United States
Environmental Protection
Agency

EPA-600/1-81-025

EPA

Research and Development

PILOT PROGRAM FOR THE NATIONAL ENVIRONMENTAL SPECIMEN BANK

PHASE I

Prepared by

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PILOT PROGRAM FOR THE NATIONAL ENVIRONMENTAL SPECIMEN BANK - PHASE I

by

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Contract No. EPA-79-D-X0105

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis and the toxicology of pesticides as well as other chemical pollutants. The Laboratory participates in the development and revision of air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data for registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is primarily responsible for providing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangerment of their health.

This report documents one aspect of an International effort in cooperation with the Environmental Agency, Federal Republic of Germany, and partially supported by EPA, to provide a comprehensive environmental monitoring program to assess the relative risk of environmental hazard to the health and well-being of our population and to aid in the improvement of our environmental quality. This program, the National Environmental Specimen Bank, will serve as an environmental warning system by providing real time chemical analysis of collected specimens. In addition, this system would permit the use of tomorrow's more sensitive and more specific methods of chemical analysis on stored samples. The advantages of such a program will permit us to assess the effectiveness of our present environmental control techniques by monitoring pollutant trends, as well as establishing environmental baseline levels of new pollutants or pollutants of current concern not previously investigate.

F. Gordon Hueter, Ph.D.
Director
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ABSTRACT

This work was performed under a joint NBS/EPA research program to develop state-of-the-art protocols for the sampling, storage, and analysis of biological and environmental-type matrices. This report summarizes the procedures used in the initial phase of a pilot program for determining the feasibility of the National Environmental Specimen Bank. A special clean-laboratory/storage facility has been completed for use in this program. Detailed protocols for sampling, storage, and analysis of human liver samples are described.

This report is submitted in partial fulfillment of EPA Interagency Agreement EPA-79-D-X0105 by the National Bureau of Standards for the 1980 contract year.

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SECTION 1

SAMPLING AND SAMPLE HANDLING PROTOCOLS FOR HUMAN LIVERS

by

Sally H. Harrison

Since the publication of the preliminary protocol for the sampling and collection of human livers in the 1979 annual report of the Specimen Bank Program (1), the details necessary for the implementation of the protocol, e.g., selection and procurement of materials for sample collection, shipping, and storage, have been finalized. Contracts for liver procurement were negotiated and awarded to three medical schools. These contractors are located in Baltimore, Maryland; Minneapolis, Minnesota; and Seattle, Washington. Each contractor is scheduled to provide 100 liver samples this year for a total of 300 samples. In Baltimore, samples are obtained from the Baltimore Medical Examiner's Office. In Minneapolis, the samples are obtained from four locations, including both medical examiner and hospital autopsy rooms. In Seattle, the samples are obtained from the county medical examiner's office and a hospital autopsy room.

To insure the quality of the samples received and to have an appreciation of the conditions under which they are collected, in-person reviews of the protocol were conducted with those persons actually collecting the samples. Some of the objectives of these visits were as follows: 1) personal contact of current NBS specimen bank personnel with the contractors; 2) assessment of the cooperation and problems involved in the interactions between contractors and autopsy personnel, e.g., medical examiners, autopsy coordinators, pathologists/pathologists' assistants, and technicians; 3) personal contact with those actually collecting and processing the samples; 4) instruction in the details of the sampling protocol; and 5) inspection of facilities, e.g., autopsy rooms, laboratory facilities, etc. These visits were made by NBS and EPA personnel soon after the contracts were awarded. Prior to the visits, however, the contractors were sent a videotape in which the techniques and procedures to be used in sample collection were demonstrated.

The autopsy room procedures are slightly different for each of the three contractors. In Baltimore, the liver is taken after evisceration and removal of the block of organs onto the dissecting table. In Minneapolis, the liver is removed and partially processed at the beginning of the autopsy. In Seattle, the intestinal organs are removed and the thoracic block cut free of the cavity. The technician then cuts the liver from these organs while they are still in the body.

In addition to the need for biological contamination control procedures, chemical contamination control was discussed with the medical personnel. The use of disinfectants, sprays and other chemicals in the autopsy room was surveyed. It was requested that this use be kept to a minimum with specimen bank cases. In addition, smoking should not be permitted in the area of sample collection and processing.

The liver sampling protocol (see Appendix I) is to be followed carefully by the contractors. The criteria for case selection (2, and Appendix I), are intended to provide samples which are a valid representation of the chemical state of the liver at the time just prior to death. Livers are excluded which are physically injured, e.g., from a car accident, diseased or infected, or chemically abnormal, e.g., drug overdose. Medical examiner's cases are more likely to satisfy these case selection requirements of the specimen bank than hospital autopsy cases. In addition, small livers, less than one kilogram total weight, are excluded because they do not provide a sufficient amount of material for the necessary multiple analyses.

The sampling data form, which contains data about both the donor and the specimen, appears in Figure 1. This form is printed on four part NCR paper. The back (orange) copy, sent with the sample, contains the information which is readily available at the time of autopsy. Later, the top two copies (white and yellow) are sent with the results of the histological study and with additional information from the autopsy findings and donor history as available. The final (pink) copy is retained by the contractor for his files.

NBS-1156
(6-80)

U.S. DEPARTMENT OF COMMERCE
National Bureau of Standards

NBS-EPA Pilot Environmental Specimen Bank

SAMPLING DATA - HUMAN LIVER

autopsy identification number

date and time of death (day/mo/yr)

date and time of autopsy (day/mo/yr)

place of autopsy

pathologist

mortuary storage date and time in

temperature °C

Patient

date of birth (day/mo/yr)

sex

residence

ethnic group

height inches

cm

weight lbs

kg

smoker yes no

occupation history

diseases

diagnosis of autopsy

Comments

Liver

weight whole g

weight section A g weight section B g

Figure 1. Sampling data form for human livers.

The protocol materials utilized in the sample collection were selected to minimize sources of contamination (and in some cases to make the procedure more convenient for the operator). These materials (listed in Appendix A) are provided to the contractor for the sampling. All personnel handling a specimen bank autopsy case should wear non-talced, dust-free gloves and have been instructed in what constitutes contamination of the gloves necessitating changing to a clean pair. The liver sample is always placed on dust-free, clean, Teflon FEP film. Based on previous evaluation (3), Teflon is one material which is acceptable for both trace element and trace organic analyses.

After removal from the body, the liver is rinsed with a commercial water produced as a high performance liquid chromatography (HPLC) reagent water. This water was analyzed for its trace element content and the results of these analyses appear in Table 1. (See Section 5 for trace organic analysis of this water) At the low levels of trace elements found in this water, contamination due to the rinsing of the liver will be negligible. After washing the whole liver, the left lobe of the liver is dissected from the whole organ and then divided into two sections using a titanium knife mounted on a Teflon handle. Titanium is an element that is currently considered to be neither toxic nor essential to man. If contamination does occur from the use of this knife, it will be with only this one element.

TABLE 1

Trace Element Analysis of HPLC Reagent Water

Element	Neutron Activation Analysis		Spark Source Mass Spec.
	Lot 1* µg/mL	Lot 2 µg/mL	Lot 1* µg/mL
Cr	<1	<1	0.3
Mn	≤2	-	-
Fe	6	<20	5
Co	0.37	<0.05	-
Ni	<4	-	1
Zn	<1	<1	0.2
As	1.0	<2.7	-
Se	<0.02	<0.04	0.1
Sr	≤0.36	-	-
Mo	0.06	0.3	0.02
Ag	<0.04	<0.03	-
Cd	<0.15	<0.06	0.1
Ba	≤0.19	-	0.5
Hg	0.35	0.09	-
Pb	<7	-	0.2

* Different bottles of the same production lot.

- Indicates no determinations for this element.

Each left lobe section is placed in a Teflon FEP bag and heat sealed. It was originally planned that the samples would be vacuum sealed in the Teflon bags. However, this procedure was found to be awkward and difficult to achieve; under vacuum the bags tend to wrinkle across the sealing area preventing an airtight seal. Heat sealing the Teflon is one of the more difficult techniques in the procedure. Each sample is placed in a second Teflon bag and sealed again. The sealed samples are then placed in cylindrical cardboard containers (57 mm O.D. x 117 mm) and quick frozen by immersion in liquid nitrogen. When frozen, the samples are transferred to nitrogen vapor cryogenic shippers (MVE models BDS 5 or BDS 12) for storage until shipment to NBS via express freight. The cryogenic shippers are generally received within 24 hours of shipment; however, if they are delayed, they have a holding time of 12 days when properly cooled with liquid nitrogen.

After each case, the titanium knife is cleaned with high purity water and alcohol to prevent cross-case contamination. After six cases and after each knife sharpening, the titanium knife undergoes a more rigorous cleaning procedure. These two knife cleaning procedures are given in Appendix II. A new silicon carbide (Crystolon) bench stone was sent to each contractor for sharpening the titanium knife. Only this stone is to be used for sharpening the knife, and only the titanium knife is to be sharpened on this stone. This restriction will prevent non-titanium metal contamination of the knife during sharpening.

To insure that samples placed in the specimen bank are free of infection, a blood sample is collected from all donors for hepatitis B antigen screening. A histological section is collected from the right lobe for examination. The liver samples remain in a temporary storage facility until the results of the hepatitis screening are available. If the test for hepatitis B is positive, the sample is sent in the cryogenic shipper to Baltimore for incineration. If the results are negative, the samples are banked into the main storage facility. Both the slides and the results of the histological examination are sent to NBS within a month of sample collection. The histological slides are also stored in the bank facility.

When received at NBS, liver samples are immediately transferred from the liquid nitrogen vapor cryogenic shipper to a liquid nitrogen storage freezer. In this freezer, they are stored in the vapor phase above the liquid nitrogen level, where the temperature ranges from -196 °C to -120 °C. The samples are never thawed until the time of analysis.

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SECTION 2

DESIGN PRINCIPLES AND CONSTRUCTION OF THE PILOT SPECIMEN BANK FACILITY

by

John R. Moody

INTRODUCTION

Although the basic design of the NBS Pilot Bank facility has been described previously (1), a further distinction needs to be made between a chemically clean laboratory and the more common clean room.

Clean rooms have been used in industry for more than 20 years. Predictably, the first applications of clean room technology in the chemistry laboratory transferred existing technology with no consideration for the unique problems inherent in handling corrosive and toxic chemicals in a clean room. As a result, the clean rooms built at NBS during the 1960's have proven to be inadequate for their intended use. The first true chemically clean laboratory at NBS was built in 1971 and the design was the result of collaboration between NBS scientists and the contracting company that built the laboratory.

In the intervening years a second generation, improved chemically clean laboratory was built at NBS (1977) and it was this laboratory which was the model for the Pilot Bank facility. The Pilot Bank facility contains the first NBS laboratory specifically designed for both trace metal and trace organic analysis. The following sections will show how these objectives have been achieved.

General Design Principles

The heart of any clean room design is the high efficiency particulate filter or HEPA filter. These filters were originally designed as containment filters for the nuclear industry, but the clean room concept evolved during the 1950's in the electronics industry with the introduction of the transistor to the market place. The efficiency of the HEPA filters is generally 99.97 percent at a particle size of 0.3 μm, the least efficient filtration being for particles of this size. Early clean room designs consisted of whole walls or banks of HEPA filters with the air flow moving horizontally across the room to an air plenum as in Figure 1.

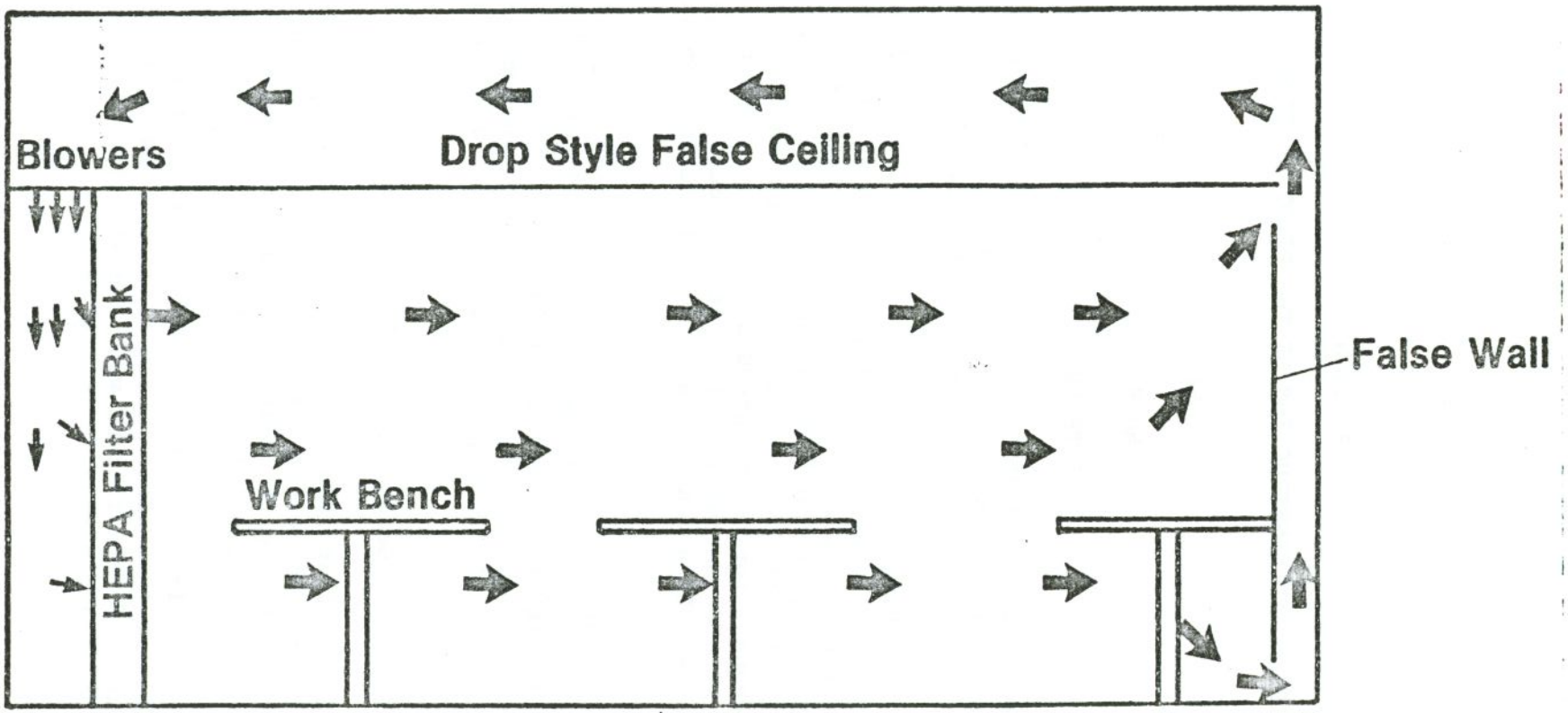

Figure 1. Horizontal-flow style clean room.

In a large room this design creates problems since dirt and particulates generated on work surfaces near the filter must travel over all other work surfaces before exiting the room. In the chemistry laboratory, this results in cross contamination between samples at different locations in the laboratory. The vertical air flow concept, Figure 2, eliminates this problem by placing the air supply in the ceiling and the exhaust in the floor. This type of design also reduces somewhat the need for special clothing, e.g., a smock can be worn instead of a shoulder to foot coverall. This is especially advantageous where chemists must enter and leave the laboratory often.

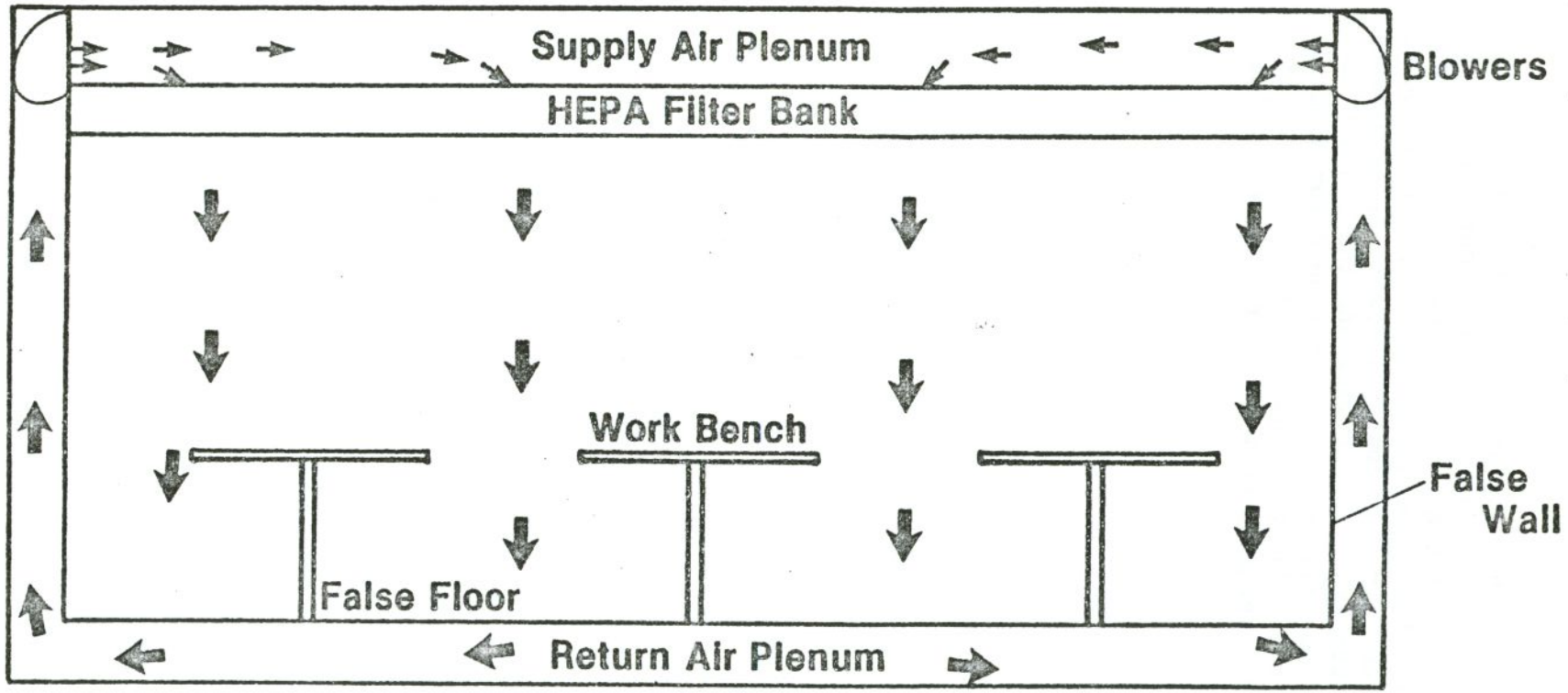

Figure 2. Vertical-flow style clean room.

There are, however, some problems with this design, not the least of which is high cost. If one were to assign a priority to the flow of clean air in a chemical laboratory it would be as follows:

Air Supply → HEPA Filters → Clean Air → Sample → Chemist → Equipment → Exhaust/Return.

In such a scheme, the sample will be in the most protected part of the laboratory, nearest to the air supply. Ideally, the total path length between air supply and air return is as short as possible and heavy particulate generators; e.g., equipment such as refrigerators, ovens, pH meters, etc.; will be located as near to the air exhaust as possible.

The NBS Design

The design employed by NBS accomplishes the desired design criteria as shown in Figure 3. The vertical flow concept is employed; however, clean air is supplied only over the work space designated for sample handling or chemistry. The air exhausting from the clean work space is used to sweep the remaining room in three paths. The upper path leads to an air return vent located in the ceiling thus eliminating dead air in the upper part of the room. The middle exhaust, which is placed behind the second work surface, serves to exhaust dirt and particulates likely to be generated by the equipment used in the laboratory. Finally, the lower air return at the floor level serves to both eliminate dead air near the floor and to exhaust dirt tracked into the room via shoes, rollers, clothes, etc.

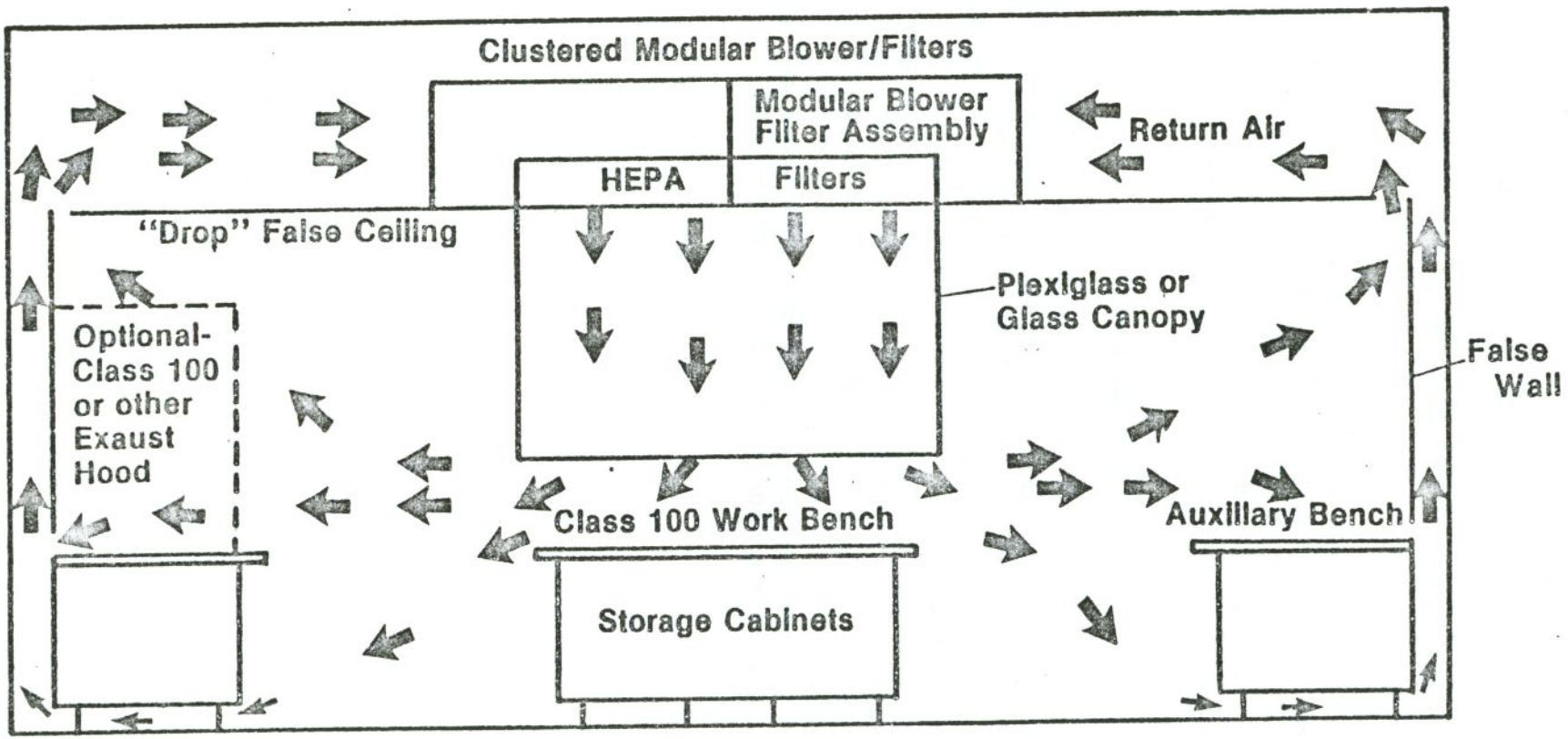

Figure 3. Basic NBS design clean room.

All air is recirculated except for that fraction of air which is exhausted through chemical fume hoods. Air conditioned make-up air is simply blown in above the false ceiling where it is picked up by the air handling modules which contain the HEPA filters. As in all clean rooms, the air circulation causes the clean lab itself to be positive in pressure relative to the air return wall, ceiling, and space immediately around the laboratory. The physical plant of the building must provide sufficient air conditioned air and fume hood exhaust air for the requirements of the individual laboratory.

Under operating conditions, the exhaust air velocity from the clean air work bench is 100 linear feet per minute. Acceptance tests of the clean lab established that the entire facility (exclusive of the storage area) met Federal Class 100 standards for clean air (<100 particles per cubic foot) under static conditions, e.g., no activity in the laboratory. Under actual usage conditions, Class 100 conditions are satisfied only under the clean air modules (sample bench), and conditions elsewhere in the laboratory are only degraded where activity is taking place, e.g., chemists standing in the aisles or equipment on the side benches. Given the proper supply of air conditioned air, the laboratory will maintain a positive air pressure regardless of the location or number of doors which are opened.

A diagram of the NBS Specimen Bank Laboratory/Storage facility is shown in Figure 4. An ante room has been retained to provide a place to change clothing and provide the normal mode of entry and exit from the laboratory. Two sets of four foot wide double doors provide a means for getting heavy equipment into and out of the laboratory. The required clothing for use within the laboratory consists of a nylon cap or full head cover for long hair styles plus a nylon smock and shoe covers. Adequate door hardware was provided for safe and easy egress in case of an emergency.

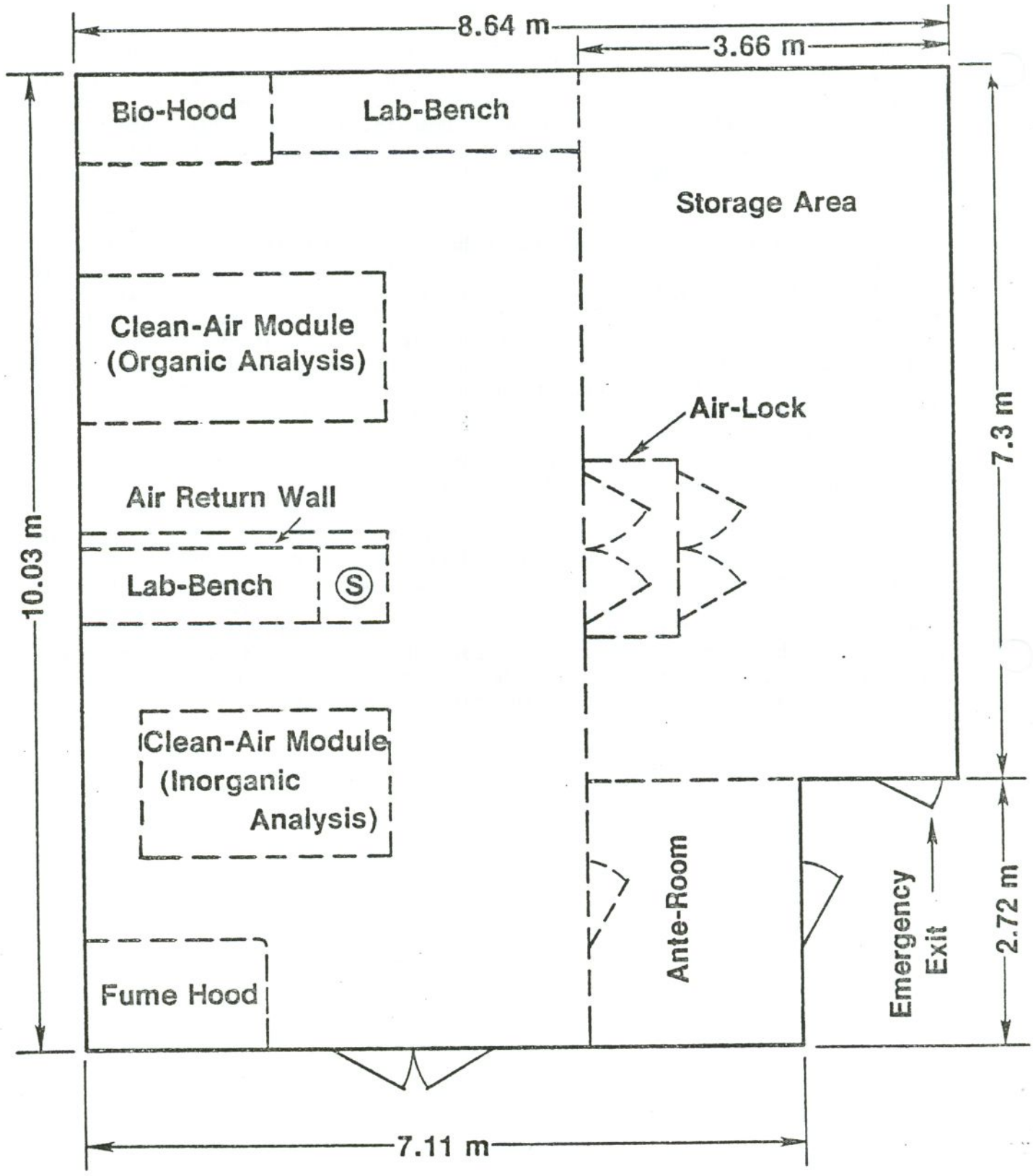

Figure 4. Specimen bank laboratory/storage facility.

Trace Inorganic Analysis Laboratory

For the trace metal chemist, the usual clean room design and specifications for clean air do not assure that the clean room will be usable for trace metal chemistry. In fact, such attention to particulate analysis diverts attention from the more serious problems in a chemistry laboratory. The single most effective philosophy in the design of the NBS clean laboratory has been the elimination of ferrous metals in particular and all metals in general from the materials used in the construction of the laboratory.

To this end, the pre-existing clean room walls were painted with polyurethane paint; a highly corrosion resistant enamel-like surface results. All new walls and ceilings were constructed of particle board panels which were coated with polyurethane enamel or a polyester resin laminate. All interior trim or joints are corrosion resistant extrusions of aluminum. All panel surfaces are laminated or coated to prevent either warpage or to provide protection from the possibility of generating particles. All cabinetry and counters were made from the same materials while doors were made from wood and the drawer slides were made from aluminum. The canopy over the working area was constructed of plexiglass.

For the laboratory sink, a seamless polyethylene sink and splash board were used together with polypropylene faucets for a corrosion-free service life. An existing fiberglass fume hood was retained although it was slightly repositioned to accomodate a new air return wall. The HEPA air filters contain no metal separators and all sheet metal and exposed extrusions are fabricated from aluminum.

Trace Organic Analysis Laboratory

The clean air facilities and concepts in the trace organic laboratory are identical with those in the trace inorganic laboratory. The primary difference has been the addition of charcoal cannister pre-filters for the HEPA air filter modules. No previous reference was found to this type of construction, usually such cannisters are used to control exhaust air, eliminate odors or fumes, etc. Air flow velocities were optimized to obtain the maximum adsorption of background organic compounds. No standards of performance were available for the design and application of charcoal filters for the production of filtered air low in trace organic content. However, the filters have been designed using the best available technology and some period of time will be required to actually determine how well the charcoal pre-filters perform; e.g., by measuring the difference in organic background between the trace organic and trace inorganic sections of the laboratory.

Other changes in the design of the trace organic laboratory include the installation of removable stainless steel work surfaces on all benches and the installation of a stainless steel biohazard-hood for the safe handling of specimens. The total amount of laminate in the laboratory was reduced by laminating only the fronts of the cabinets, not the interiors or tops. The air canopy is constructed of glass rather than plexiglass as in the inorganic laboratory. Taken altogether these changes reduce the chances for the production of background organic compounds thus aiding and working with the

activated charcoal filters. Class 100 conditions are the same as for the trace inorganic laboratory.

Freezer Storage Room

This section of the facility contains an air lock to reduce the amount of potentially humid air entering the storage area. Space has been provided for a number of mechanical freezers (set at -25 C° and -80 °C) as well as the liquid nitrogen freezers. A passive air pressure regulator in the emergency exit door will permit the adjustment of the amount of recycled air within the room while admitting air conditioned fresh air. This becomes important since it will be necessary to adjust these air flows so that the nitrogen levels in the room do not build up to intolerable levels.

Fewer clean air modules and a less sophisticated air return system are used in this room, and therefore, Class 100 conditions are not met. Class 100 conditions do exist for several feet below the HEPA filters and particle counts in the rest of the room are drastically reduced, e.g., from ∿500,000 per cubic meter in a normal laboratory to several hundred per cubic meter in the >0.3 micrometer size region. Lower air quality is permissible in this room since samples will already be packaged. However, the humidity requirements are more strict since frost build-up in the freezers is undesirable.

Several safety features have been incorporated in the design of the facility to protect both the workers and the samples. Smoke detectors and rate of rise fire detectors were incorporated into the safety design of the laboratory. An oxygen monitor is located in the storage facility to indicate if a hazardous level of nitrogen exists. The four compressor type freezers are equipped with a liquid nitrogen back-up system. This system is activated and the freezers are flooded with liquid nitrogen vapor if the temperature of the freezer increases above a pre-determined level. As an additional safety feature to protect the frozen samples, an automatic dialing system will call selected telephone numbers and indicate a fault in freezer temperatures or the storage room temperature.

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1. Gills, T. E., Rook, H. L., and Durst, R. A., eds. The National Environmental Specimen Bank Research Program for Sampling, Storage, and Analysis. EPA-600/1-79-017, U. S. Environmental Protection Agency, 1979.

SECTION 3

SPECIMEN COLLECTION AND STORAGE

by

Rolf Zeisler and Sally H. Harrison

The success of the National Environmental Specimen Bank (NESB) will be determined in a large part by the ability to preserve the chemical integrity (i.e., organic, inorganic, and organometallic species) of the samples during long-term storage. Changes in the forms and concentrations of the numerous environmentally important substances in specimens stored for extended periods may occur in several ways. Processes such as surface adsorption and sample degradation may change the concentrations of various components. Continued biochemical and enzymatic activity may produce species which may not have been present in the original sample. Contamination of specimen fluids could lead to apparent increases in trace substance concentrations. In addition, the rates of these processes will be affected by such factors as container material, contact time and area, storage temperature, pH, and initial species concentration.

The EPA/NBS Pilot Specimen Bank Program will evaluate the effects of temperature, sample handling, container materials, etc. on the chemical integrity of the sample. The unique requirements of sample integrity for both trace elements and trace organics are to be considered in the choice of container materials and the storage conditions. Container materials have been studied extensively, the results are summarized below. The evaluation of the effects of long-term storage is part of the current pilot bank program and the plans and means for this evaluation are outlined in this contribution.

Sample Collection Scheme

The scheme for the collection of human liver samples is illustrated in Figure 1. The three contractors are located in three different geographical regions in order to evaluate possible logistical problems in receiving samples. The proximity of the Baltimore contractor to NBS is advantageous for frequent interactions to evaluate the protocol procedures and for possible studies, involving fresh samples, e.g., comparison of very fresh (unfrozen) sample to an equivalent sample which has been frozen and shipped to NBS. Each contractor is to provide 100 samples/year which satisfy the case selection criteria and which are collected according to the prescribed protocol (see Appendix A). The samples are generally received at NBS within 24 hours after shipment.

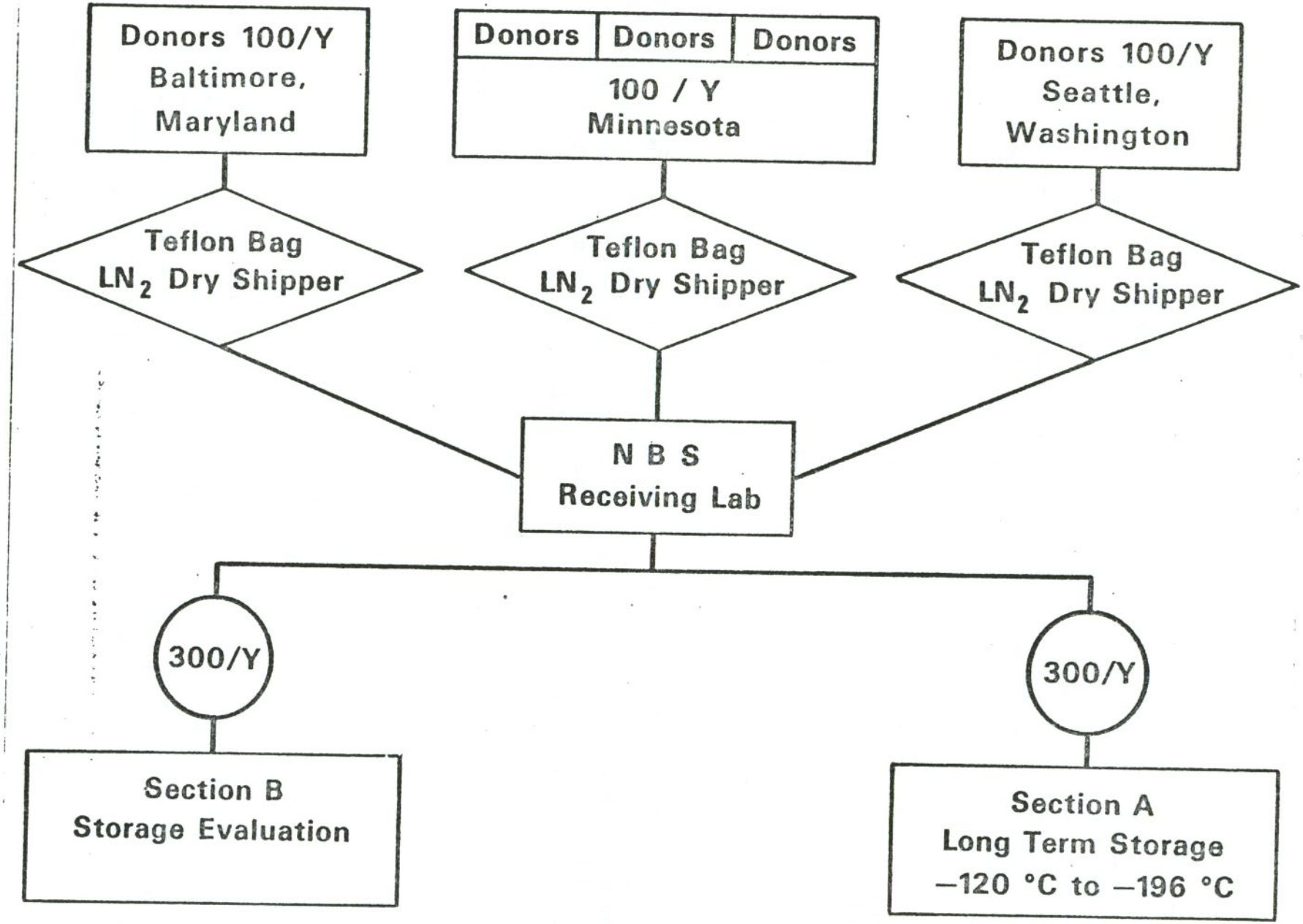

Figure 1. Sample collection scheme for human liver samples.

The liver specimens are received as two duplicate sections of the left lobe and arbitrarily identified as sections "A" and "B". The samples are placed in temporary storage (liquid nitrogen freezer) until results of the hepatitis screening are received. At that time the sections are stored separately; all "A" sections are placed in liquid nitrogen freezers for long-term storage, and the "B" sections are used for storage evaluation, i.e., real-time or immediate analysis and long-term storage evaluation at different conditions. The "A" sections are reference samples which may be used to re-evaluate results on the corresponding "B" section. At the end of the pilot program, the "A" sections stored at liquid nitrogen temperatures will represent a valuable bank of validated samples available to the scientific community. In addition, a large quantity of data, from the analyses of the "B" sections, will be available on many of these samples.

Storage Container

In a recent study, twelve different plastics were examined by gravimetry to evaluate the extent of water loss and by isotope dilution mass spectrometry and neutron activation analysis to determine the levels of impurities present in the plastic and the quantities of trace elements leached from the plastics with acids (1). From this study, Teflon was found to be suitable after proper cleaning, for the storage of samples intended for trace element analysis.

Storage Evaluation

At present, only limited data are available on the long-term storage of biological materials. For the storage of biological tissues and fluids, rapid freeze drying immediately after sampling has been recommended, but suffers from the disadvantage that some volatile components may be lost. In freeze-dried materials, trace element concentrations are stable over an extended time period even if stored at room temperature, as has been shown in various NBS Standard Reference Materials (2). Another approach is the immediate freezing of the sample or sub-sample to the lowest conveniently attainable temperature. This approach serves two purposes: 1) it reduces or stops both chemical and biological processes which could result in sample changes, and 2) it reduces the mobility of constituents in the sample and/or container material, thereby lessening the possibility of contamination and/or losses due to absorption/adsorption or volatility.

The subsampling, storage, and analysis program is designed to lead to a complete evaluation of the storage processes with respect to the long term preservation of the originally present trace constituents over a four year storage time. To solve the question of the most appropriate temperature for sample storage, the specimens are being stored under four different conditions: freeze dried at room temperature, frozen at -25 °C, at -80 °C, and in liquid nitrogen vapor at -120 °C to -190 °C. The concentrations of trace constituents found after storage under those conditions will be compared to the data found in the "real time" analysis of each specimen (Figure 2).

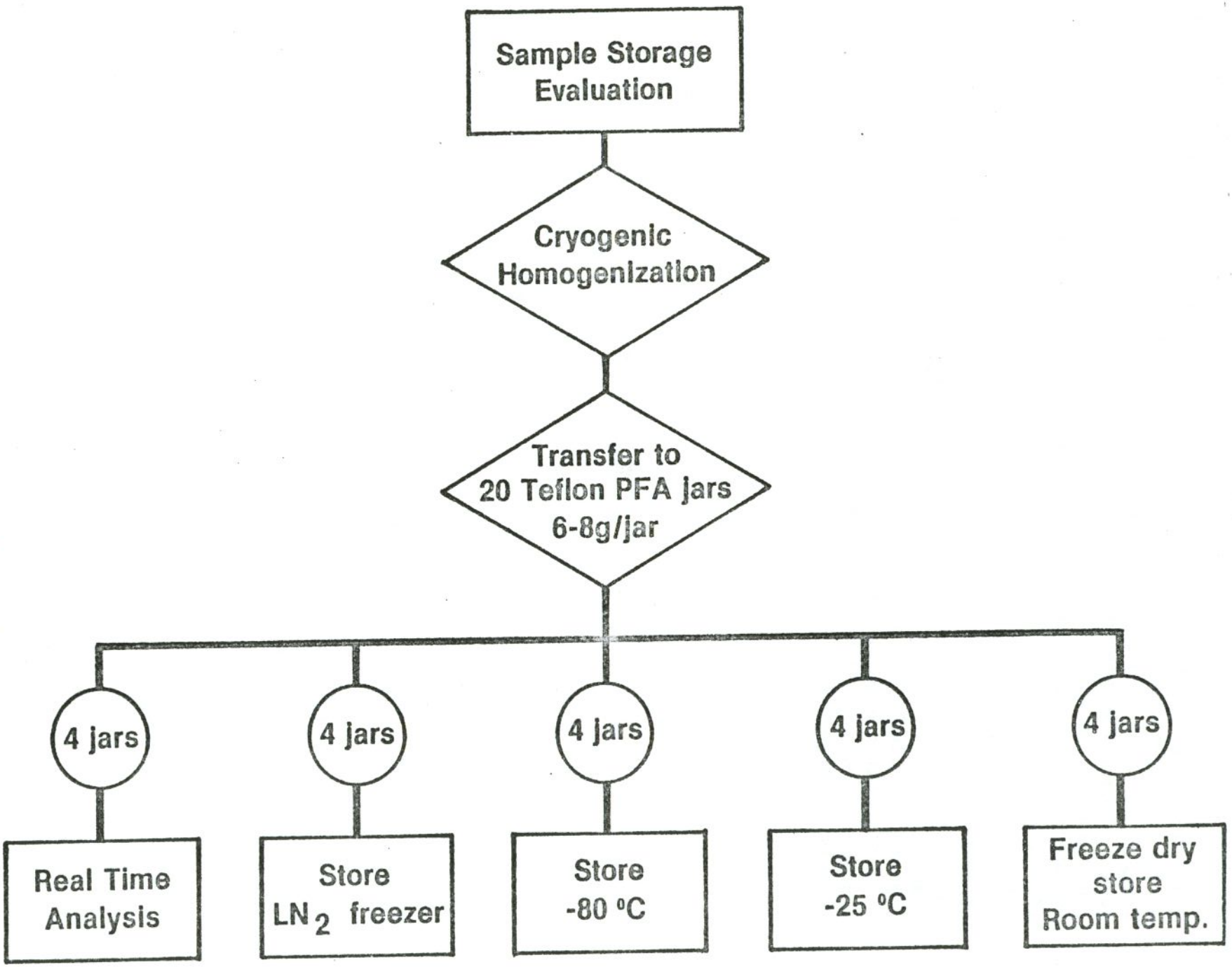

Figure 2. Allocation of subsamples for storage evaluation.

The probability for the discovery of changes in the concentrations of trace constituents during storage has been assessed by statistical treatment of the expected experimental parameters, e.g., number of specimens analyzed, homogeneity of subsamples, and analytical error (3). Figure 3 consists of a series of curves generated from the experimental parameters of 5 percent inhomogeneity and 5 percent analytical error. These curves determine the probability of detecting a particular percent change in concentration for a given number of liver samples analyzed. For example, we would have a 98 percent probability of detecting a 6 percent change (Point A) and a 78 percent probability of detecting a 4 percent change in concentration (Point B) if 30 livers are analyzed. If the sample inhomogeneity or analytical error increased to 10 instead of 5 percent, the percent change detectable at Points A and B would be 12 and 8 percent, respectively. Based on these curves, the number of samples to be analyzed was set at 30. Analyses of a smaller number of samples would significantly reduce the probabilities of detecting small changes, whereas increasing the number of samples analyzed would not increase greatly the probability of detecting changes.

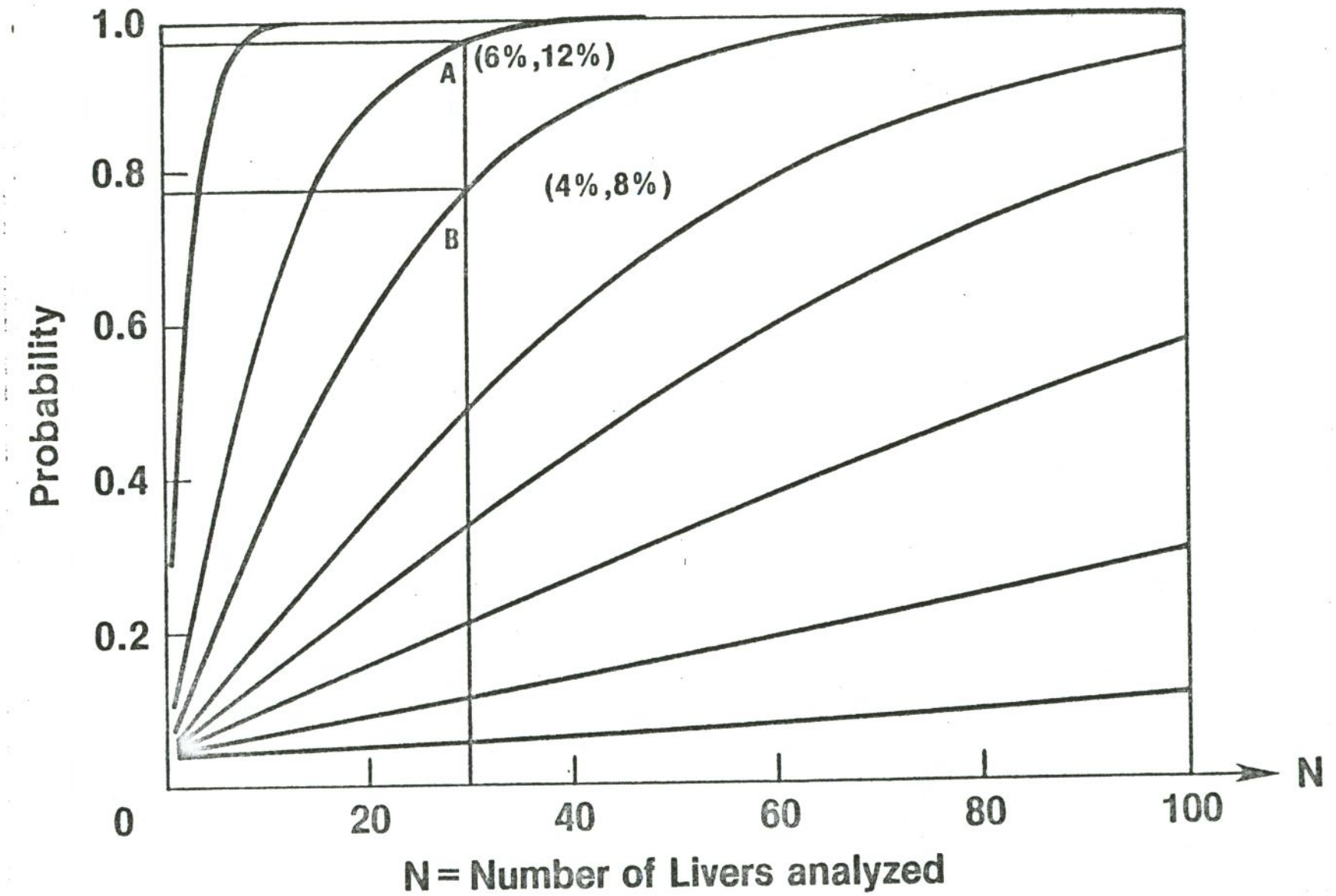

Figure 3. Probability of detecting change in the concentration due to storage conditions.

The 30 specimens used for the storage evaluation represent approximately 10 percent of the specimens collected per year. The limitation to this number is primarily the present analytical capacity. In order not to exceed the currently available analytical capability, most of the analytical work is deferred to the year 1984, when the program should have a larger analytical capacity (i.e., contract laboratories). For the evaluation of the storage of human livers, Table 1 gives the analytical needs as number of analyses to be conducted by each method.

TABLE 1. NUMBER OF LIVERS TO BE ANALYZED FOR STORAGE EVALUATION STUDY

Year		80	81	82	83	84
Real Time Analysis		36	30	30	30	
1 Year Storage	LN_2		12			30
	-85 °C	Samples	12		Samples	30
	-25 °C	from '80	12		from '83	30
	RT		12			30
2 Years Storage	LN_2					30
	-85 °C				Samples	30
	-25 °C				from "82	30
	RT					30
3 Years Storage	LN_2					30
	-85 °C				Samples	30
	-25 PC				from '81	30
	RT					30
4 Years Storage	LN_2					24
	-85 °C				Samples	24
	-25 °C				from '80	24
	RT					24
Livers Analyzed/Year		36	78	30	30	456

Storage System

The storage area of the pilot facility contains three liquid nitrogen freezers (LN_2) (500 L capacity each), four compressor type biological storage freezers (382 L capacity each) and a shelf cabinet for storage at room temperature. The total storage capacity is in excess of 3000 specimens.

The liver specimens are received at NBS in cylindrical paper tubes (57 mm O.D. x 117 mm). These containers provide a standard geometry for specimen storage. These samples are stored in the LN_2 freezers in larger cylindrical tubes (6.35 cm O.D. x 64.8 cm), with five specimens in each tube. Each LN_2 freezer will hold 170 tubes or 850 specimens. Each tube is uniquely identified and labeled beginning with ØA to ØZ, 1A to 1Z, etc. In the compressor freezers, whole liver sections are stored in trays containing 16 samples each, three trays per shelf, six shelves per freezer for a total of 288 samples per freezer. Sample storage location is identified by freezer, shelf, tray, and position.

Homogenized subsamples (see Section 4 for details) are stored in 15 mL-Teflon PFA jars. The lids of these jars have been modified to contain removable cardboard inserts on which the sample identification number is written. The Teflon jars are placed in square cardboard boxes (140 mm x 140 mm x 50 mm) which are labeled with a three-digit number embossed on the side. Each box will hold eight jars. These boxes are stored in racks in the LN_2 and compressor freezers.

When samples are banked, a storage file is created which is presently on hard copy (Figure 4). This form includes the following information: sample identification number, storage identification number, weights, and storage dates in and out. The storage identification number has three fields: an alphabetic freezer identification code (one digit) a shelf/rack code (two digits), and a box/position code (three digits).

Three compressor type freezers will provide sufficient storage space for the evaluation of the -80 °C storage. One freezer is designated for the evaluation of -25 °C storage.

NBS-1160
(7-80)

U.S. Department of Commerce
National Bureau of Standards

NBS-EPA Pilot Environmental Specimen Bank

Sample Storage

	Storage ID	Wt(g)	Date In	Date Out	by (Initials)
Section A					
Section B					

Comments— _____

_____

Subsamples

		Storage ID	Wt(g)	Date In	Date Out	by (Initials)
LN_2	B001					
	B006					
	B011					
	B016					
— 80°	B002					
	B007					
	B012					
	B017					
— 25°	B003					
	B008					
	B013					
	B018					
F.D.	B004					
Dry Wt. Factor	B009					
	B014					
	B019					
Real Time	B005					
	B010					
	B015					
	B020					

Figure 4. Sample storage form.

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SECTION 4

TRACE ELEMENT ANALYSIS

by

Rolf Zeisler

Recent research in trace element analysis has resulted in the improvement of existing methods and the development of new procedures emphasizing higher sensitivity for the majority of the elements. The goal of this program is to include all elements of environmental concern, denoted as first priority elements, in the analytical scheme. However, as the discussion of first priority elements tends to be open ended, the development of methods and procedures will be a continuous process, allowing more elements to be included in the analytical scheme.

The current analytical protocol is directed towards the determination of most of the elements on a list of first priority elements, which has been proposed by the International Workshop on Monitoring Environmental Materials and Specimen Banking (1). Additional elements occurring as biological trace elements in the samples are included in the protocol because their levels might supply information about the "normal" state of the specimen. Data for these elements are available from the analytical protocol without requiring additional analyses. The elements under consideration and their concentration in human livers (from reference 2) are compiled in Table 1. The techniques and procedures to be used in the inorganic analysis of NESB human liver samples are voltammetry — anodic stripping voltammetry (ASV) or linear sweep voltammetry (LSV), atomic absorption spectrophotometry (AAS), and neutron activation analysis — instrumental analysis (INAA) , and radiochemical analysis (RNAA). The flow chart in Figure 1 demonstrates the distribution of the subsamples to the different principal techniques for analysis and the elemental coverage obtained by applying this scheme.

TABLE 1. ANALYSIS OF TRACE ELEMENTS IN HUMAN LIVER

Element	Concentration (μg/g)[a]	Analytical Method[b]	Category[c]
Be	ND	AAS	1
F	0.06 - 1.4		1
Al	1.6 - 2.6	ASV/LSV	2
V	< 0.007 - 0.09	R	1
Cr	0.005 - 0.27	INAA	1
Mn	0.5 - 1.9	INAA	1
Fe	70 - 210	INAA	2
Co	0.017 - 0.16	INAA	1
Ni	0.009 - 0.32	ASV/LSV	1
Cu	3.2 - 14.7	ASV/LSV, RNAA	3
Zn	31 - 80	ASV/LSV, INAA	1
As	0.006 - 0.46	RNAA	1
Se	0.097 - 0.68	INAA, RNAA	1
Rb	7 - 12	INAA	2
Sr	0.01		3
Mo	0.4 - 1.6	RNAA, INAA	1
Pd	ND		1
Ag	0.006 - 0.07	INAA	2
Cd	0.5 - 4.9	ASV/LSV, RNAA	1
Sn	0.08 - 0.65	R	1
Sb	0.01	INAA, RNAA	2
Ba	0.01		3
Pt	ND	R	3
Hg	0.005 - 0.25	AAS	1
Tl	0.001 - 0.009	R	1
Pb	0.8 - 2.3	ASV/LSV	1

a Fresh weight, range of concentrations from reference 2.
b Analytical method to be used at NBS in the pilot specimen bank program.
c 1) First priority element (1);
2) Trace elements available with applied methods to monitor "normal" body concentrations for non-pollutants.
3) Trace elements of environmental importance not included in first priority list.
R Research initiated ND No data available

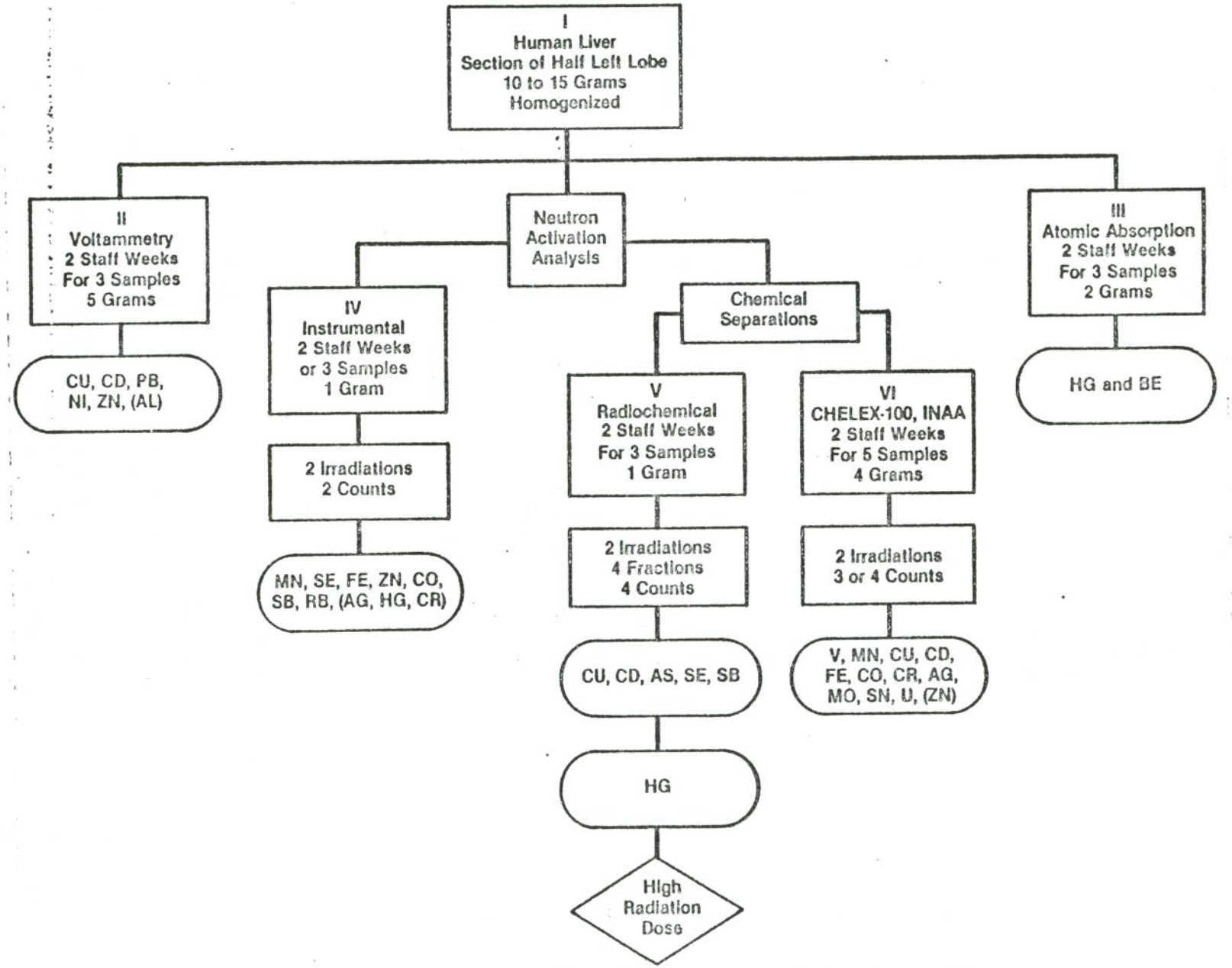

Figure 1. Analytical methods for analysis of human livers.

The details of the different analytical techniques which will be applied routinely on the specimen bank samples are currently being evaluated. A quality assurance program is built into the inorganic analytical protocol by determining a number of elements with two techniques as well as duplicate analyses for all samples. This approach is commonly used in the NBS Standard Reference Materials certification program. The techniques applied will be documented in a format related to the ASTM procedures. This will enable analysts in other laboratories to make results intercomparable.

The subsamples distributed to the analysts are obtained by the homogenization of a half left lobe of a human liver (Section B). The homogenization is achieved by the previously investigated cryogenic homogenization (brittle fracture) technique. The procedure has been demonstrated to produce homogenous subsamples from five to fifteen grams of biological materials (3). A prototype brittle fracture machine with capacity up to 250 g has been developed at NBS. The performance, the homogeneity of the samples, and the exclusion of contamination during processing is currently being investigated. The high capacity is needed to provide twenty subsamples, six to eight grams each from the half left lobe of a human liver. These subsamples are contained in Teflon jars and distributed for real time analysis and storage evaluation.

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SECTION 5

TRACE ORGANIC ANALYSIS

by

Stephen A. Wise

FY '79 was the first year of funding for organic analyses related to the Environmental Specimen Bank. However, the Organic Analytical Research Division has been involved for a longer time in related research concerned with the development of analytical methods for the determination of trace-level hydrocarbons in marine biota, particularly mussels, oysters, and clams. This work was partially funded by the National Oceanic and Atmospheric Administration as part of a baseline study in the Prince William Sound of Alaska. Highlights of the accomplishments of this program which relate to the Environmental Specimen Bank include:

1) The development of a dynamic headspace sampling technique to determine trace-level hydrocarbons in marine tissue (1,2).

2) The development of a high-performance liquid chromatographic (HPLC) method for the removal of polar biogenic compounds and the isolation of the hydrocarbons (1).

3) The initiation of an interlaboratory comparison exercise between eight laboratories as part of a quality assurance program for hydrocarbon measurements in mussel homogenates (2,3).

4) A hydrocarbon stability study of a mussel tissue homogenate stored over a 15-month period at -10 °C (2).

The experience gained during this work will be useful in dealing with trace level organics in any tissue matrix, but particularly in the second year of the Pilot Specimen Bank Program when an aquatic accummulator, such as mussels or oysters, will be collected, analyzed, and archived.

Analysis of Protocol Materials

Initial efforts in trace organic analysis for the Environmental Specimen Bank have emphasized the determination of possible trace organic contamination from the sample handling protocol and the storage containers.

Several sources of water (i.e., a commercially prepared water and high-purity water prepared at NBS for use in trace element analysis, and water prepared at NBS for use in organic analysis) were investigated to determine a suitable water for rinsing of the livers during the sample collection. The rinsing step in the protocol is very important to remove any contamination introduced during the autopsy (i.e., dust particles, dirt, bone chips, etc.). The commercial water (Baker HPLC water) for use in high performance liquid chromatography (HPLC) was analyzed by GC and HPLC and found to be relatively clean for organics when compared to the two NBS sources. This water was also analyzed for trace element content and found to be satisfactory.

The HPLC analyses consisted of concentrating about 500 mL of the water on an HPLC column using a technique known as trace enrichment (4). A gradient of 0-100 percent acetonitrile in water was used to elute the organic compounds from the column. Ultraviolet absorption (uv) at 254 nm is utilized for detection. The comparison of the HPLC chromatograms for the Baker water (a) and the NBS high-purity water for trace element analysis (b) are shown in Figure 1. UV detection at 254 nm does not respond to all organic compounds. Thus, gas chromatographic analyses using a universal flame ionization detector were also performed to assure the purity of the water with regard to organic contamination. These analyses indicated that the Baker HPLC water was a satisfactory water for use in the sampling protocol. In addition, the convenience of purchasing a commercial water for the contractors, rather than supplying it from NBS, was also important in the selection of this source.

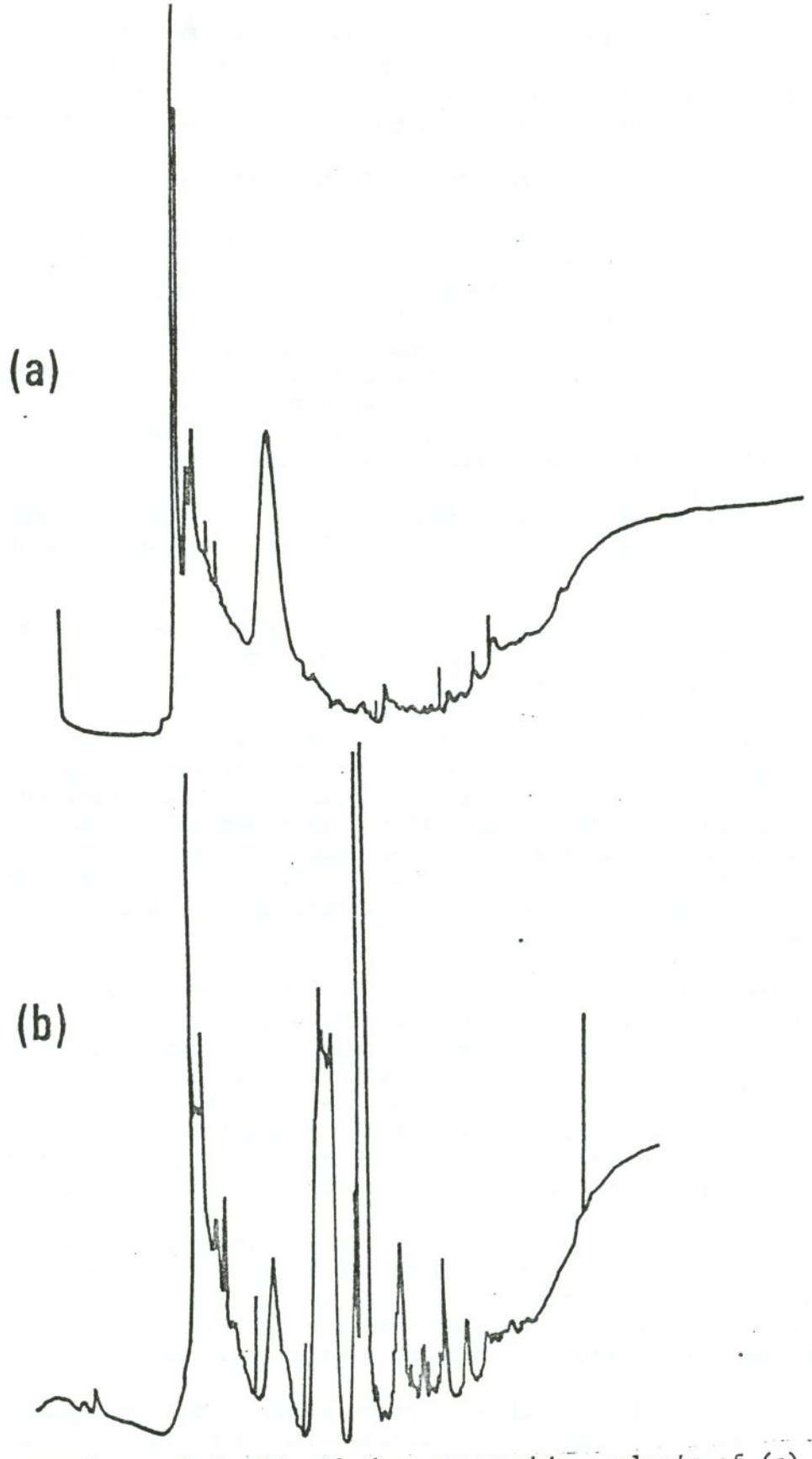

Figure 1. Comparison of the liquid chromatographic analysis of (a) HPLC water and (b) NBS high-purity water for trace element analyses.

In a preliminary investigation of the suitability of the Teflon bags for tissue storage, no organics (by GC) were extracted from the bags by water. Several additional Teflon bags and containers which contained water for over 100 days have been analyzed and no significant leaching was found.

Analytical Techniques

Based on a literature survey of organic pollutants in liver tissue, we decided to focus initially on the determination of organochlorine compounds (i.e., aldrin, dieldrin, chlordane, 1,1,1-trichloro-2,2-bis(pentachlorophenyl)-ethane (DDT), 1,1-dichloro-2-bis-(p-chlorophenyl)ethylene (DDE), endrin, heptachlor, etc.) and polycyclic aromatic hydrocarbons (PAH). Both of these classes of organic compounds are contained in the EPA Priority Pollutant list. A significant amount of work on organochlorine pesticides in human tissue has been reported and is summarized in the recent EPA report on "Chemicals Identified in Human Biological Media, A Data Base" (5). However, most of the reported data are for adipose tissue, blood, and urine, and only limited data are presented for human liver tissue. Several reports of the determination of these compounds in human liver have been published in Europe (6-8) and Japan (9-11).

Kraul and Karlog (6) reported levels of DDT and metabolites of 0-22 µg/g extractable fat (mean of 6 µg/g) for 82 samples in Denmark. Bjorseth, Lunde, and Dybing (7) reported polychlorinated biphenyls (PCB), hexachlorobenzene (HCB), and DDE in human liver at average levels of 1.9, 0.15, and 0.8 µg/g of extractable fat by GC analysis on 10 samples in Norway. They analyzed the same samples for total chlorine by neutron activation analysis and found levels of 2.1 µg/g extractable fat. Hattula et al. (8) reported levels of total DDT at 0.22 ppm and PCB at 0.15 ppm in human livers in Finland. Sasaki et al. (9) reported analyses on 21 liver samples with average levels of DDE, DDT, and dieldrin levels in liver of 4.4, 0.82, and 0.33 ppm. The levels in liver and kidney were reported as the highest in any of the organs sampled, i.e., spleen, brain, kidney, and liver (9-11).

In general, the analytical methods for these compounds employ: (a) extraction with acetonitrile, (b) liquid/liquid partition into hexane or benzene, (c) column chromatography to isolate the compounds of interest, and (d) gas chromatographic (GC) analysis with selective electron capture detection (ECD). This general procedure has been used for the initial investigation of a liver sample. An HPLC method for the removal of the polar biogenic compounds was employed rather than the classical column chromatography. The HPLC method previously mentioned for isolating hydrocarbons in mussel tissue was modified for the isolation of organochlorine pesticides. This method is more rapid and reliable than classical column chromatography. A comparison of the GC analyses before and after the HPLC clean-up procedure indicated that many large interfering GC peaks were removed using this technique. GC analysis revealed numerous components in the sample responding to the ECD indicating possible presence of organochlorine compounds.

GC methods utilizing high efficiency glass capillary column, have been developed at NBS to separate 16 pesticides listed on the U.S. Environmental Protection Agency Priority Pollutant list. In addition, normal-phase and

reverse-phase HPLC separations of a number of organochlorine pesticides have been developed for use in the analysis of liver samples.

Polycyclic aromatic hydrocarbons have been reported in human tissues at very low levels (0.2 µg/100 g dry substance) (12). NBS has developed methods for the analysis of PAH in various matrices including marine tissue (13). The application of this methodology to human liver samples will be investigated.

As funding permits or as priorities change, we may develop methodology for other types of organic compounds on EPA's Priority Pollutant list, i.e., phenolic compounds, phthalate esters, etc. The multitude of organic compounds emphasizes an important aspect of the Specimen Bank concept; namely, we cannot measure every organic pollutant, but we have the ability to perform retrospective analyses for pollutants that might be unknown or poorly determined at present.

The development of analytical methodology for quantitative analysis of trace organics in human liver tissue will continue in FY'80. During this development phase, the following areas will be addressed:

(1) Selection of appropriate internal standards for quantitation and recovery studies of these standards from the liver matrix.

(2) Selection of the most efficient extraction method for removal of compounds of interest from the tissue.

(3) Evaluation of freeze drying for loss of volatile organic components.

(4) Homogeneity of liver samples for organic compounds and suitability of cryogenic brittle fracture homogenization for organic analysis.

(5) Weight basis for reporting results of analyses — wet vs. dry weight or suitable alternative.

(6) Comparison of organochlorine content in livers as determined by gas chromatography and total chlorine content as determined by neutron activation analysis.

Quality Assurance

An important part of any program involved in the quantitation of trace level organics in biological samples, such as the Environmental Specimen Bank or current pollution monitoring programs, is assessing the reliability of the data generated. At present it is difficult to determine the accuracy of trace organic analyses and there is usually only limited knowledge of the comparability of data from different laboratories. In the Pilot Environmental Specimen Bank Program, interlaboratory comparison of data will be necessary to evaluate the reliability of data and analytical methods. Sample splits and interlaboratory comparison exercises with the German Specimen Bank will be extremely valuable. In addition, the third year of the Pilot Program calls for the initiation of contracts for chemical analyses by outside

laboratories. At this point, comparability of data will be of utmost importance, otherwise previously generated data will have only limited value for evaluating storage stability, for monitoring trends, etc.

Recently, several interlaboratory comparison exercises have indicated the difficulties in quantitating trace organics in natural matrices such as sediments and biological samples (2,3,13,14). Two intercomparison exercises for trace level hydrocarbons in sediments and mussel tissue were initiated by NBS. In the first study (12), the results for sediment analyses from eight laboratories varied by one to two orders of magnitude. In the second study involving two mussel homogenates (2,3), results for hydrocarbon levels varied by factors of three to four. These results, and those of other similar studies, should serve as a warning against over interpretation of trace organic measurements currently generated in monitoring programs. Participation in interlaboratory comparisons is necessary until reference materials or reference methods are available to assess the accuracy of these measurements.

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SECTION 6

SAFE HANDLING OF HUMAN TISSUE SAMPLES

by

Stephen A. Wise

The handling of human liver samples carries the potential risk of exposure to infectious diseases, particularly hepatitis B virus or "serum hepatitis." As a result, certain precautions are necessary to insure protection of the analysts involved in sample processing and analysis.

To eliminate potentially infectious liver specimens from the specimen bank samples, a blood sample is removed from the donor at the time of autopsy to be used for hepatitis B screening. In addition, liver specimens from the right lobe are removed for preparation of histological slides. These slides are examined by the pathologists for evidence of infectious diseases.

Samples received at NBS (contained in double Teflon bags and a cardboard cylinder) are transferred from the LN_2 shippers to temporary storage in a LN_2 freezer. (Freezing at LN_2 temperatures does not destroy hepatitis virus, but rather preserves it.) After negative results are received from the hepatitis B screening, the samples are transferred into the specimen bank storage facility. If hepatitis results are positive or if the integrity of the sample is jeoparidized, i.e., ruptured inner and outer bags, the sample is sent to Baltimore for incineration. Samples are selected for homogenization only after both the hepatitis and histological results are received.

The sample handling steps in the homogenization procedure, i.e., removal of Teflon bags, transfer to brittle fracture apparatus, and subsampling of the frozen homogenate from brittle fracture apparatus to Teflon jars, are performed in the biohazards hood. Analysts involved in these procedures wear complete clean lab clothing with disposable mask and surgical gloves for protection.

It is estimated that one out of every 200 people is a carrier of hepatitis B; however, the actual number of infected samples that the specimen bank program will encounter should be even less due to the prescreening described above. Even though the samples are prescreened for hepatitis B virus, there is a slight possibility that the virus could go undetected. In addition, other types of hepatitis viruses exist which are difficult to detect. Therefore, safety protocols and precautionary measures will be followed closely to insure protection of the specimen bank personnel. In addition to transmission

of the virus by parenteral routes such as inoculation or blood transfusions, the most critical, in relationship to the specimen bank handling, is hand-to-mouth contact.

A Safety Advisory Committee has been established to advise the Center for Analytical Chemistry at NBS concerning suitable safety precautions for the handling and processing of these liver samples. This committee consists of Michael Greene, M.D. (NBS physician), Robert Purcell, Ph.D. (Chief of Hepatitis Section, National Institutes of Health) and Dr. Thomas Koch, Ph.D. (Department of Pathology, University of Maryland). Recommendations were made by the Committee regarding procedures to safeguard personnel.

1) Attitude -- All personnel should regard and handle every tissue sample as potentially infectious.

2) Training -- All personnel should attend a biohazards training course.

3) Procedural precautions to be followed to avoid infection:

   a) Avoid hand-to-mouth contact in the lab. Eating, drinking, and smoking are prohibited in the laboratory.

   b) Never pipette by mouth.

   c) Wear protective garments when handling samples, i.e., surgical gloves, disposable gowns, disposable mask when possibility of splattering exists.

   d) Work areas and instruments should be cleansed and disinfected/sterilized after use using such procedures as: (1) boiling water for 10 min., (2) autoclaving, (3) chemical disinfectants, e.g., 0.5 to 1.0 percent sodium hypochlorite in methanol, chlorox, or acid.

   e) Make periodic inspection of work procedures to assure that all safety procedures are adhered to.

   f) Limit access to the clean room/storage facility to those personnel familiar with these safety procedures.

4) Personal Hygiene -- Good personal hygiene is essential for protecting against hepatitis B infection. Washing of the hands after handling of samples and prior to leaving the lab is the most important single precaution.

5) Medical Precautions -- All staff should receive medical examinations prior to working with the tissue samples. Serological tests should be performed on all staff handling tissue every three to six months depending on the frequency and nature of the handling.

The Safety Advisory Committee will be requested to annually review the procedures of the specimen bank program.

SECTION 7

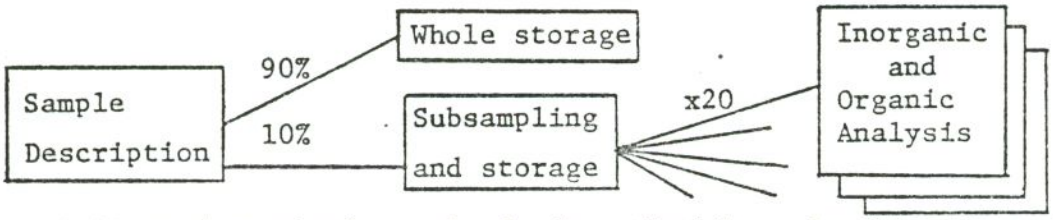
DATA HANDLING -- PROBLEMS AND PROSPECTS

by

Richard M. Lindstrom

An essential component of the Pilot NESB project is the orderly handling of large quantities of data related to the samples collected. Over the life of the project as many as 3000 samples need to be cataloged and their subsampling, storage, and analysis histories recorded. As analytical data about the samples become available, correlations will be sought with these histories. The volume and nature of the data base requires computer management.

An estimate of the scale of the data base may be made from the quantity of data on the paper record sheets in current use to describe the flow of samples:



Each liver (or other) sample is described by a Sampling Data sheet (see Section 1, Figure 1) which contains up to 500 characters of information. Ten percent of the samples are divided, each into 20 subsamples. The history of each sample and its subsamples is subsequently described by a Sample Storage sheet (NBS1160, see Section 3, Figure 4), with room for 800 characters of information. Each subsample may be analyzed for as many as thirty constituents, which will generate (assuming replicate analyses) as many as 1500 characters of information. The size of the data base, then, may be estimated as 500 + 800 + (0.1 x 20 x 1500) = 4300 characters per average sample which, assuming 3000 samples, makes a total of 13×10^6 characters, or 13 megabytes.

This estimate is dominated by the amount of analytical data assumed for each subsample. With modern data base management techniques, room need not be left in the data files for blank data elements, and the storage requirements become very much less.

If 13M bytes of data were to be entered through a keyboard, the time required at 40 words/minute (200 characters/minute) would be 270 staff weeks. Consideration must be given to entering the results of analyses, which dominates the quantity of data anticipated, into the data base in a semi-automated manner.

For the purely curatorial activities such as logging samples into and out of storage, a desirable system will feature data entry and inquiry at a terminal adjacent to the sample storage facility with response times of a few seconds or less.

One of the major objectives of the pilot bank project is to define necessary and sufficient sampling, subsampling and storage procedures, as judged by searching for excess variability in the analytical data that can be correlated with such variables as time in storage, temperature, and sample treatment. Another use of the data is to search for correlations, among samples shown to be otherwise equivalent, between trace element composition and occupational or medical history. With such a large, complex, multi-variant data base in prospect, considerable thought should be given to these end uses in the selection or design of software.

The resources necessary to accomplish the work just outlined include a substantial minicomputer with a 20M byte disk, two terminals, and a complete and sophisticated software set for creation, editing, interrogation and statistical interpretation of a multimegabyte data base.

APPENDIX I

LIVER SAMPLING PROTOCOL

I Case Selection

Autopsy coordinator or equivalent is contacted to determine if a case is available which satifies the following criteria:

a. Liver is expected to be normal and uninjured.
b. No known history of alcoholism, hepatitis, drug abuse, gross sepsis, long-term treatment for disease, tuberculosis, cirrhosis, liver carcinoma, chronic circulatory failure, or congestion.
c. Death not due to chemical or drug overdose or exposure. Exception — CO poisonings are acceptable.
d. Autopsy will be performed less than 24 hours after death.
e. Donor has not been embalmed or frozen.
f. Livers with a total weight greater than one kilogram.

II Materials needed for a single case

a. 4 or more pairs non-talced, dust-free gloves*
b. 4 or more dust-free Teflon FEP bags*
c. 3 or more dust-free Teflon FEP sheets*
d. 2 or more gummed, crack and peel, sample identification labels*
e. 1 titanium knife*
f. 1 or more sample data forms*
g. 2 500-mL Teflon bottles containing high purity water*
h. 1 histology vial or equivalent
i. A sample transport container with lid, if necessary
j. Washing board or washing pan, if necessary
k. Syringe, needle, and vacutainer or equivalent

* These protocol items are provided by NBS.

III Donor and Sample Information

The following information is recorded on the sample data form at the time of autopsy:

a. Autopsy identification number.

Preface each with the one letter location identification as follows:

B = Baltimore Medical Examiners Office, Md.
R = St. Paul - Ramsey Hospital, St. Paul, Minn.
H = Hennepin County Hospital Minneapolis, Minn.
M = Mayo Clinic, Rochester, Minn.
U = University of Minnesota Hospital, Minneapolis, Minn.
S = Seattle Medical Examiner's Officer, Seattle, Wash.
V = Harborview Medical Center, Seattle, Wash.

b. Date of death, recorded in order of day, month (numerical), year.
c. Time of death, using 24 hour time designation rather than a.m. and p.m. (It is recognized that the time of death may not be known exactly, so give a best estimate. Example: after 18:00.)
d. Date of autopsy, recorded in order of day, month (numerical), year.
e. Place of autopsy. Example: Baltimore Medical Examiner's Office.
f. Pathologist, attending or responsible physician.
g. Mortuary storage date, time in, and temperature. (We want to know how long the donor was kept under controlled conditions after death.)
h. Date of birth. If not known at the time of autopsy, give best age estimate above the block for date of birth.
i. Sex.
j. Ethnic group. Examples: black, caucasian, oriental, hispanic, etc.
k. Height of donor, inches and centimeters.
l. Weight of donor, pounds and kilograms.
m. Diagnosis of autopsy. At the time of autopsy, give best estimate of the probable cause of death.
n. Comments. Use this space to describe any deviations from the protocol or anything special about the case.
o. Liver weights, whole and separate sections. Indicate how many figures are significant.

IV Sample Handling

Sterile, cleaned, non-talced vinyl gloves are provided and should be used by all personnel involved in liver removal and handling. Extreme precaution must be taken throughout the autopsy procedure to reduce the risk of chemical contamination of the liver sample. Contamination may result during the autopsy from the donor, the individual(s) performing the autopsy, the atmosphere, the surgical instruments, and any chemicals used in cleaning or disinfecting the autopsy room. A list of chemicals used in the autopsy room should be sent tc NBS with an indication of the frequency and type of use. Smoking is not allowed in the vicinity of the autopsy or during the sample processing in the laboratory.

V Sample Collection Procedure

a. The liver should be removed as close to the beginning of the autopsy as possible. The person(s) removing and dissecting the organs should wear the dust-free vinyl gloves provided by NBS. Ligaments may be cut using surgical scissors which have been rinsed in water to remove any disinfectant or other cleaning chemical. If the membrane covering the left lobe is ruptured, the sample is no longer valid. The liver should not at any time during the sampling procedure be placed on any surface other than the cleaned-Teflon sheets provided by NBS.

b. The excised liver is placed on a Teflon sheet for washing. The liver, and most importantly the left lobe, should not be allowed to slip off the Teflon sheet. Approximately 250 mL or more of high purity water from the Teflon bottle is poured over the liver to wash off blood and other fluid. The liver is picked up and, while held in the air, the Teflon surface is rinsed with the water. The liver, with the unwashed side facing up, is placed back on the Teflon sheet. Another 250 mL of water is used to rinse this side. The liver is allowed to drain for several minutes.

c. The liver is transferred to a clean Teflon sheet resting on the autopsy room scales. The weight of the whole liver is read and recorded.

d. The Teflon sheet holding the liver is removed from the scales and placed on a flat surface for dissection. Using the titanium knife, the left and right lobes are separated by a cut just to the left of the falciform ligament. If the weight of the left lobe is estimated to be greater than 400 g, make the dissection cut somewhat into the left lobe to reduce the amount sampled to less than 400 g. The right lobe is returned to the autopsy personnel.

e. Using the titanium knife, the left lobe is divided into two sections, one will be designated as section A and the other as section B. The left lobe is divided by a cut which divides the specimen into anterior and posterior portions of roughly equal size.

f. Each section is placed in a dust-free Teflon bag provided by NBS, being careful to keep blood away from the outside of the bag and the sealing area of the inside of the bag. If the sample must be transported from the autopsy room to another laboratory for sealing of the Teflon bags, the unsealed bags are placed in a sample transport container with lid, preferably glass and preferably lined with the dust-free Teflon (styrofoam is unacceptable). The bags should fit snuggly so that the liver sections do not move about during transportation.

g. A sample of blood (generally from within the heart) is collected for a hepatitis B antigen screening and a liver section is removed from the right lobe for preparation of histological slides. One slide is prepared with H&E stain. The paraffin block should be available to NBS personnel for preparation of a second slide should this become necessary.

h. The Teflon bags containing the liver sections are heat sealed. It is important to seal the bag with a minimum amount of air remaining inside. The air may be squeezed out or vacuum applied to a partially sealed bag. Another technique is to heat seal the bag with as little air remaining as possible, then squeeze the bubble of remaining air to a corner, puncture this corner with the titanium knife, squeeze out the remaining air and reseal the corner. However, it is particularly difficult to get a good seal where fluid has come in contact with the Teflon surfaces. The seal can be tested by gently squeezing the bag to see if pressure holds. A second seal is made slightly away from the first and parallel to it to provide a double seal.

i. The liver sections are weighed using an empty bag to tare the weight of the Teflon bag. The weights are recorded on the sampling data form and the sample labels. The labels are affixed to the sample bags.

j. Each sample is then placed in a second bag and sealed again as in h above.

k. The double-bagged samples are placed in the cylindrical cardboard containers provided and immersed in liquid nitrogen* for freezing. (The LN_2 shipper should not be used for freezing down the samples but only for shipping.) Sampling should remain in LN_2 for 10 minutes to insure thorough freezing.

l. The back (orange) copy of the sample data form is rolled up and placed in the inner cylinder of the LN_2 shipper. The bagged, boxed, and frozen liver sections are then transferred to this cylinder and placed in the LN_2 shipper for storage.

m. The LN_2 biological cryogenic shipper should be filled with LN_2 for at least 24 hours to fully prepare it for shipping (see instructions received with shipper for proper filling to maintain cryogenic capacity).

n. The liver specimens should be stored no longer than 10 days. The small, single sample shippers should be shipped within 48 hours of sample collection.

*Precautions should be observed when working with liquid nitrogen. Liquid nitrogen should not be stored in sealed containers. Personnel handling liquid nitrogen are cautioned to wear boots, cuffless trousers, non-absorbant apron, loose, insulating gloves and face shields.

o. The samples are shipped to NBS using a 24 hour express package service (NBS will pay for the shipping expenses).

p. The hepatitis results are called into NBS as soon as available (no longer than two weeks). The histological slides and/or paraffin blocks are sent to NBS with results of the histological examination as well as the two top copies of the sample data form. At NBS the samples are not processed for analysis until histological results are received. Therefore, it is important that these results are received at NBS as soon as possible, preferably within two weeks. This form should now contain as much of the requested information as available from the case. If more time is required to obtain donor history information, call histological results into NBS, and retain Donor Information Form until completed. The pink copy of the form is retained for your files.

NOTES CONCERNING CONTAMINATION SOURCES

The control of contamination requires diligent attention to detail. The success of the Specimen Bank program hinges on this point. A lack of contamination control has made existing banks of samples useless for general trace constituent analyses (1). Since it is impossible to point out every source of contamination that may by encountered, some of the most obvious sources will be described to provide a general awareness of the problem. The following example will illustrate the effect of contamination. A 10 µg flake of stainless steel contains approximately 1 µg of nickel. The natural occurrence of nickel in human liver is at levels of 0.01 to 0.2 µg of nickel per gram of sample. As we generally use about 1 gram of sample for analysis, a tiny flake of stainless steel in our sample could produce an analytical result which was 100 times higher than the true value.

In the autopsy room, the air, the implements, counter tops, and working personnel are sources of contamination. The air may contain trace vapors of formalin, xylene, and hair spray. It may also contain particles: dust, cigarette ash, and wear particles from motors. Implements and working surfaces may be contaminated with chemicals used for cleaning and salt and oils from human contact (via hands, etc.). A common contaminate introduced by human hands is gold from jewelery. Cigarette ash contains relatively high amounts of cadmium and organic compounds. Cigarette smoke contains trace quantities of hundreds of organic compounds.

The dust-free gloves should be used liberally as they are easily contaminated. Picking up a pen to record a weight contaminates them, grasping the Teflon bottle to wash the liver contaminates them, adjusting eyeglasses, touching your face, touching the outside of the bag which contains the clean Teflon sheets or bags contaminates the gloves.

Recent research has pointed out other sources of contamination. One trace organic study found some interesting and unusual compounds in human tissue. They were later found to have been introduced when the sample was cut on a styrofoam board. In another case, the calcium content of a liver was found to be unusually high. It was traced back to contamination from the

contents of the stomach. Liquid nitrogen contains oil and dust. The two sealed bags are provided to protect the sample as it is frozen in the LN_2.

The previous are just a few examples which will give the reader an appreciation of the chemical contamination problem.

APPENDIX II

PROCEDURES FOR CLEANING TITANIUM KNIFE

The only reusable item in the liver sampling protocol is the titanium knife. There are two cleaning procedures for the knife: the first is to be completed after each use and the second is used only after approximately six cases and after each sharpening. The titanium knife should be sharpened only with the new silicon carbide stone provided and only the titanium knife is to be sharpened on this stone.

Cleaning Procedure I

After placing liver sections in bags and before leaving the autopsy room, the knife should be rinsed using the high-purity water. While rinsing, and with gloved hands, run fingers over the blade and handle to help remove any adhering blood or tissue. This is best done before any fluid or tissue has a chance to dry on the knife. In the laboratory the knife should be rinsed again, as above, with water and then with ethanol. The knife is then placed in a clean glass container, covered with the high-purity water and boiled for 10 minutes. The knife is removed and placed on a clean surface (do not touch the blade) and allowed to air dry, preferably in a laminar flow hood. The knife should then be placed in a Teflon bag, made from the Teflon sheets, for storage and transportation to the next sampling case. The knife should at no time be touched with ungloved hands.

Cleaning Procedure II

This procedure should be applied after the knife has been used in approximately six cases since last cleaning and always after the knife is sharpened. Rinse the knife in the autopsy room as described in Cleaning Procedure I. In the laboratory the knife is placed in a clean glass container and covered with reagent grade chloroform. It should remain in the solvent for one hour or longer. The chloroform is poured off and the knife covered with reagent grade ethanol for one hour or longer. The ethanol is removed and the knife is rinsed with high-purity water. The knife is covered with dilute hydrochloric acid for two hours (made up using one part reagent grade acid and two parts high-purity water). The acid is removed and the knife rinsed with high-purity water. The cleaning is repeated with dilute nitric acid for two hours (made up using one part reagent grade nitric acid and two parts high-purity water). The acid is removed and the knife rinsed once with high-purity water, once with ethanol, and then twice with high-purity water. The knife may be dissassembled to clean if necessary. The knife is removed from the washing container and placed on a clean surface to air dry, preferably in a laminar flow hood. Only the knife handle should touch the drying surface. The clean, dry knife is stored in a Teflon bag made from Teflon sheets.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3. RECIPIENT'S ACCESSION NO.

4. TITLE AND SUBTITLE
PILOT PROGRAM FOR THE NATIONAL ENVIRONMENTAL SPECIMEN BANK - PHASE I

5. REPORT DATE

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
Sally H. Harrison, Rolf Zeisler and Stephen A. Wise

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Center for Analytical Chemistry
National Bureau of Standards
Washington, D.C. 20234

10. PROGRAM ELEMENT NO.
CCHH1A

11. CONTRACT/GRANT NO.
EPA-79-D-X0105

12. SPONSORING AGENCY NAME AND ADDRESS
Health Effects Research Laboratory RTP,NC
Office of Research and Development
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA-600/11

15. SUPPLEMENTARY NOTES
Project Officer: George M. Goldstein

16. ABSTRACT

This work was performed under a joint NBS/EPA research program to develop state-of-the-art protocols for the sampling, storage, and analysis of biological and environmental-type matrices. This report summarizes the procedures used in the initial phase of a pilot program for determining the feasibility of the National Environmental Specimen Bank. A Special clean-laboratory/storage facility has been completed for use in this program. Detailed protocols for sampling, storage, and analysis of human liver samples are described.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
RELEASE TO PUBLIC	UNCLASSIFIED	61
	20. SECURITY CLASS *(This page)*	22. PRICE
	UNCLASSIFIED	

EPA Form 2220-1 (9-73)