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RECENT ADVANCES IN DOPING ANALYSIS (4)

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E. NOLTEERNSTING, I. MATONDO, E. MURWISI, W. SCHÄNZER:
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Brief Report of the Doping Analyses during the 6th All Africa Games in Harare 1995 (September, 13th - 24th)

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1. Prelinimary Remark

The doping analyses were eclipsed by the death of Prof. Dr. Manfred Donike (Head of the Institute of Biochemistry, German Sport University Cologne and Secretary of the subcommission "Doping and Biochemistry of Sport" of the IOC Medical Commission) during the last preparing trip to Harare. He passed away in the early morning of August, 21st, three weeks before the beginning of the 6th All Africa Games on board an aircraft. It was a difficult task for all the people involved to continue the initiated work without him.

2. Accreditation

The All Africa Games in Harare, Zimbabwe were the 6th since the beginning in 1965 in Brazzaville, Congo. The Games are now firmly established and take place in a four year cycle since 1987.

In the past doping analyses have never been performed on-site and consequences due to positive results could be effected only with a long delay. To avoid these inconveniences the Zimbabwe Regional Drug Control Laboratory (Ministry of Health and Child Welfare) and the Ministry of Sports and Culture asked the Institute of Biochemistry, German Sport University Cologne to take over the patronage for the first on-site dope analyses during All Africa Games. The International Olympic Committee (IOC) granted temporary accreditation to the IOC accredited laboratory in Cologne to carry out doping analyses at the Zimbabwe Regional Drug Control Laboratory (ZRDCL) in Harare for the period of the All Africa Games. After the tragic death of Prof. Dr. Manfred Donike the IOC immediately accepted Dr. Wilhelm Schänzer as Head of the IOC accredited laboratory in Cologne.

The ZRDCL expected to analyse 362 samples within 10 days (maximum 49 samples per day) taken from about 4000 participants from 48 African countries.

3. Laboratory

The ZRDCL is a joint project between the World Health Organisation and the Ministry of Health, Zimbabwe to serve Zimbabwe and the other countries in the Subregion III. Its purposes are

- to test drugs and allied substances for various purposes
- to serve as a regional training centre for drug analysts
- to develop a databank and furnish drug regulatory information for the Subregion
- to carry out accelerated stability studies as requested by clients and other joint projects.

The ZRDCL is headed by Ian Matondo (director) and the the anlyses were carried out in an extension situated in 73 Dunmore Avenue, Queensdale, Harare managed by Ellison Murwisi.

3.1. Equipment and analytical instruments

Instruments 3.1.1, 3.1.2, 3.1.3 and 3.1.6 were supplied by the Ministry of Sports and Culture, instruments 3.1.4 and 3.1.5 were supplied by Hewlett Packard on a loan contract and instrument 3.1.7 was supplied by the University of Zimbabwe - Medical School.

3.1.1. GC/NPD 1

GC:	HP5890E Series II with 2 split/splitless injection ports
Detector:	FID and NPD
ALS:	HP7673
ChemStation:	Vectra PC with DeskJet
Software:	Rev.A.03.01
Column:	Fused silica capillary column, 30m HP MS5,0.25mm i.d.,0.25µm film thickness

3.1.2. GC/MS-System 1

GC:	HP5890E with split/splitless injection port and one channel of EPC
MS:	HP5972A
ALS:	HP7673
ChemStation:	Vectra PC with LaserJet 4L
Software:	G1034A Ver C.03.00
Column:	Fused silica capillary column, 30m HP MS5,0.25mm i.d.,0.25µm film thickness

3.1.3. GC/MS System 2

GC:	HP5890E with split/splitless injection port and one channel of EPC
MS:	HP5972A
ALS:	HP7673
ChemStation:	Vectra PC with LaserJet 4L
Software:	G1034A Ver C.03.00
Column:	Fused silica capillary column, 17m HP Ultra1,0.2mm i.d.,0.11µm film thickness

3.1.4. GC/MS System 3

GC:	HP6890E with split/splitless injection port and two channel of EPC
MS:	HP5972A
ALS:	HP7673
ChemStation:	Vectra PC with DeskJet
Software:	G1700AA Ver.A.00.00
Column:	Fused silica capillary column, 17m HP Ultra1,0.2mm i.d.,0.11µm film thickness

3.1.5 GC/NPD 2 (in standby for emergency cases)

GC:	HP5890E Series II with 2 split/splitless injection ports
Detector:	FID and NPD
ALS:	HP7673
ChemStation:	Vectra PC with DeskJet
Software:	Rev.A.03.01
Column:	Fused silica capillary column, 30m HP MS5,0.25mm i.d.,0.25µm film thickness

3.1.6 HPLC (in standby for caffeine confirmation)

Pump:	HP79852A (quarternary gradient pump system)
Detector:	HP79853C (programmable variable wavelength detector)
ChemStation:	Vectra PC with DeskJet
Software:	G2170AA
Column:	LiChropher® RP18, 5µm, 125x4mm I.D. (Merck,GER)

3.1.7. IMx System from Abbott

(for hCG Screening, located in University of Zimbabwe
- Medical School, Department of Clinical Biochemistry)

3.2. Staff

The staff consisted of co-workers from the ZRDCL from various laboratories in Harare, from the IOC accredited laboratory in Cologne, Germany and from the IOC accredited laboratory in Bloemfontein, South Africa. It was considered to be very useful to include also the South African laboratory in the dope analyses of the All Africa Games to acquire experiences for the next Games taking place in South Africa.

3.2.1 Staff from the IOC accredited laboratory in Cologne, Germany

Mr. PD Dr.Wilhelm Schänzer (head of laboratory)
Mr. E. Nolteernsting (certifying chemist; Pre Anal., Scr. II, Scr. V, Scr. VII)
Mrs. Dr. U. Mareck-Engelke (certifying chemist; Scr 4)
Mr. G. Sigmund (certifying chemist; Scr 1)
Mrs. U. Schindler (senior chemist; Scr. 4)

3.2.2. Staff from the IOC accredited laboratory in Bloemfontein, South Africa

Mr. Dr. P.J. van der Merwe (certifying chemist; hCG)
Miss E.Kruger. (senior chemist; Scr 4)

3.2.3. Staff from Harare, Zimbabwe

3.2.3.1 Staff from the ZRDCL

Mr. Prof. T. E. Chagwedera (Ph. D., conlsultant)
Mr. I. N. Matondo (M. Sc., Anal. Chem., Lab. Director)
Mr. E. Murwisi (Advanced Dipl. Anal. Chem. and Biochem., Lab. Manager)
Mr. G. Jambaya (B.Sc. Chem. Biochem.,Senior Analyst; Scr IV)
Mrs. C. Manyika (B. Sc. Chemistry, Senior Analyst; Scr VII)
Mr. W. Maduku (B. Sc. Applied Chem.; Pre Anal.)
Mr. D. Charangwa (B. Sc. Applied Chem.; Scr I)
Mr. O. Thanda (National Certificate Biol. Sciences; Scr V)
Mr. J. Takarupiwa (Advanced Dipl. Anal. Chem. and Biochem.; Scr II)
Mrs. C. Kanjere (Advanced Dipl. Microbiol. and Biochem.; Pre Anal., hCG)
Mr. A. Ganga (National Certificate Chemistry; Scr IV)

3.2.3.2. Staff from theGovernment Analyst Laboratory, Harare

Mr. M. L. Musiambiri (B. Sc. Chem. and Biochem.; Scr I)

3.2.3.3 Staff from the University of Zimbabwe , Institute of Pharmacy

Mr. P. Chirisa (B. Sc. Biochem. and Biological Sciences;Pre Anal. Scr IV)

Mr. J. Mutenure (Advanced Dipl. Biological Sciences; Scr I)

Mr. T. Tsawayo (National Certificate Chem. and Biochem.; Scr II)

3.2.3.4. Staff from the Forensic Laboratory , Harare

Ms. R. Dururu (B. Sc. Chem.; Scr I)

Mr. B. Saidi (B. Sc. Chem.; Scr V)

4. Sample Reception and Pre-Analysis Procedure

The samples were collected by the local collecting team under the assistance of a team from the Norwegian Confederation of Sports headed by Rune Andersen. The samples taken in Harare and Chitungwiza were transported by members of the collecting team directly to the laboratory. The samples taken in Bulawayo were transported by members of the collecting team on the next available aircraft to Harare Airport and afterwards directly to the laboratory.

After arrival in the laboratory the seals of the bags were checked, the code numbers were noted and compared with those on the delivering forms. Then the A- and B-containers were unpacked, the code numbers were noted and compared with those on the doping control forms. The A-containers were opened and prepared stickers with the laboratory numbers were placed on the A-bottles, the B-containers and on the corresponding forms. The approximate volume of each sample was estimated and noted.

The B-containers were stored immediately at 4°C in lockable refrigerator in separate locked room.

The A-bottles were opened and the pH (pH-meter) and the specific gravity (refractometer) were measured. The samples were aliquoted and directly stored in a lockable refrigerator at 4°C. All information regarding the samples and the batches obtained from the according forms were entered into a computer (database).

5. Doping analyses procedures

The sample were prepared according to the following procedures

5.1. Screening I (stimulants)

To 5 ml of urine are added:
ca. 200 mg of K_2CO_3/$NaHCO_3$ 1:2, pH 9.6,
1.5 ml of diethyl ether,
25 µg of N,N-diisopropyl-n-dodecane as internal standard and
ca. 3 g of anhydrous sodium sulphate.
The mixture is shaken for 20 minutes, centrifuged at 3000 rpm. The ether layer and 0.3 ml methanol are transferred into vials for an automatic liquid sampler
3 µl of the solution are injected.
GC-parameters (instrument 3.1.1) :
carrier gas: helium , head pressure: 20 psi , split: : 10ml
temperature program: 115°C, +22°C /min, 5 min at 320°C
injector: 280°C; detector: 300°C

5.2. Screening II (heavy volatile stimulants and narcotics)

To 3 ml of urine add:
100 mg of cysteine,
300 µl of 6 N HCl,
30 µl of norephedrine in 0.06 N HCl (0.1 mg/ml) as an internal standard
and mix for 10 seconds.
Heat this acidified urine at 100 °C for 30 minutes (acidic hydrolysis).
cool to room temperature and add 3 ml diethyl ether.
Shake for 10 minutes, centrifuge at 3000 rpm for 5 minutes
and discard the organic layer.
Add: 150 µl of 2.7 M boric acid in 10 M KOH,
200 mg of Na_2CO_3 / $NaHCO_3$ 1:3 (w/w),
3 ml ethyl acetate,
50 µl N,N-diisopropyl-pentadecyl-amin (DIPA 15) in methanol (0.1 mg/ml) and
2 g anhydrous sodium sulphate.
Shake for 15 minutes, centrifuge at 3000 rpm for 5 minutes,

transfer the organic layer to a test tube, evaporate to dryness.
The residue is reconstituted in 100 µl MSTFA and heated for 15 min at 60 °C
3 µl of the solution are injected.
GC/MS parameters: (Instrument 3.1.4)
carrier gas: helium , head pressure: 14 psi , split: : 8.6 ml
temperature program: 120°C, +20°C /min, 2 min at 320°C
injector: 300°C; transfer line: 300°C
Scan-acquisition parameters: m/z 50 - 550.

5.3. Screening IV (anabolic agents)

2 ml (if s.g. >= 1.01 g/cm^3) , 4 ml (if s.g. < 1.01 g/cm^3), 8 ml (if s.g. < 1.005 g/cm^3) or 16 ml (if s.g. < 1.025 g/cm^3) of urine are added to Amberlite XAD-2 columns. The columns (pasteur pipette, closed with a glass pearl, bed height ca. 2 cm) are washed with 2 ml of bidistilled water and eluted with 2 ml of methanol.

Add 20 µl of a methanolic solution of internal standards containing

50 ppm	17α-methyltestosterone,
50 ppm	[2,2,4,4-$^2H_4$]-etiocholanolone,
9 ppm	[16,16,17-$^2H_3$]-testosterone,
1.5 ppm	[16,16,17-$^2H_3$]-epitestosterone and
24 ppm	[2,2,4,4-$^2H_4$]-11ß-hydroxyandrosterone

to the methanolic eluate evaporate to dryness and dissolve the residue in 1 ml of 0.2 M sodium phosphate buffer pH 7.
Add to the buffer solution 50 µl of beta-glucuronidase from E.coli and hydrolyse the mixture for 1 h at 50°C. The buffer solution is alkalised with 250 µl of 20% K_2CO_3/$KHCO_3$ 1:1 (w/w) to pH 9 - 10 and extracted with 5 ml of diethyl ether on a mechanical shaker for 5 minutes. After centrifugation the etheral layer is transferred and evaporated to dryness in vacuum.
The dry residue is derivatised with 100 µl of MSTFA/NH_4I/ethanethiol-TMS 1000:2:3 (v:w:w) and heated for 15 min. at 60°C.
3 µl of the solution are injected.
GC/MS parameters: (Instrument 3.1.3.)
carrier gas: helium , head pressure: 18 psi , split: : 10 ml
temperature program: 181°C, 3°C/min - 230°C, 40°C/min - 310°C, 2min
injector: 300°C; transfer line: 300°C
SIM-acquisition.

5.4. Screening V (diuretics)

2 ml of urine and 0.5 µg of mefruside in methanol are added to Amberlite XAD-2 columns. The columns (pasteur pipette, closed with a glass pearl, bed height ca. 2 cm) are washed with 2 ml of bidistilled water and eluted with 2 ml of methanol.
The eluate is evaporated to dryness and to the residue about 50 mg of anhydrous potassium carbonate, 170 µl of acetonitrile and 30 µl of iodomethane are added. The tightly sealed mixture is heated at 60°C for at least 3 hours.
3 µl of the solution are injected
GC/MS parameters: (Instrument 1.1.2.)
carrier gas: helium , head pressure: 25 psi , split: : 10 ml
temperature program: 190°C, +30°C /min, 4.5 min at 320°C
injector: 300°C; transfer line: 300°C
SIM-acquisition.

5.5. Screening VII (beta-blockers)

To 3 ml of urine add:
30 µl of toliprolol/bupranolol (0.02 mg/ml),
500 µl of 1 M sodium acetate buffer
and mix for 10 seconds.
If the pH value is higher than 5.5 add acetic acid until pH is between 5.0 and 5.5-
and add 50 µl of ß-glucuronidase/arylsulfatase from Helix pomatia (serva).
Heat mixture for 3 hours at 50°C ,
cool to room temperature,
add 200 mg of K_2CO_3 / $NaHCO_3$ 1:2 (w/w),
3 ml ethyl acetate and
2 g anhydrous sodium sulphate.
Shake for 15 minutes, centrifuge at 3000 rpm for 5 minutes,
transfer the organic layer to a test tube, evaporate to dryness.
The residue is reconstituted in 100 µl MSTFA and heat for 15 min at 60 °C
3 µl of the solution are injected.
GC/MS parameters: (Instrument 1.1.4)
carrier gas: helium , head pressure: 14 psi , split: : 8.6 ml
temperature program: 120°C, +20°C /min, 2 min at 320°C
injector: 300°C; transfer line: 300°C
Scan-acquisition parameters: m/z 50 - 550.

5.6. Screening for hCG

General description:
200 µl of urine are transferred to the reaction cells in the carousel. The following reaction kits were used: IMx hCG Reagent Pack (No.3A63-20) from Abbott.

6. Organisation of Analyses and Reports

The dope analyses of the All Africa Games in Harare were performed in accordance to the rules of the IOC. There was no technical problem within the analytical tests in the laboratory. All analytical instruments were ran with sufficient stability allowing sensitive and reproducible gas chromatographic / mass spectrometric analyses.
The urine samples arrived from the different control stations every evening between 6 and 12 p.m. Samples from Bolawajo arrived the next morning. Sample preparation started in the night followed by analyses of the first urine samples. Confirmations and reanalyses of suspicious A-samples were achieved within the following day. Results of the urine-samples were presented within 24 hours after the arrival of the samples at the laboratory and reported to the Medical Commission of the All Africa Games 95.
In general the Medical Commission met every evening between 9 and 10 p.m.. The Medical Commission was represented by the chairman Dr. Thomas Chaita, Zimbabwe, David Mutambara, Zimbabwe and Dr. Roald Bahr, Norway.
B-analyses of positive A-samples were performed on the following day after presenting the results of the first test to the Medical Commission.
There was no time delay to this procedure during the games.

7. Results

In total 352 samples were taken and analysed during the 6th All Africa Games (table 1). All samples were tested for stimulants, heavy volatiles/narcotics, anabolic agents and hCG. 134 were tested for diuretics and 24 were tested for ß-blockers.
12 samples were tested positive, 3 test samples introduced by the Medical Commission and 9 "real" positive samples (table 1 and table 2). All 9 positive cases were also confirmed positive in the analyses of the B-samples.

8. Acknowledgment

Acknowledgment and gratefulness have to be given to all the people and institutions who helped to complete this difficult task successfully .

Analyzed doping control samples at the All Africa Games 1995, Harare, Zimbabwe, 13-23.Sep.1995												
Sport	14.9.	15.9.	16.9.	17.9.	18.9.	19.9.	20.9.	21.9.	22.9.	23.9.	Total	Positive
Athletics	15	18	18	14	19						84	3
Basketball								4	4		8	
Boxing						6	8		4		18	
Cycling		6			6				7		19	2
Diving	4	4	4								12	
Football										4	4	
Gymnastics						4	4	3	3		14	
Handball									8		8	
Hockey									4		4	
Judo	4	5	5	4							18	
Karate	6	5	6	2							19	
Shooting		3		2	2	3	2				12	
Swimming			6	7	5		8	6	5		37	
Lawn Tennis							2				2	
Table Tennis				2				2	2		6	
Taekwondo			4	4	4	4					16	
Volleyball									8		8	1
Weightlifting						8	8	8	16		40	2
Wrestling				2	2	8		2	3	6	23	1
Total	29	41	43	37	38	33	32	25	64	10	352	9

Table 1: **Table of doping control samples analysed during the 6th All Africa Games separated by sports and dates**

Day	Substance	Event	Code No.	Lab. No.
1	Dextropropoxyphene	Athletics	000 290	12
2	Clostebol	**Control**	000 180	56
3	Fencamfamine	**Control**	000 262	85
3	Nandrolone	Athletics	000 272	87
3	Salbutamol	Athletics	000 363	92
3	Pethidine	**Control**	000 259	105
4	Nandrolone	Wrestling	000 371	150
7	Nandrolone	Weightlifting	000 428	233
9	Nikethamide	Cycling	000 237	291
9	Nikethamide	Cycling	000 243	293
9	Dextropropoxyphene	Volleyball	000 263	286
9	Nandrolone	Weightlifting	000 693	322

Table 2: Table of positive A-samples analysed during the 6th All Africa Games