Reprint from

RECENT ADVANCES IN DOPING ANALYSIS (5)

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Sport und Buch Strauß, Köln, 1998

S.Horning,W.Schänzer,B.Sample:
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In: W. Schänzer, H. Geyer, A. Gotzmann, U. Mareck-Engelke (eds.) Recent advances in doping analysis (5). Sport und Buch Strauß, Köln, (1998) 329-337

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HRMS Analyses Performed at the 1996 Summer Olympic Games

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Introduction

Gas chromatography, high resolution mass spectrometry (GC/HRMS) using multiple ion detection (MID) via electric field scanning is an unmatched analytical method for trace level detection of anabolic steroids. Basic principles of high resolution electric field scanning and steroid screening have been discussed in previous Workshop contributions [1-3]. Here, the use of HRMS at the 1996 Summer Olympic Games is described. This includes (i) the installation of three Finnigan MAT 95S mass spectrometers at the SmithKline Beecham Clinical Laboratories in Atlanta, (ii) IOC accreditation of screening procedure IVb and HRMS related procedures prior to the Games and (iii) a report of the HRMS analyses performed during the Games. As it is the responsibility of the head of the laboratory to report on the analytical findings, this information is not included here.

The first use of GC/HRMS for steroid screening was the 1993 World Championships in Track and Field held in Stuttgart, Germany. The first use of HRMS for steroid screening during an Olympic Games was in Oslo, Norway, 1994. In the Cologne laboratory, the Finnigan MAT 95 instrument has been used for routine screening of anabolic steroids since 1993. The instrument has proven to be highly reliable, highly sensitive, highly accurate and highly stable. Per year more than 5,000 samples have been run on this instrument. Due to proper care (and some luck), little maintenance has been necessary to keep the instrument in good condition (since the instrument arrived in February 1993, the ion source has been cleaned on two occasions, June 1995 and December 1996). Since implementation of HRMS for routine screening analysis there has been a substantial increase in the number of positive steroid cases. For example, in 1995, of the 116 positive cases, 75 were identified only by HRMS. In 1996, only 7 of the 28 reported metandienone positives were identified by

conventional quadrupole GC/MS, the remainder were detected only by HRMS screening. Similar improvement was found for other banned substances such as clenbuterol and stanozolol.

The decision of the IOC to use HRMS at the 1996 Summer Olympic Games was based on part on the excellent performance of the MAT 95 mass spectrometer in the Cologne laboratory and the recommendation of the International Weightlifting Federation (IWF), which has been using HRMS for control at their major competitions. After more than three years of routine use, the HRMS screening method was well-established and the long-term stability and high up-time of the instrument was proven. Operation of the MAT 95 instrument is fully automated, it is self-calibrating (even while acquiring data), and it is highly stable so that it can be used even under the highly demanding workload conditions of an Olympic Games. Moreover, no specialized sample preparation needs to be performed for HRMS analyses. The same samples, the same quality controls, and the same blanks can be run on the quadrupole GC/MS and the GC/HRMS instrument. Finally, specialized sample work-up procedures had been developed using HPLC and immunoaffinity chromatography to confirm HRMS screening results [4], so that there was no question that the HRMS method was fully established and could be used at the 1996 Games.

Steroid screening using HRMS allows for long-term retrospectivity of steroid misuse. For administration of metandienone, screening with HRMS offers a 3-fold increase in the time over which the 17β-methyl-5β-androst-1-ene-3α,17α-diol metabolite can be detected [5]. The related metabolite, 18-nor-17,17-dimethyl-5β-1,13-dien-3α-ol, can be detected for even a longer period of time (the base peak EI fragment ion (m/z 253.1956) is intense and virtually free from background chemical noise). In the case of a negative finding by HRMS screening there is the added sense of assurance that steroid misuse has not occurred recently, as nearly all of the banned steroids can be detected at extremely low levels. Finally, because of the great deal of press review the HRMS method received prior to the Olympic Games, there was increased pressure on the athletes and federations to avoid doping well before the start of these Games.

Discussion

In August 1995, shortly after Professor M. Donike passed away, the plans he had for implementing HRMS at the 1996 Summer Olympic Games were discussed with Prince de Merode of the IOC and Professor Don Catlin, the head of the Los Angeles IOC accredited laboratory, who later was named in charge of anabolic screening at the Games. In December, 1995, Richard Pound of the IOC executive board announced that HRMS would be used in Atlanta. This announcement took several by surprise, as no plans for HRMS had been made by ACOG (the Atlanta Committee for the Olympic Games) or SmithKline Beecham Clinical Laboratories. In January, 1996, the following groups began planning for HRMS in Atlanta: Cologne laboratory (S. Horning and W. Schänzer), SmithKline Beecham Clinical Laboratories (B. Sample), Los Angeles laboratory (D. Catlin), members of ACOG and Finnigan MAT (Bremen and USA). As the IOC decided that all samples had to undergo HRMS analysis, an order was placed for three MAT 95S instruments.

In March, 1996, SmithKline Beecham began modification of their laboratory to allow for placement of three MAT 95S instruments. As they completed their work at the end of May, the instruments were shipped from Bremen, Germany to Atlanta, with the first arriving on May 30, the second on June 10, and the third on June 22. The first two instruments were installed and tested, and by June 17, 1996, were ready for use.

Testing of the MAT 95S instruments was performed using standards and supplies from the Cologne laboratory. These included GC insert liners, septa, silylation reagents, spiked urine samples, as well as worked-up and derivatized urine blanks and positive urine samples, which had been analyzed in the Cologne laboratory. Screening analyses were performed on both instruments for several days to test their performance and stability. This included more than 30 urine samples, quality control samples and low level quality control sample (QC5 and HQC, see Table 1 and Figure 1). The MAT 95S instruments passed all tests and performed as well as (actually better than) the MAT 95 instrument in the Cologne laboratory.

On June 25, 1996 temporary IOC accreditation of the SmithKline Beecham took place. After the samples were prepared they were analyzed by GC/HRMS and quadrupole GC/MS (HP 5972 MSD). Four positive samples were detected by GC/HRMS. The screening results

were confirmed by HPLC fractionation and immunoaffinity chromatography techniques and the HRMS method, as well as the entire laboratory, received accreditation.

Samples found positive by GC/HRMS during IOC accreditation of the Atlanta laboratory.

Sample	Substance
1	metabolites of metandienone
2	clenbuterol and metabolites of nandrolone
3	metabolites of methyltestosterone and stanozolol
4	high T/E ratio

After accreditation was completed, the third MAT 95S instrument was installed and underwent testing. Up to the time of the start of the Games the following individuals were trained to operate the MAT 95S instruments and to read the screening data: Dr. Ray Kazlauskas, Dr. Michel Becchi and Michael Sekera.

As the samples arrived during the Games they were held until a sufficient number were available to form a batch. In each batch, one blank urine and one HQC urine were prepared. Batches were worked-up and derivatized by staff from the Los Angeles laboratory. As soon as the samples were ready for analysis they were split into subgroups and analyzed by GC/HRMS and GC/MS. The MAT 95S instruments often ran without pausing. In case of confirmation, only two MAT 95S instruments were available and this caused some sample back up. There was no down time due to instrument failure (there were power outages due to storms) and only routine GC maintenance needed to be performed. On one instrument, the ion volume was exchanged prior to running confirmation analyses.

- **A total of 3251 analyses (2847 during the Games) were performed on the MAT 95 instruments.**

  Instrument A: 1208
  Instrument B: 1156
  Instrument C: 887

- **No more than 18 hours passed between the time the samples arrived in the laboratory and HRMS analysis.**

- **All three MAT 95S instruments performed fully to expectation based on HQC standards prepared with each batch of samples.**

In Figure 1 a set of ion chromatograms from HQC138 measured on instrument A on August 5, 1996 (analysis 1193) is shown. One can easily identify all the substances in HQC, norandrosterone (NORAND), clenbuterol (CLENB), 3'- and 4β-hydroxystanozolol (STAN METAB), 17β-methyl-5β-androst-1-ene-3α,17α-diol (EpiMetenediol), 5a- and 5b-17α-methyl-androstane-3α,17β-diol (THMT) and 18-nor-17,17-dimethyl-5β-1,13-dien-3α-ol (18-Nor-EMD). This figure is representative of the performance of all three MAT 95S instruments through the entire Games.

In Figure 2 the sample load on each instrument for each day of the Games is shown. On occasion an instruments ran for more than 2 days (more than 150 analyses) with stopping for GC or MS maintenance (e.g., changing the GC septa, establishing that the ion source tune is optimized, etc.). Performance was judged on the basis of the HCQ sample which was run with every batch of samples. The Finnigan MAT 95S mass spectrometer was highly stable and highly reliable even under these extreme conditions. At no time did any of the high resolution mass spectrometers suffer from technical failures. As a point of interest, the first indication for the cases of bromatan misuse was noted by Dr. Ray Kazlauskas while examining data obtained from the high resolution mass spectrometer.

References

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5. Schänzer W., Geyer H. and Horning S., Long-term determination of metandienone and mestanolone, in W. Schänzer (Editor), *Recent Advances in Doping Analysis (5) - Proceedings of the 15th Cologne Workshop on Dope Analysis 1997*, Sport und Buch Strauß, Köln 1998.

Table 1. HQC urine used to control HPLC performance.

Substance	Concentration [ng/mL]	Concentration in derivatized sample [ng/µL]
Clenbuterol	1	0.02
18-nor-17,17-dimethyl-5β-1,13-dien-3α-ol	2	0.04
Norandrosterone	2	0.04
17β-methyl-5β-androst-1-ene-3α,17α-diol	1	0.02
Noretiocholanolone	1	0.02
17α-methyl-5α-androstane-3α,17β-diol	1.5	0.03
17α-methyl-5β-androstane-3α,17β-diol	3	0.06
3'-hydroxystanozolol	2	0.04
4β-hydroxystanozolol	2	0.04

The HQC urine was prepared in the Cologne laboratory and designed to test the performance of the GC/HRMS instrument.

2 mL urine were worked-up according to the standard procedure for steroids and derivatized with 100 µL MSTFA/TMIS. GC/HRMS analyses were performed using a Hewlett Packard 5890 GC fitted with a HP Ultra 1 column (17 m x 0.2 mm i.d., 0.11 µm film thickness, with He carrier gas, 11 psi head pressure). A 2 µL aliquot of sample was injected in the split injection mode (20 mL/min split flow, 2 mL/min septum vent flow). Temperature program: initial 185°C, 5°C/min to 235°C, 20°C/min to 310°C.

HMRS analyses were performed with a Finnigan MAT 95S mass spectrometer using 65 eV EI ionization (ion source temperature 240°C) in the electric scan mode using MID (multiple ion detection mode) analysis at ca. 3,000 resolution. Perfluorophenanthrene (FC-5311) was used for mass locking and calibration [3].

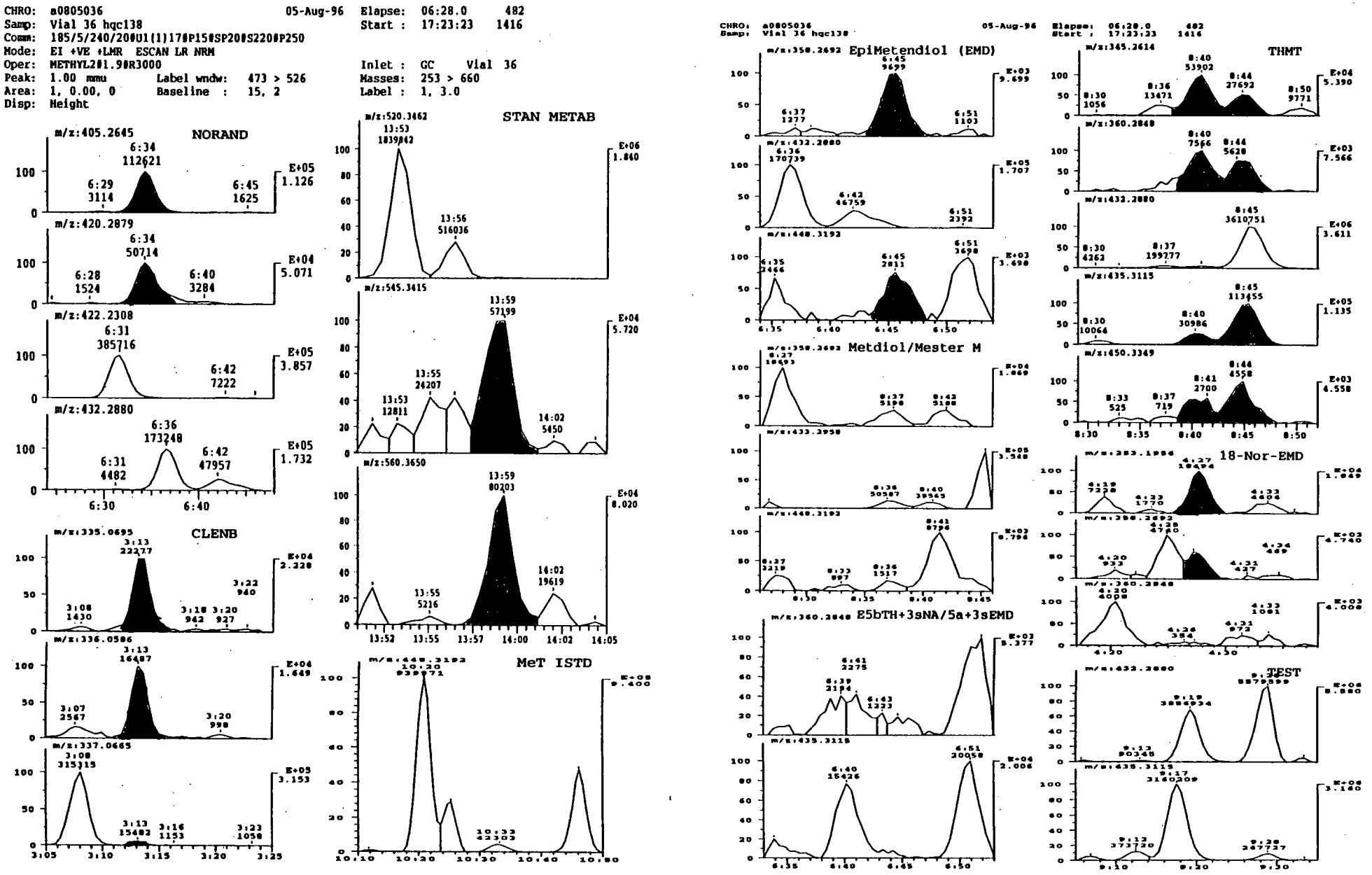

Figure 1. Set of ion chromatograms from HQC138 measured on instrument A (analysis 1193).

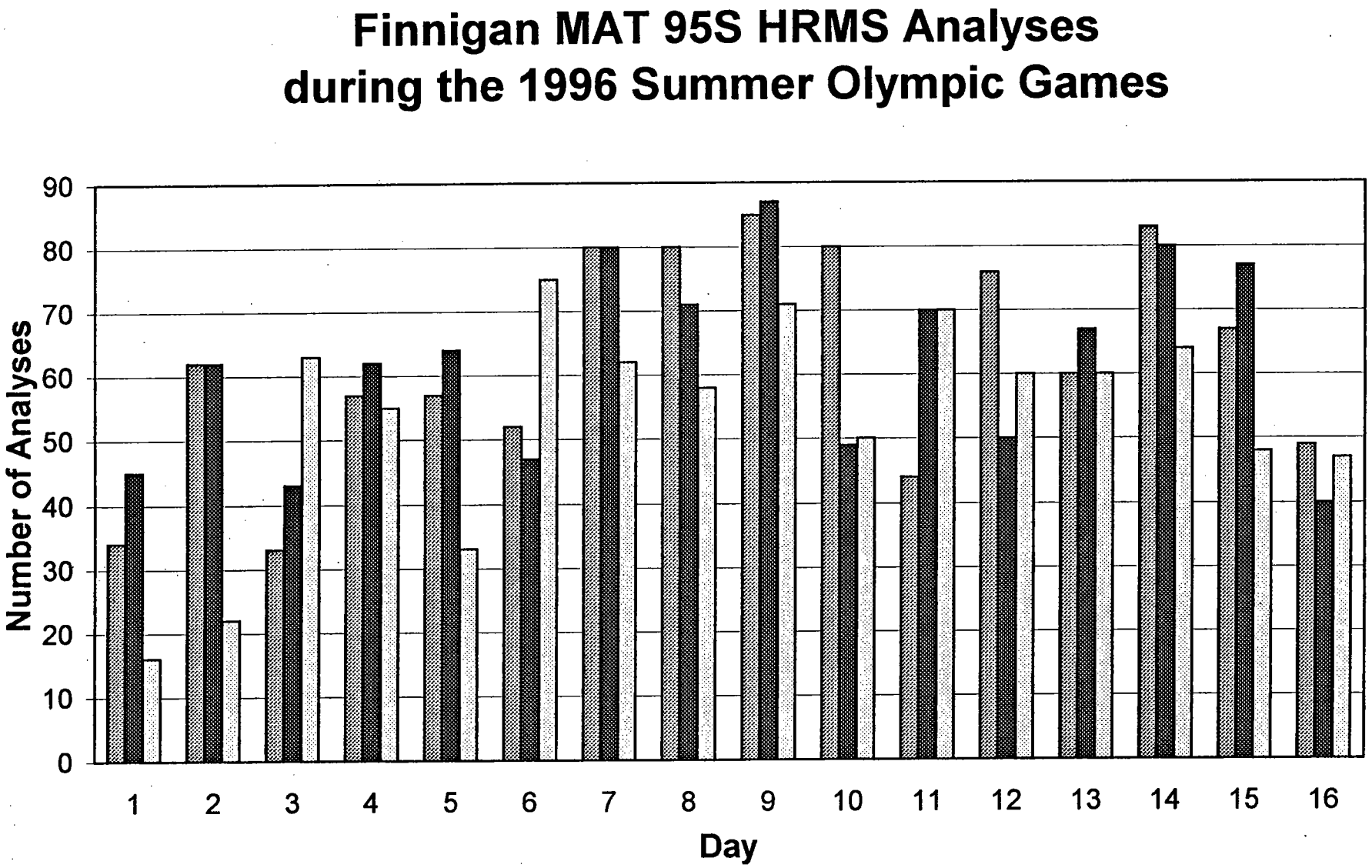

Figure 2. Sample distribution on the three MAT 95S over the period of the Games (gray: Instrument A, black: Instrument B, white: Instrument C).

History of the three MAT 95 S instruments used during the Centenial Olympic Summer Games in Atlanta 1996

Date		MAT 95 S - A	MAT 95 S - B	MAT 95 S - C
Apr. week 14		Order	Order	Order
Apr. week 15				
Apr. week 16		Shipment to UCLA		
Apr week 17		Installation		
May week 18		Installation		
May week 19				
May week 20		On-Site Training		
May week 21			Shipment to Atlanta	
May week 22			Installation	
Jun. week 23			Installation	Shipment to Atlanta
Jun. week 24		De-Installation		Installation
Jun. week 25		Shipment to Atlanta	Test measurements by Stevan	Horning
Jun. week 26		Installation	Accreditation done 24 - 26	Accreditation done 24 - 26
Jul. week 27				
Jul. week 28				
Jul. week 29 Jul. week 30 Jul. week 31		XXVI Olympic July 19	Summer Games to August 4 in	in Atlanta 1996
Aug. week 32		De-Installation	De-Installation	De-Installation

Figure 3. Order and installation plan of MAT 95S instruments in Atlanta. Kindly provided by Dr. H. Münster, Finnigan MAT, Bremen, Germany