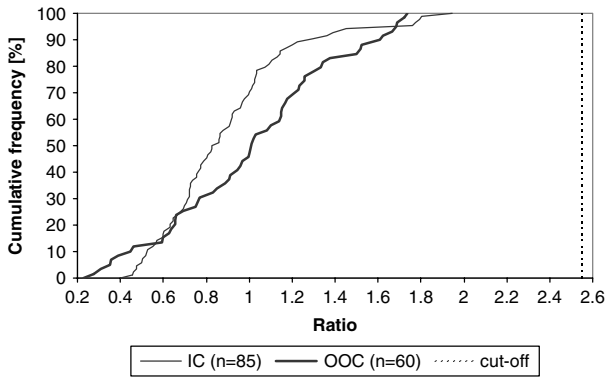

Figure 9. Cumulative frequency distribution of the confirmation hGH "REC" to "PIT" ratios in the in- and out-of-competition samples during the EURO 2008.

The density of testing was comparably high; 286 out of 368 players (i.e. 77%) were subjected to anti-doping tests. No adverse analytical findings were reported for IC or OOC testing.

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