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An overview of doping control analysis during Commonwealth Games 2010, New Delhi

National Dope Testing Laboratory, New Delhi, India

1.0 Introduction

The XIX Commonwealth Games (CWG), held at New Delhi, India from October 3-14, 2010 included 6,081 athletes from 71 Commonwealth nations, who competed in 21 sports and 272 events. It was the largest International multi-sport event staged in New Delhi, India.

The Organizing Committee Commonwealth Games (OC-CWG) was responsible for out-of-competition (OC) and competition testing of the aforesaid games. The OC testing was performed before and throughout the games, as a full panel in competition (C) testing as defined by the organizing committee. A total of 1482 urine samples and 188 blood samples were collected as per the agreement signed between National Dope Testing Laboratory (NDTL) and CWG organizing committee, which reflects the comprehensive anti-doping program followed during the said games. This report briefly illustrates the procedure, instrumentation, staff organization and statistical data carried out at NDTL referred to the event.

2.0 Accreditation, scope, personnel and result management

All analytical and managerial procedures were accredited as per ISO/IEC 17025 by National Accreditation Board for Calibration and Testing Laboratory (NABL), and by World Anti Doping Agency (WADA) as per WADA International Standard for Laboratories (ISL v 6.0)[1,2]. The laboratory was staffed with 56 members viz scientists (8), analysts (4), research fellows (16), trainee analysts (17) and administrative staff (11). In addition, seventeen international experts/scientists were invited from five WADA accredited laboratories (advisors-05 and certifying scientists -12) (Table 1).The laboratory was functional round the clock during games period. The turn around time as per the contract with testing authority is given in table- 2. The security arrangement at NDTL was controlled by the security staff on duty for 24 hour basis. The access control system, using electronic cards and an electronic database involved three levels of access: reception, operational and highly controlled zones given as per the work profile of the staff at various levels.

Table 1. Experts from various WADA accredited laboratories during CWG 2010

WADA accredited Laboratory	Number	Experts	Areas of expertise of certifying scientists
Drug Control Center, London	03	Scientific Advisor – 01 Certifying scientists - 02	Growth hormone-01 GC-C-IRMS-01
Cologne, Germany	04	Scientific Advisor – 01 Certifying scientists - 03	Steroids (GC-MS)-01 GC-C-IRMS-01 LC-MS/MS-01
Anti-Doping laboratory, Rome	05	Scientific Advisor – 02 Certifying scientists – 03	Blood transfusion -01 LC-MS/MS-02
ASDTL, Sydney	01	Scientific Advisor – 01	-
Austria Doping Laboratory	02	Certifying scientists - 02	EPO-01
Tokyo Doping Lab	02	Certifying scientists - 02	Stimulants & Narcotics (GC-MS)-01 Steroids (HRMS & GC-MS/MS)-01

Table 2 Turn-around time for sample test reporting during CWG 2010

Sample Type	Test conducted	A Sample		B Sample
		Negative	Positive	
Urine testing	**Drugs as per WADA 2010 prohibited list**	24 hr	48 hr	24 hr (In case of morphine 48 hr)
	EPO	72 hr	6 days	3 days
	IRMS Analysis	4 days	4 days	3 days
Blood testing	**hGH Testing**	24 hr	48 hr	24 hr
	CERA	24 hr	5 Days	3 days
	Blood Transfusion & HBOCs	48 hr	72 hr	48 hr
	Blood parameter	24 hr		NA

3.0 Sample Collection and Reception

The collection of urine and blood samples and its delivery at the lab (in two shifts) was the responsibility of Commonwealth Games Federation Medical Commission (CGF-MC). A total of 1482 urine samples were collected out of which 121 samples (OC-8 & C-113) were for EPO. The blood samples (188) were collected for Human Growth Hormone (93), Continuous Erythropoetin Receptor Activator (CERA) (23), Haemoglobin Based Oxygen Carriers (HBOCS) (40), Blood parameters (58) and blood transfusion (58) testing. The distribution of samples (day wise and discipline wise) is given in Fig. 1 & 2.

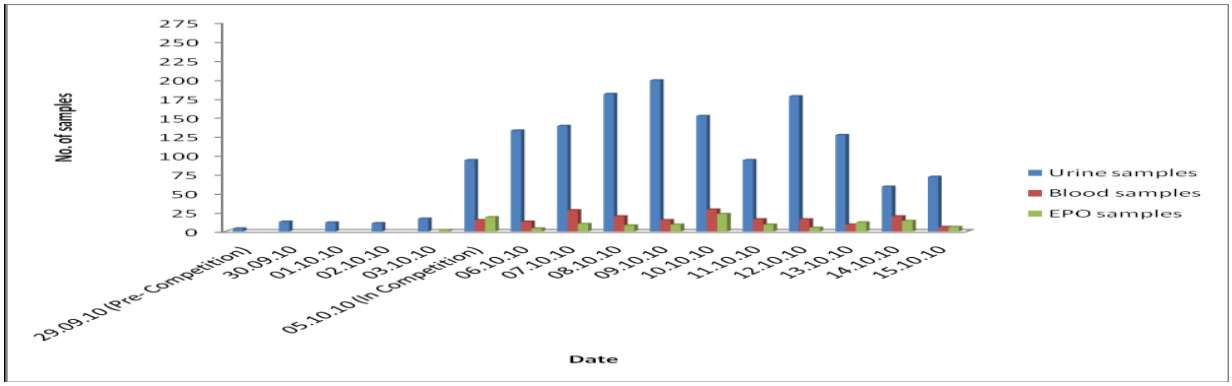

Fig. 1 Distribution of No. of samples received per day

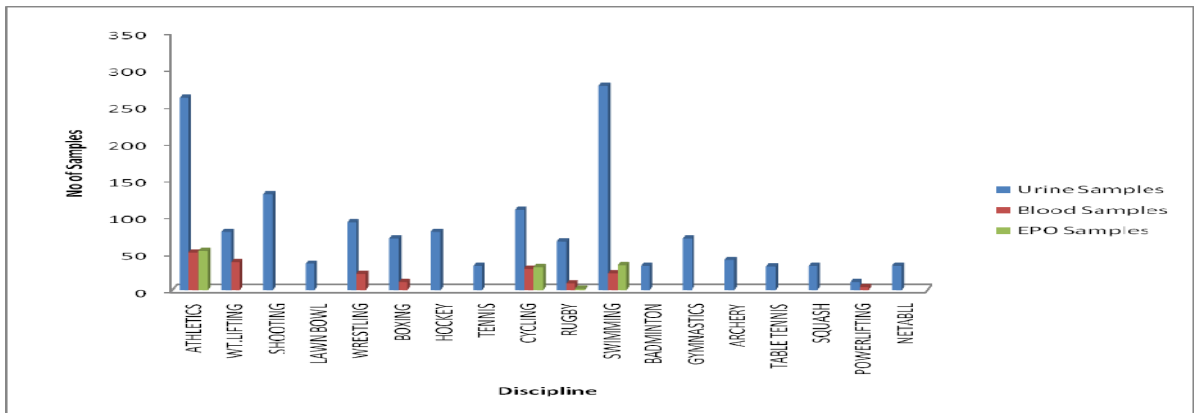

Fig. 2 No. of samples received from various disciplines

4.0 Experimental

4.1 Arrival of samples and preliminary analysis:

The handling of samples, preparation of aliquots for testing, pre-analysis, screening and confirmation procedures, result management, documentation and reporting process were in accordance with the WADA ISL. On arrival of sealed carry bags containing the samples, the same were inspected, code number noted and compared with those on the chain of custody forms. Then the A and B samples were unpacked, checked for non-conformance (NC), registered and the lab code were assigned. The non-conformances were reported within 1-3 hours of sample reception to CGF-MC. Total number of NC's in urine samples were 33 (out of which 28 tested, 5 not tested), and in blood samples were 14 (out of which 11 tested and 3 not tested)[3.] .

The ‘B’ samples were stored at -20^{0}C in cold room and for ‘A’ samples, the volume, pH and specific gravity were measured and recorded. The samples (blood and urine) were distributed in aliquots to the analysts for different screening procedures.

4.2 Screening and confirmation strategy

All urine samples were evaluated through five (05) screening procedures and were analyzed employing various analytical techniques viz. gas chromatography mass spectrometry (GC-MS), gas chromatography tandem mass spectrometry (GC-MS/MS), gas chromatography-nitrogen phosphorous detection (GC-NPD), liquid chromatography tandem mass spectrometry (LC-MS/MS) and immunoassay. The screening procedure for EPO analysis was applied to 121 samples. Each sample was reprocessed and confirmed prior to reporting an Adverse Analytical Finding (AAF). A separate confirmation procedure based on gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS) was used for endogenous steroids. Blood samples were analyzed for specific tests as requested.

4.3 Quality control strategy

To ensure the quality of screening in the chromatographic procedures, negative and positive quality control samples were included in all batches and were treated in the same way as the test samples. The quality controls were blank urine samples spiked with reference materials at the minimum required performance limit (MRPL) or the threshold values (for threshold substances). Hydrolysis Control (HC) and Derivatization Control (DC) were also included in screenings IV (procedure for anabolic agent and other drugs).

4.4 Analytical Resources

A total 38 instruments for different screening procedures were operational to meet the turn around time (TAT) as per the agreement with CWG-OC.

5.0 Results and Discussion

Urine Analysis

During the entire games period, 1482 samples were analyzed from 17 different sports disciplines. The highest numbers of samples analyzed in one day were 199. The results were reported to the Testing Authority as per the agreement (Table-2). A total of 11 samples undergone GC-IRMS analysis, out of which 03 showed high T/E ratio (>4.0) , 05 showed high Androsterone (>10,000 ng/ml) and/or high etiocholanone (>10,000 ng/ml), 02 showed high DHEA (>100 ng/ml) and 01 showed high 5-b androstanediol (> 200 ng/ml). After confirmation analysis on GC-IRMS, only 01 sample with high androsterone and etiocholanone was found to be of synthetic origin, and was reported as AAF for Testosterone or its prohormone. The remaining were found to be of endogenous origin, hence reported negative.

The Table 3 gives the details of eleven (11) samples reported as AAF to Chairman, CGF-MC 2010. Out of these, 4 "B samples" were analyzed, the two were performed during games period (Methylhexaneamine and Norandrosterone) in the presence of athlete, and another 2 post game period (Testosterone Prohormone and Norandrosterone). Two documentation packages (Testosterone Prohormone and Norandrosterone) were prepared by the laboratory on request and submitted to Chairman, CGF-MC. Out of 121 samples tested for EPO, there was mild to moderate shifting of bands of uhEPO to the basic region in 70 samples though it did not fulfill the WADA evaluation criteria for rhEPO. Further confirmation of these samples with double immunoblotting after SDS-PAGE found them stable and showed only uhEPO[4]. The shift of bands to basic region leading to confirmation of samples may be due to proteinuria after competition.

Table-3: Details of Adverse Analytical Findings (AAF) reported by NDTL during CWG 2010.

5 AAFs were resolved pursuant to the CGF-ADS	• **19- Norandrosterone -** 03 female cases with concentration of 47 ng/ml, 3.2 ng/ml, 6.2 ng/ml and presence of Norethisterone
	• **d-Amphetamine** with Valid TUE- 01 case
	• **Triamcinolone** with TUE- 01 case
6 AAFs referred to adjudication stage	• **Methylhexaneamine** (02 cases)
	• **19- Norandrosterone** - 02 cases with concentration >25 ng/ml
	• **Marijuana metabolite(** THC) – 01 case with concentration of 32 ng/ml)
	• **Testosterone Prohormone** – 01 case (GC-IRMS-Exogenous)

Blood Analysis

188 blood samples collected for Human Growth Hormone (93), CERA (23), HBOCS(40), Blood parameters (58) and blood transfusion (58) were tested and were found negative.

6.0 Conclusion

The drug testing for the CWGs was accomplished successfully and the XIX Commonwealth Games could be referred as drug free games (percentage of AAF was only 0.7%), which may be due to stringent rules and regulation applied by CGF-MC.

7.0 References

1) International Standard of laboratories(ISL), version 6.0,2009 http://www.wada-ama.org/Documents/World_Anti-Doping_Program/WADP-IS-Laboratories/ISL/WADA_Int_Standard_Laboratories_2009_EN.pdf (access date 27.09.10)
2) Beotra A, Jain S, Kaur T, Lal R, Ahi S, Dubey S, Garg T, Kashif M, Haseen J, Singh A. (2011) Doping Control analysis during the 1st Singapore Youth Olympic Games at National Dope Testing Laboratory, India. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) Recent Advances in Doping Analysis (19), Köln, Under publication.
3) Kaur T, Gotzmann A, Jamal H, Singh Ashok, Kumar Yogesh, Lal R, Joshi M , Beotra A, Jain S. (2011) Experience in Sample Receiving during Commonwealth Games 2010. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) Recent Advances in Doping Analysis (19), Köln, Under publication.
4) Jain S, Reichel C, Benetka W, Lal R, Raj A, Tyagi D and Beotra A. (2011) Competition testing of Erythropoietin (EPO) during Commonwealth Games – 2010. In: Schänzer W, Geyer H, Gotzmann A, Mareck U. (eds.) Recent Advances in Doping Analysis (19), Köln, Under publication.

8.0 Acknowledgements

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