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Part II. Automation, Reporting and the Local Area Network
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Introduction

The Department of Pharmacology and Toxicology, *Institut Municipal d'Investigació Mèdica*, has been performing doping control analysis since the beginning of 1986, the year in which it was also accredited as an IOC antidoping laboratory. This accreditation, combined with the decision to award the 1992 Olympic Games to Barcelona, resulted in the laboratory being chosen to conduct antidoping tests for this event. However, in order to screen the almost 2000 urine samples expected during 15 days of Olympic competition, the capacity of this laboratory will be considerably expanded using the support of the *Comité Organizador de los Juegos Olympicos de Barcelona* (COOB'92) and of the *Institut Municipal d'Asistència Sanitària* (IMAS). This expansion will occur in a number of ways, including finance for the purchase of a substantial amount of additional analytical and computing equipment and software.

The budget for the purchase of analytical and computing equipment is divided into three parts - (1) Analytical Instruments, (2) Computing Equipment and (3) Software. Analytical instruments have been purchased or loaned from different companies depending on the purpose: **Scharlau (Barcelona, Spain)** HI 8418 for pHmeters, **PAAR (Graz, Austria)** DMA 48 for densimeters, **ABBOTT Laboratories (Dallas, Texas, USA)** TDx and IMx for the analyses based on fluorescence polarization immunoassays or microparticulate enzime immunoassays, and **Labsystems (Barcelona, Spain)** for ELISA tests. Chromatographic instruments are being purchased through a significant contract signed with **Hewlett-Packard (Palo Alto, California, USA)** for several GC, GC/MS, GC/MS/LC

and HPLC instruments and for the loan of a similar number during the Games period. Other computing equipment purchases include a medium sized HP 9000/832 Unix system and the loan of a 9000/425 Unix workstation - the 9000/832 to be used to support a Laboratory Information Management System and **Oracle** data base, and the 9000/425 for file management and additional storage during the Games. The most significant software purchase will be a Spanish developed LIMS known as **LABiX (Central de Procesos Informaticos S.A., Valladolid, Spain)** which includes an **Oracle** data base run-time license, and that has been specially adapted to the needs of a modern laboratory in a joint effort between CPI and the Barcelona Laboratory staff (see Part I of this document). Considerable effort has also been directed towards developing a new system of "neo-macros" for initial data processing and report generation as described below.

Network Configuration

The expected maximum daily volume of samples, data and reports during the Games is shown in ***Figure II.1.***

Resource	Volume
Samples	200
Aliquotes	3000
Data files	1600
Megabytes (data)	800
Report pages	8200
Megabytes (reports - current)	1000
Megabytes (report - neomacros*)	100

* described in later sections

Figure II.1 Anticipated Maximum Daily Volumes

A total of 26 workstation controlled chromatographic instruments will be used for screening and confirmation procedures of these samples (according to the definition shown in ***Figure II.2***), and 12 **X Windows** and ASCII terminals for additional data entry and analysis of generated results. The inventory of computer based modules is shown in ***Figure II.3.***

Procedure	Description
1A	Stimulants, and some narcotics
1B	Strychnine (concentrated extract)
1C	Pre-confirmation by GC/MSD using the concentrated 1B extract
2A	Narcotics, ß-blockers, stimulants and metabolites
3A	Pemoline, caffeine, cortisol and cortisone
4A	Anabolic steroids (free fraction)
4B	Anabolic steroids (combined fraction)
5A	Diuretics, probenecid and mesocarb

Figure II.2 Definition of Chromatographic Analytical Procedures

Instrument	Model	Number
GC/MS/LC (Unix)	HP 5890A/5989A/1090L	1
MS/LC (Unix)	HP 5989A/1050	1
GC/MSD (Unix)	HP 5890A/5970A	3
GC/MSD (Unix)	HP 5890A/5971A	10
GC/NPD (MS-DOS)	HP 5890A	5
HPLC/DAD (Pascal)	HP 1090M	6
L.I.M.S. (Unix)	HP 9000/832	1
File Server (Unix)	HP 9000/425	1
X Terminal	HP 700/RX	4
X Terminal	HP 700/X	5
ASCII Terminal	HP 700/92	4 *
PC (MS-DOS)	AT/386/486	10

* directly connected to HP 9000/832

Figure II.3 Instrument and Terminal Inventory

All chromatographic instruments as well as non-instrument computing systems will be networked in an Ethernet based Local Area Network (LAN) (***Figure II.4***). This network will be connected to the existing IMIM LAN using technology (**XYPLEX MAXserver 3010** Bridge) which ensures data security within the laboratory.

A summary of the distribution of analytical instruments by screening procedure appears in ***Figure II.5***.

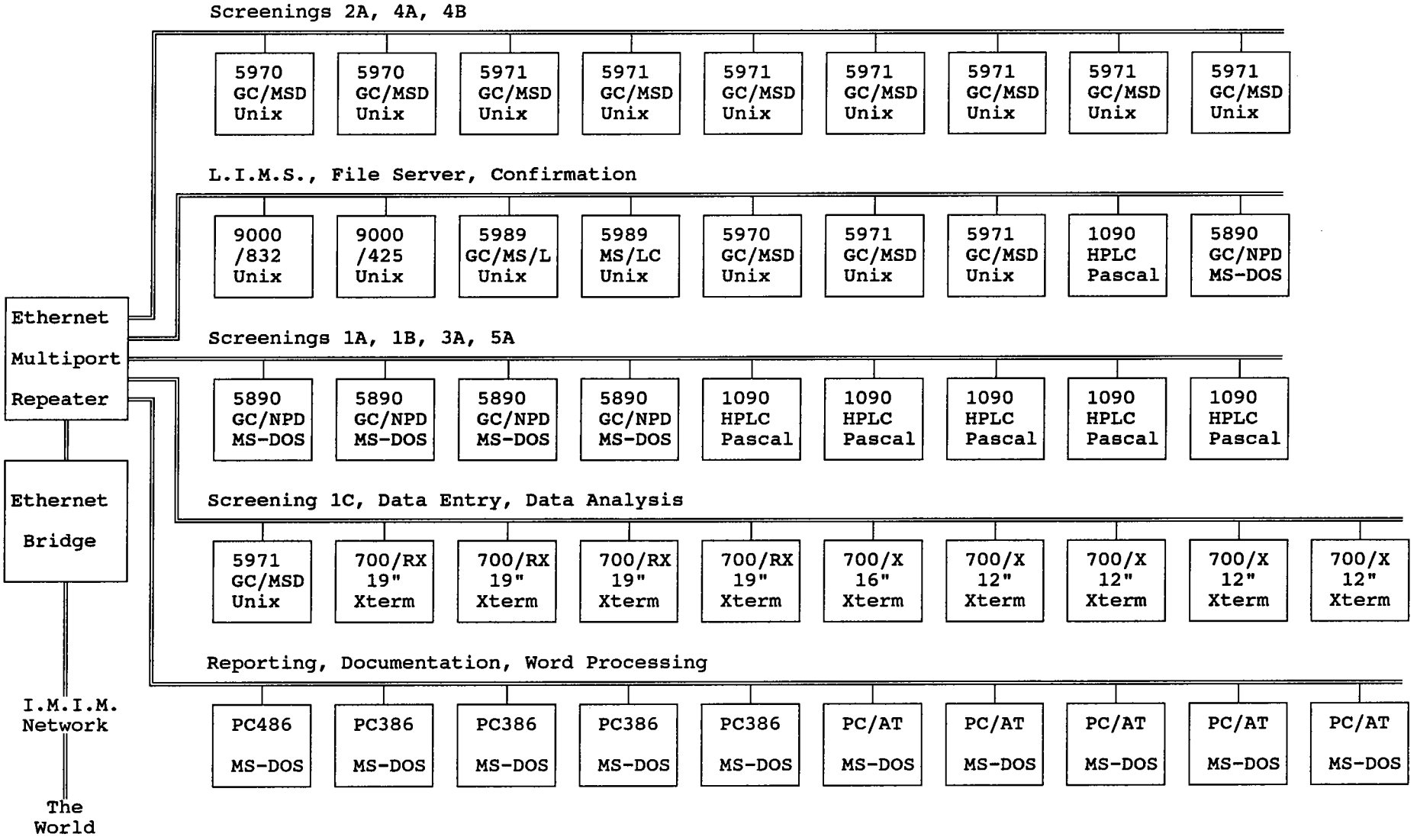


Figure II.4 Local Area Network Configuration

Procedure	Instrument Type	Operating System	Number Inst	Disk Size	Percent Samples	Samples/ Per Hour	Data File Size	Maximum Data/Hour
1A (scr)	5890 GC/NPD	MS-DOS	3	80 mb	**100	4	20 kb	240 kb
1B (scr)	5890 GC/NPD	MS-DOS	1	80 mb	100	12	10 kb	120 kb
1C (pre)	5971 GC/MSD	Unix	1	600 mb	20	4	2500 kb	2000 kb
1 (conf)	5890 GC/NPD	MS-DOS	1	80 mb	10	4	20 kb	8 kb
	5971 GC/MSD	Unix	1 *	600 mb	10	4	2500 kb	1000 kb
2A (scr)	5971 GC/MSD	Unix	3	600 mb	100	4	1500 kb	18000 kb
2 (conf)	5971 GC/MSD	Unix	1 *	600 mb	10	4	1500 kb	600 kb
3A (scr)	1090 HPLC	Pascal	2	40 mb	100	6	60 kb	720 kb
3 (conf)	1090 HPLC	Pascal	1 *	40 mb	10	6	60 kb	36 kb
	5971 GC/MSD	Unix	1 *	600 mb	5	4	1500 kb	300 kb
	5989 GC/MS/LC	Unix	2 *	600 mb	5	4	2500 kb	1000 kb
4A (scr)	5970 GC/MSD	Unix	2	600 mb	100	8	100 kb	1600 kb
4A (conf)	5970 GC/MSD	Unix	1 *	600 mb	10	8	2500 kb	2000 kb
4B (scr)	5971 GC/MSD	Unix	4	600 mb	100	3	200 kb	2400 kb
4B (conf)	5970 GC/MSD	Unix	1 *	600 mb	10	3	2500 kb	750 kb
5A (scr)	1090 HPLC	Pascal	3	40 mb	100	5	150 kb	2250 kb
5 (conf)	1090 HPLC	Pascal	1 *	40 mb	10	5	150 kb	75 kb
	5971 GC/MSD	Unix	1 *	600 mb	10	3	2500 kb	750 kb
	5989 GC/MS/LC	Unix	1 *	600 mb	5	3	2500 kb	375 kb

* instrument shared between procedures
** 100% is taken for the total numer of screening samples for each procedure
scr: screening conf: confirmation pre: preconfirmation

Figure II.5 Equipment Distribution by Screening Procedure

The use of a LAN has aided the development and installation of a Laboratory Information Management System and associated data base. This system will be installed on a dedicated Hewlett-Packard 9000/832 computer, and will be used to control many aspects of the reception, distribution and analysis of samples, and the reporting and archiving of data. In addition, a HP 9000/425 workstation will be used as a File Server, thereby offloading some of the workload of the LIMS computer system.

The relationship between a typical instrument, the LIMS and the File Server is shown in ***Figure II.6***. A detailed description of the design of the LIMS is presented as Part I of this document.

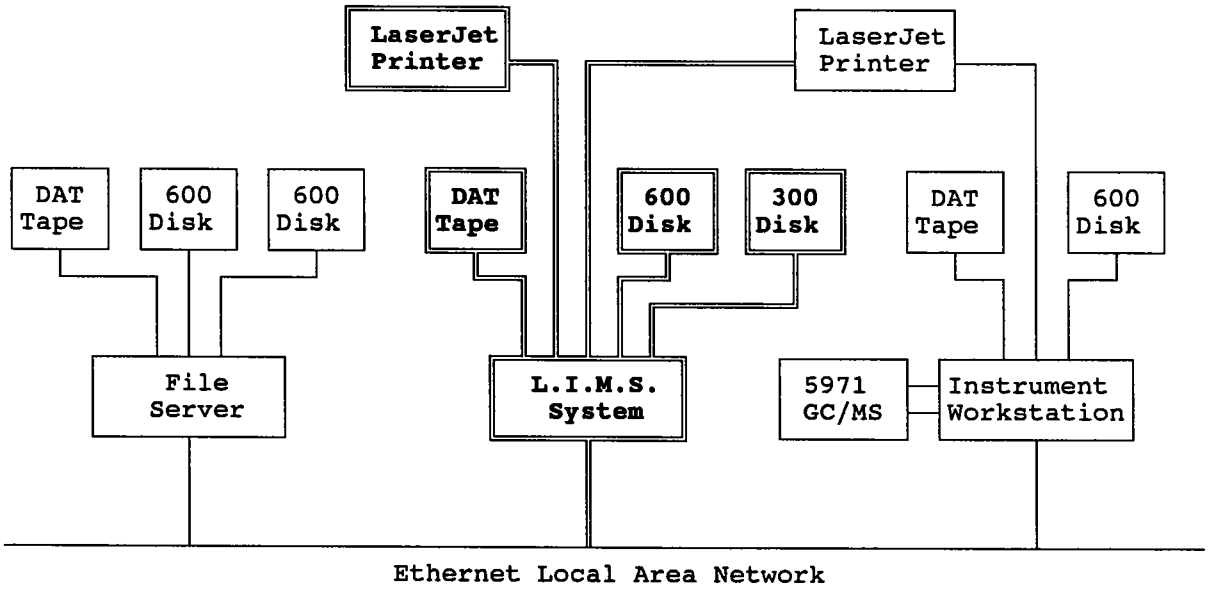


Figure II.6 Instrument, L.I.M.S. and File Server

The LIMS and Instrument Systems

Apart from supporting the Spanish developed Laboratory Information Management System **LABiX**, the LIMS computer performs a number of other functions in the control of samples and their data. Communication between the LIMS and each instrument is fundamental, and the logical relationship between these is illustrated by ***Figure II.7***.

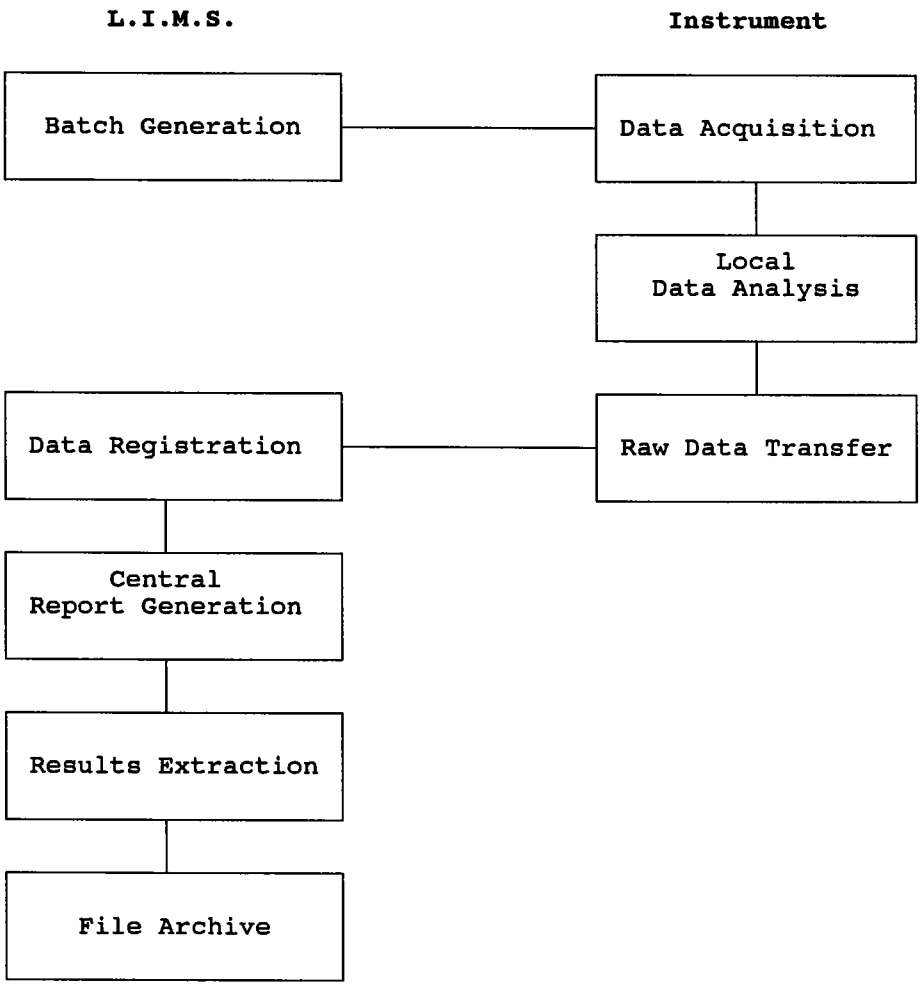


Figure II.7 LIMS and Instrument Functions

Batch Generation

Batch (or lot) generation will be defined by the Certifying Scientist in conjunction with the LIMS, with each batch consisting of about 20 samples plus controls, blanks and other quality assurance samples. At the time of batch creation a set of worksheets and bar code labels will be printed to accompany the batch of sample aliquotes through the analysis procedures. Each batch is assigned a unique code (***Figure II.8***), and a subdirectory is created on the LIMS disk for later storage of data related to samples in the batch (***Figure II.9***).

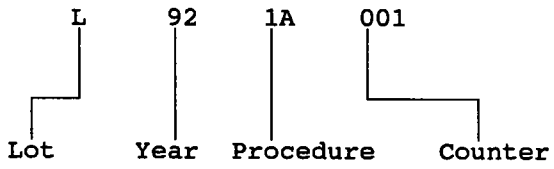


Figure II.8 Batch Code Format

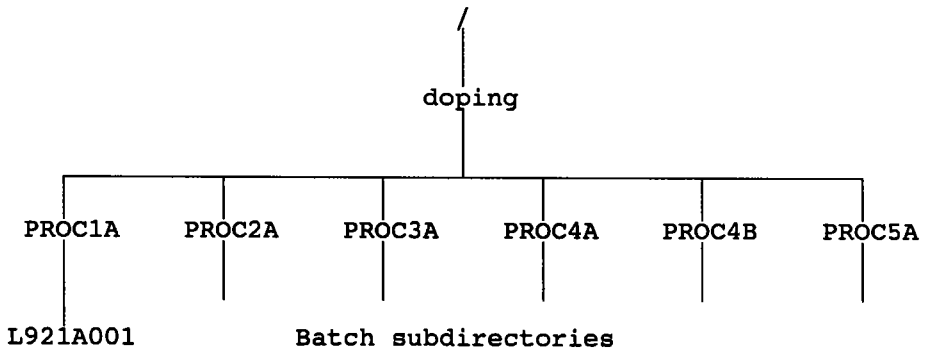


Figure II.9 Batch Subdirectory Structure

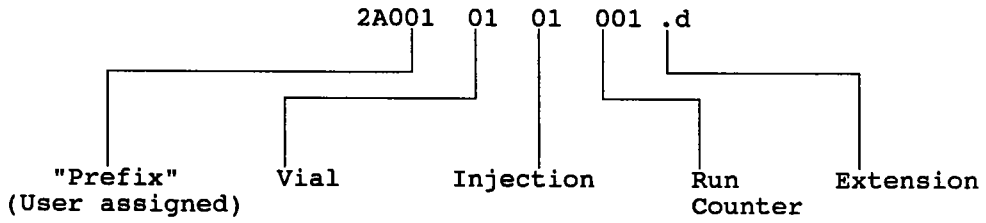
Data Acquisition

Using the accompanying worksheets and bar code labels (see Part I) the analyst will prepare extracts for batches of samples. Sequence parameter and sample tables will then be entered using the worksheet and a manual bar code reader (wand). The use of bar code technology will avoid possible operator error which can easily occur when entering a large number of sample codes. At this stage the analyst will start the prepared sequence and inject the samples on the corresponding analytical instrument.

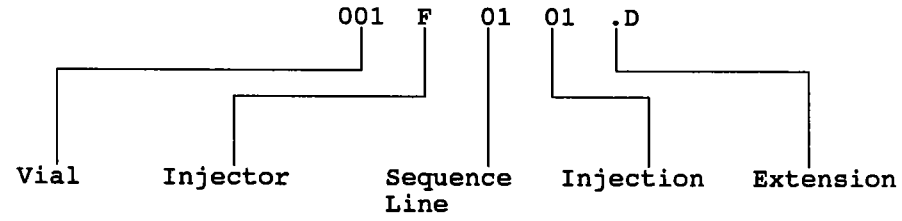
Data Analysis

Data files of varying sizes (10,000 to 2,500,000 characters) and name formats will be generated for each sample in a batch (***Figure II.10***). At the same time a report including graphical and textual information will also be generated. Because the graphical information contained in standard reports is stored in bitmap format, the resulting large files will be discarded and only the hard copy report saved. More sophisticated reporting will be provided by the centralized LIMS system at a later stage.

GC/MS (Unix):



GC (MS-DOS):



HPLC (Pascal):

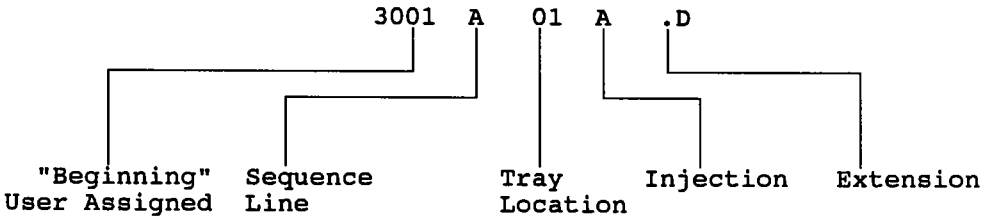


Figure II.10 System Dependent File Name Formats

(NB. File names do not provide for inclusion of sample codes).

Data Transfer

The use of the HP **ChemLAN** product will allow each data file to be sent automatically to its predefined batch subdirectory on the file server. The typical ChemLAN configuration specifies a single Unix Workstation (eg. GC/MS) to act as server for MS-DOS and Pascal systems. However, in the case of the Olympic Games, the file server system will be configured as the ChemLAN server, and each of the instrument workstations (including HP-UX Unix systems) will be configured as clients.

The analyst will predefine data file transfer as part of each method and when generated, data files will be immediately copied into the subdirectory previously allocated on the file server. By sharing file server disks with the LIMS computer this essentially means that the LIMS system itself will have immediate access to data files as they arrive from the instrument clients.

Data Registration

A separate LIMS module has been developed to detect the arrival of new data files in the file server subdirectories. On arrival, a data file will be identified by the sample code in its header, "registered" by the LIMS, and the header information extracted for inclusion in the LIMS data base. The extracted sample code will be used as the name of a new subdirectory level containing all files pertaining to the particular sample (***Figure II.11***).

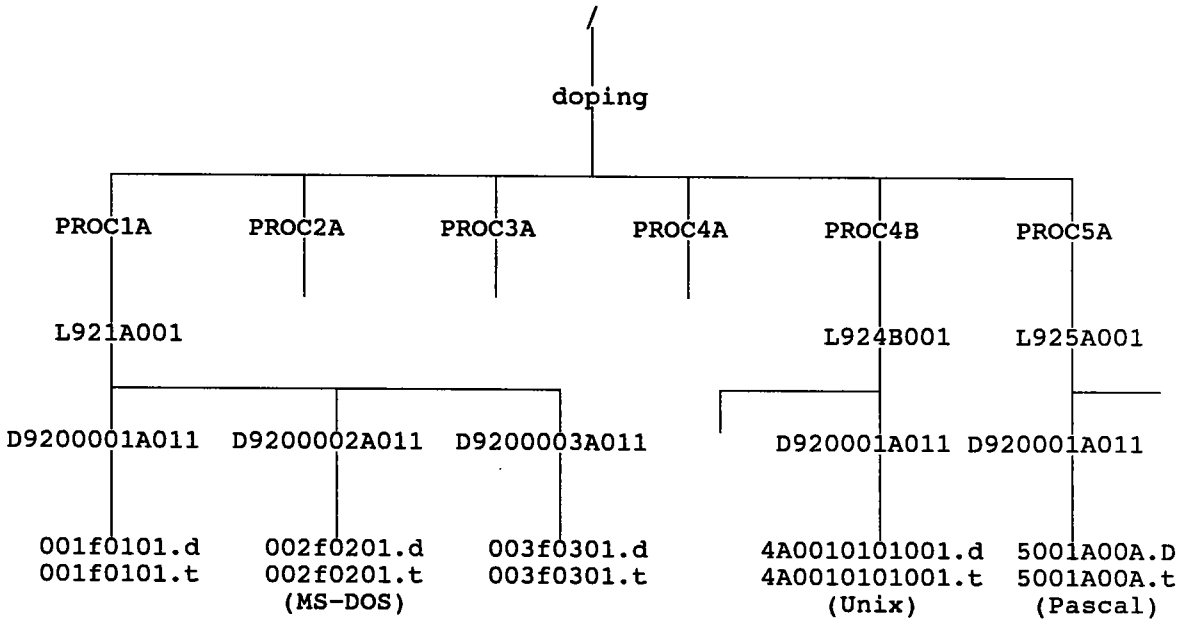


Figure II.11 Creation of Sample Subdirectory

Report Generation

Another function of this LIMS module is the generation of reports. After identifying a particular sample, it will start a reporting program that is processed on the LIMS system but that prints a high quality laser beam printer document locally on the instrument which generated the data file (or on any other redirected printer in the network).

This reporting will be performed by a special system of "neo-macros" developed at IMIM. The main features of this system are:

- It uses a set of procedures especially designed for Antidoping control target analysis.

- Higher resolution print quality is used to improve the detail and design possibilities of hard copy reports.

- The use of the standard HPGL printer graphics language rather than screen dumps (bitmap format) reduces report sizes (measured both in pages and megabytes of disk space).

- Control of search algorithms improves peak identification and integration.

- The use of a separate modifiable parameter file for each procedure and instrument combination improves target identification.

- Report formats and components are standardized for all screening procedures.

- Reports generation is centralized and offline thereby eliminating some previous bottlenecks.

Examples of reports for each of the screening procedures appear in ***Appendix II.1.*** Approximate data and report file sizes for these procedures are presented in ***Figure II.12.***

Screening Procedure	Data File *Disk Space	Standard Reports *Disk Space	Pages	Neo-Macro Reports Disk Space	Pages
1A	20 kb	17 kb	2	9 kb	2
1B	10 kb	17 kb	2	4 kb	1
2A	1500 kb	270 kb	6	34 kb	3
3A	60 kb	50 kb	2	8 kb	1
4A	100 kb	180 kb	6	14 kb	2
4B	200 kb	530 kb	15	50 kb	3
5A	150 kb	400 kb	12	41 kb	4

* approximately

Figure II.12 Typical Data File and Report Sizes

Results Extraction

The neo-macro reporting system also generates a summary table file of peaks and their integration. This information will be captured by the LIMS module and all information in the table included in the data base. In essence, this will avoid an otherwise messy interface between the LIMS and graphics files.

An example summary table file from a Screening 4B reference sample is shown in ***Figure II.13***.

SUBSTANCE	R.T. (min) Exp.	Real	RESPONSE FACTOR	AREA	HEIGHT	CONC. (ng/ml)
Methyltestosterone	15.35	15.35		169308	47467	500.00
Testosterone	13.56	13.57	22.11	24069	6205	43.18
Epitestosterone	12.68	12.70	26.51	26693	8140	57.42
D3-Testosterone	13.53	13.53		12323	3145	20.00
Androsterone	10.84	10.92	577.80	631939	120196	4225.31
Etiocholanolone	11.13	10.05	442.03	408912	113813	2091.64
D4-Etiocholanolone	10.93	10.96		86416	21626	500.00
11-OH-Androsterone	13.77	13.77	225.60	164423	45353	1029.21
11-OH-Etiocholanolone	13.94	13.93	283.03	27513	7420	216.06
D4-11-OH-Androsterone	13.74	13.72		36041	7388	240.00
Tetrahidrocortisol	19.42	19.29	2383.08	253416	149698	3566.94
Testo./Epitesto.				0.90	0.76	0.75
Andro./Etio.				1.55	1.06	2.02
Testo./Andro.				0.04	0.05	0.01
Testo./Etio.				0.06	0.05	0.02

Figure II.13 Example Summary Table File

Data Archive

Since all data and table files will be accessible by both the LIMS and file server systems, two high volume DAT tape units will be available for file back up procedures. It is expected that at least one copy of the dedicated Antidoping disk (600 megabyte capacity) will be required each day. The frequency of such copies will increase as the need arises. They will be deleted from the server after reporting to the I.O.C., and only when at least two copies have been taken. In addition, short term copies of all data files will remain on the local disk of the instrument which generated them. Files will be deleted on the instrument disk only when space is required.

Conclusion

The Barcelona '92 Antidoping Laboratory will be one of the most complex and sophisticated of its type in the world. The sheer volume of data and reports produced will require advanced computing hardware and software to ensure the integrity of this data, and to ensure that its management and analysis be trouble free.

With the assistance of the Hewlett-Packard corporation and the *Central de Procesos Informaticos* (**C.P.I.** - developers of basic **LABiX**), the Laboratory has been able to mount a system which it believes can cope more than successfully with the expected 2000 samples in the 15 days of Olympic competition. Moreover, we have developed a system of reports which are of considerably higher design and presentation quality than it has been able to achieve using standard macro techniques.

In summary, it is believed that the Computing component of the Barcelona '92 Antidoping Laboratory will be a major innovation with respect to that of previous Olympic Games.

Appendix II.1 **Report Format of Procedure 1A and 1B.**

Procedure 1A:

Title:	Stimulants
Definition:	Volatile nitrogen containing compounds excreted free in urine.
Sample:	Spiked urine containing a set of reference standards.
Page 1:	• Standard sample header information, plus batch code and instrument network name. • Height scaled signal (NPD) chromatogram. • Height truncated signal chromatogram used to visually amplify small peaks. • Internal Standard identification window showing the integration of the identified peak and its real retention time.
Page 2:	• Reduced header information. • Report table showing presumptive substance metrics. Peaks matching entries in the reference substance table are named. Unidentified peaks are indicated by '?'. Response factors and concentrations are only calculated for quantifiable substances.

Procedure 1B:

Title:	Strychnine
Definition:	Special instrumental conditions for the analysis of strychnine.
Remarks:	Not presented. Follows similar report format to Procedure 1A.

Appendix II.2 **Report Format of Procedure 2A.**

Title: Narcotics, ß-blockers, stimulants

Definition: Heavy volatile compounds excreted conjugated in urine.

Sample: Spiked urine containing a set of reference standards.

Page 1:

- Standard sample header information, plus batch code and instrument network name.
- Height scaled total ion chromatogram (TIC).
- Four height scaled selected ion chromatograms (m/z 284, 86, 292 and 344) to trace the presence of ß-blockers . The updated expected retention times and the names of some of these are shown. The spiked urine contains alprenolol, metoprolol, pindolol, acebutolol, levobunolol, carteolol and sotalol.
- Two height scaled selected ion chromatograms to trace the presence of codeine and morphine. The integrations and the real retention times of the identified peaks are shown. The spiked urine contains both codeine and morphine.
- Two reference substance identification chromatograms showing the integration of the identified peaks and their real retention times. D3-Codeine is used for the updating of expected retention times, and as internal standard for quantitation. D5-MDMA is added as a marker for N-TFA derivatization.
- Report table showing the integrated substance metrics. Response factors and concentrations are calculated for codeine and morphine.

Page 2:

- Reduced header information.
- Time window ion chromatograms to monitor the various narcotics. The updated expected retention times and their systematic names are shown. The spiked urine contains none of these substances.

Page 3:

- Reduced header information.
- Height scaled total ion chromatogram (TIC)
- Ten height scaled selected ion chromatograms to trace the presence of stimulants. The updated expected retention times and the names of some of these are shown. The spiked urine contains norephedrine, ephedrine, propylehexedrine, chlorphentermine, methoxyphenamine, amphetamine, methylphenidate, ritalinic acid, fenfluramine, fencamfamin and pholedrine.

Appendix II.3 **Report Format of Procedure 3A.**

Title: Caffeine, pemoline

Definition: Detection of pemoline and quantitation of caffeine, cortisol and cortisone.

Sample: Spiked urine containing pemoline and 12.55 μg/mL of caffeine.

Page 1:

- Standard sample header information, plus batch code and instrument network name.
- Two height scaled chromatograms at a selected wavelength (216 nm) to trace the presence of pemoline. The second of these corresponds to an amplification of the time window where this substance appears with the indication of its updated expected retention time.
- Two height scaled chromatograms at a selected wavelength (280 nm) to trace the presence of caffeine. The second of these corresponds to an amplification of the time window where this substance appears showing the integration of the identified peak and its real retention time. The full ultraviolet spectrum of that peak is also presented below to avoid any false identification. The spiked urine contains 12.55 μg/mL of caffeine.
- One height scaled chromatogram at a selected wavelenght (246 nm) to trace the presence of cortisol and cortisone. The real retention times and the integrations of the identified peaks are indicated. The spiked urine contains naturally ocurring cortisol and cortisone.
- Internal Standard identification window showing the integration of the identified peak and its real retention time. Used as the reference substance for the updating of expected retention times.
- Report table showing the integrated substance metrics. Response factors and concentrations are calculated for caffeine, cortisol and cortisone.

Appendix II.4 **Report Format of Procedure 4A and 4B.**

Procedure 4A:

Title:	Steroids (free fraction)
Definition:	Anabolic steroids excreted free in urine.
Sample:	Spiked urine containing a set of reference standards.
Remarks:	Not presented. Follows similar report format to Procedure 4B except that only the internal standard is integrated.

Procedure 4B:

Title:	Steroids (combined fraction)
Definition:	Anabolic steroids excreted both free or conjugated in urine. Only those excreted conjugated are actually monitored.
Sample:	Spiked urine containing a set of reference standards.
Page 1:	• Standard sample header information, plus batch code and instrument network name. • Height scaled total ion chromatogram (TIC). • Four internal standard identification chromatograms (m/z 446, 435, 438 and 526) showing the integration of the identified peaks and their real retention times. Methyltestosterone is used as the reference substance for the updating of expected retention times. • Four height scaled selected ion chromatograms (m/z 432, 434, 522 and 636) to trace the presence of some endogenous steroids. The integrations and the real retention times of the identified peaks are shown. • Report table showing the integrated substance metrics. Response factors and concentrations are calculated for the selected endogenous steroids. Deuterated compounds, where these exist, are used to quantify their analogues.
Page 2:	Not presented. It has the same format as page 3.
Page 3:	• Reduced header information. • Time window ion chromatograms to monitor the various exogenous anabolic steroids. The updated expected retention times and their systematic names are shown. The spiked urine contains all substances appearing on this page with the exception of 16ß-OH-stanozolol and clenbuterol.

Appendix II.5 **Report Format of Procedure 5A.**

Title:	Diuretics
Definition:	HPLC analysis of diuretics, mesocarb and probenecid.
Sample:	Spiked urine containing one of a set of reference standards.
Page 1:	• Standard sample header information, plus batch code and instrument network name. • Two height scaled chromatograms at selected wavelengths (270 and 350 nm) to trace the presence of diuretics. • Internal standard identification set containing the ultraviolet reference spectrum (from a library), height scaled chromatogram at selected wavelength (270 nm) showing the integration of the identified peak and its real retention time, and the actual ultraviolet spectrum of that peak. • Report table showing the integrated substance metrics of the internal standard.
Page 2:	Not presented. It has the same format as page 3.
Page 3:	• Reduced header information. • Three columns containing, for each substance, the ultraviolet reference spectrum (from a library), one or two height scaled chromatograms at selected wavelengths showing the updated expected retention times, and the actual ultraviolet spectra of those peaks found in the first chromatogram. Of the substances appearing on this page the spiked urine contains conjugated p-OH-mesocarb and the two isomers of cyclothiazide.
Page 4:	Not presented. It has the same format as page 3.