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Trace Metal Cleanroom

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Foreword

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The National Exposure Research Laboratory - Cincinnati (NERL-Cincinnati) conducts research to:

- Develop and evaluate analytical methods to identify and measure the concentration of chemical pollutants in drinking waters, surface waters, groundwaters, wastewaters, sediments, sludges, and solid wastes.

- Investigate methods for the identification and measurement of viruses, bacteria and other microbiological organisms in aqueous samples and to determine the responses of aquatic organisms to water quality.

- Develop and operate a quality assurance program to support the achievement of data quality objectives in measurements of pollutants in drinking water, surface water, groundwater, wastewater, sediment and solid waste.

- Develop methods and models to detect and quantify response in aquatic and terrestrial organisms exposed to environmental stressors and to correlate the exposure with effects on chemical and biological indicators.

This publication, "Trace Metal Cleanroom," was prepared as a part of the Regional Applied Research Effort (RARE) Program at the request of EPA Region II. This publication documents current cleanroom designs, specifications and protocols for ultra low level analysis of arsenic, lead, mercury and selenium. It provides guidance for regional environmental laboratories in the special practices used in the analysis of ultra low trace metal. NERL-Cincinnati is pleased to provide this manual and believes that it will be of considerable value to many public and private laboratories that wish to perform trace metal analyses in water matrices for regulatory or other reasons.

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Abstract

Accurate chemical analysis of inorganic elements and their species at ultra-trace concentrations is essential for understanding and modeling environmental systems and determining health effects. In order to maintain sample integrity and prevent contamination during sample preparation or analysis, a high purity cleanroom or clean zone is required. Proper design, construction, use, and maintenance of a trace element cleanroom are necessary to achieve a high cleanliness environment, but guidelines and protocols specific to trace element cleanrooms are not available in federal or international cleanroom standards or in published literature. This work developed practical design and construction options and use and maintenance protocols based on several sources: review of existing guidelines and standards, tours of six trace element cleanrooms, interviews with designers and users, and experiences with the trace element cleanroom suite at Research Triangle Institute. Several design options are possible depending on technical factors such as the elements being analyzed and the sample preparation and analysis methods used, and non-technical factors such as the number of samples and analysts. Some of the most important design features of any trace element cleanroom are the use of high efficiency air filters, laminar air flow, acid-tolerant and low particulate construction materials, and vents for removal of acid-laden or toxic vapors. However, it is equally important that the cleanroom have its access restricted to essential technical staff only, and that the technical staff be thoroughly trained in cleanroom use, behaviors, and maintenance.

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List of Abbreviations and Symbols

Abbreviation	Meaning
ACS	Analytical and Chemical Sciences (unit of RTI)
AsH_3	arsine
ASV	anodic stripping voltammetry
CEN	Comite' European de Normalisation
D	downstream particle concentration (particles/unit volume).
EMSL*	Environmental Monitoring Systems Laboratory
GFAA	graphite furnace atomic absorption
HCl	hydrochloric acid
HDPE	high density polyethylene
HEPA	High Efficiency Particulate Air
Hg(0)	metallic mercury
HNO_3	nitric acid
H_2O_2	hydrogen peroxide
H_2SO_4	sulfuric acid
HVAC	Heating, ventilating, and air conditioning
ICP	inductively coupled plasma
ICP-MS	inductively coupled plasma-mass spectrometry
IES	Institute for Environmental Sciences
MΩ	mega ohm
mph	miles per hour
NA	Not applicable
NaOCl	sodium hypochlorite
NIST	National Institute of Standards and Technology
NS	Not specified
ppb	part per billion
ppm	part per million
ppt	part per trillion
PVC	poly(vinyl chloride)
RTI	Research Triangle Institute
SOP	Standard Operating Procedure
U	upstream particle concentration (particles/unit volume)
ULPA	Ultra Low Penetration Air
µm	micron; 10^{-6} meters

* EMSL-Cincinnati was reorganized in 1995 and is now part of the National Exposure Research Laboratory (NERL-Cincinnati).

Section 1.0

Introduction

In order to develop an accurate understanding and model of trace element speciation and cycling in the environment, it is necessary to protect environmental samples from contamination during sample preparation and analysis stages. In many cases, air-borne particulates have high metal concentrations and are a major source of sample contamination. In order to eliminate this problem, specially designed trace metal cleanrooms are used. Cleanrooms are rooms that have a high flow rate of purified air which continuously blankets samples and materials in a clean atmosphere. They are equipped with High Efficiency Particulate Air (HEPA) filters and/or Ultra Low Penetration Air (ULPA) filters that remove almost all particles from the air. These cleanrooms are continually flushed with purified air so that samples can be processed without atmospheric contamination. Use of cleanrooms has enabled metal quantitation in the parts per trillion range for some elements.

The Environmental Monitoring Systems Laboratory - Cincinnati (EMSL - Cincinnati)* requested cleanroom design specifications and use protocols for cleanrooms to be used for analysis of arsenic, lead, mercury, and selenium. The sample types include water, wastewater, seawater, and related matrices, and the expected elemental concentrations are in the parts per trillion to parts per billion range. The specifications and protocols will ultimately be used by EMSL - Cincinnati to provide guidance to contract laboratories that perform inorganic environmental analyses at trace and ultra-trace concentrations. Thus the goals of the work presented in this report were to evaluate existing options and standards for cleanroom designs and materials, develop functional specifications for trace metal cleanrooms, and develop protocols for cleanroom use and reagent purification procedures.

This report contains a summary of technical activities undertaken, an overview of cleanroom theory and design options, a summary of existing and proposed standards issued by the federal government and private organizations, recommendations for design, use, and maintenance of a trace metal cleanroom, and protocols developed for use in the Research Triangle Institute (RTI) Inorganic Cleanroom Facility.

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Section 2.0

Summary and Conclusions

The goals of the Work Assignment were to develop functional specifications for trace metal cleanrooms, cleanroom use protocols, and reagent purification protocols. In order to accomplish this, a review of existing cleanroom standards and recommendations was performed, six trace metal cleanrooms were visited, and interviews with the laboratory designers, principal users, and authors of cleanroom standards were conducted. In addition, we installed and started to use our own trace metal cleanroom at RTI during the period of this Work Assignment.

One important conclusion of this report is that even with a field as narrowly defined as trace element environmental analytical chemistry, there are many different needs and acceptable solutions for cleanroom designs. No one design will satisfy the needs of all users, in part because not all users analyze the same suite of elements. The differences in design requirements stem from technical factors such as the chemical behavior of different elements, and the different preparation and storage procedures required to best analyze those elements, and from non-technical factors such as the size of the facility, number of analysts, local safety and utility codes, etc.

Federal standards are available for cleanroom class designation and particle counting methods, but they are not available for cleanroom designs, construction materials, personnel garments, activities, or training, etc. Guidelines are available from the Institute for Environmental Sciences (IES) in several of these areas, but they are designed to be general guidelines that are not specific to any one industry or application. As a result, the guidelines represent a starting point for trace element cleanroom considerations, but do not provide information that can be comprehensively applied. This report presents design, construction, use, and maintenance options with specific application to trace element cleanroom laboratories used for environmental analytical chemistry. Advantages and disadvantages for each cleanroom design option are presented so that the reader may have guidance for their own application. Finally, standard operating procedures used at RTI, Florida International University, Battelle Pacific Northwest, and Frontier Geosciences are included as appendices to provide specific protocols used at those facilities.

Section 3.0

Recommendations

This report contains practical cleanroom design options and Standard Operating Procedures (SOP) with specific application to trace element analysis for environmental samples. Theoretical aspects of air filtration and dynamics are also presented as background information for the reader. The primary recommendation of this report is that the reader use the design options presented, or modify them for their application in a manner that is consistent with efficient air filtration and laminar air flow dynamics.

The Standard Operating Procedures (SOP) included in the appendices provide specific guidance in areas of cleanroom use, maintenance, operations, personnel, garments, and reagent purification procedures. These documents are the most recent versions available but are continuously revised to include the most appropriate procedures. In some cases, such as recommended cleanroom garment use or frequency of maintenance activities, recommendations are made in an heuristic manner. It should be recognized that as standard procedures are rigorously applied and correlations made between cleanroom cleanliness levels and specific procedures, the procedures will be modified as needed. Initially, it is prudent to err on the side of excess restrictions and over-zealous contamination control rather than to assume a more casual approach that could jeopardize sample integrity. Thus, a secondary set of recommendations are to track the performance of the laboratories using the various design options and SOPs presented, and determine the effect of specific procedures and designs on the cleanliness levels of the laboratories and ultimately on the contamination levels of the samples.

Section 4.0

Technical Activities

In order to accomplish the goals listed in Sections 1 and 2, several technical activities were performed. The following list summarizes the activities; they are briefly described in this section and a more complete discussion of the results of each activity is presented in subsequent sections of this report.

1. Review of federal standards and Institute of Environmental Sciences (IES) standards for cleanroom use;
2. Tour of six trace metal cleanrooms;
3. Consultation with cleanroom laboratory designers and users;
4. Review of current cleanroom practices, sample handling procedures, and reagent purification procedures for inclusion in protocols; and
5. Preparation of protocols.

Review of Standards

Because there was no document available to describe the unique design and material considerations required for a trace metal cleanroom, only general guidance could be gleaned from the guidelines published in Federal Standard 209E (FED-STD-209E; 1992) for cleanrooms. Similarly, the Institute of Environmental Sciences "Recommended Practice" documents and the draft European standard, CEN/TC 243/WG 1 [Geilleit, 1992], which is based on Federal Standard 209, were also reviewed for definitions and general guidelines.

Tour of Laboratories

Six laboratories were toured to learn how cleanroom guidelines have been implemented and how successful they are. The six laboratories were:

1. Florida International University, Miami, FL
   Dr. Ronald Jones, Laboratory Designer and Director
   Class 100 mercury analysis facility
2. National Institute of Standards and Technology, Gaithersburg, MD
   Dr. John Moody, Laboratory Designer and Director
   Class 100 general metal analysis facility
3. University of North Carolina, Chapel Hill, NC
   Dr. Steven Goldberg, Laboratory Designer and Director
   Class 100 general metals analysis facility
4. Brooks Rand Corp., Seattle, WA
   Mr. Richard Brooks, President
   Dr. Lian Liang, Laboratory Director
   Class 100 mercury and general metals analysis facility
5. Frontier Geosciences, Seattle, WA
   Mr. Nicolas Bloom, Laboratory Designer and Director
   Mercury analysis facility
6. Battelle Pacific Northwest, Sequim, WA
   Ms. Brenda Lasorsa, Laboratory Director
   Mercury analysis facility

Consultation with Designers and Users

Each of the individuals listed above was interviewed at length regarding the design considerations of their laboratory and the procedures used to avoid sample and reagent contamination. In some cases, additional workers in the laboratory were also interviewed to provide information about other sample handling and preparation procedures used. When possible, protocols and/or publications were collected that detail the sample collection, preparation, and analysis procedures, the cleanroom operation and maintenance procedures, and reagent and labware purification procedures.

Review of Currently Used Procedures

The sample handling and preparation procedures provided by each of the consultants were reviewed along with procedures available in the literature in order to assess the relative utility of various techniques. Several of these were then experimentally assessed in the RTI/Analytical and Chemical Sciences (ACS) Inorganic Clean Lab Facility. Any modifications suggested by our experience were incorporated into protocols and recommendations.

Preparation of Protocols

The following standard operating procedures (SOP) were prepared for use in the RTI/Analytical and Chemical Sciences Inorganic Clean Lab Facility; copies of the protocols are contained in Appendices A through D:

RTI-ACS/SOP-174-001
ACS[a] Inorganic Class 100/10,000 Clean Lab Facility
RTI-ACS/SOP-174-002
Cleaning Labware in the ACS[a] Inorganic Class 100/10,000 Clean Lab Facility

RTI-ACS/SOP-174-005
Monitoring and Maintaining Cleanliness of the ACS[a] Inorganic Clean Lab Facility

RTI-ACS/SOP-174-007
Purification of Reagents in the ACS[a] Inorganic Clean Lab Facility for Trace/Ultratrace Metal Analysis

[a] ACS = Analytical and Chemical Sciences unit of Research Triangle Institute.

Section 5.0

Cleanroom Theory and Standards

Definitions

A general definition of a cleanroom is a room which has a gentle shower of highly filtered air for the purposes of transporting airborne particulate contaminants away from sensitive samples or products, and maintaining a clean environment with low particle concentrations. A cleanroom is defined in U.S. Federal Standard 209E as "a room in which the concentration of airborne particles is controlled and which contains one or more clean zones." A clean zone is defined as "a defined space in which the concentration of airborne particles is controlled to meet a specified airborne particulate cleanliness class." Thus a cleanroom may have one or more regions within the room in which the concentration of airborne particles is maintained less than or equal to specified limits.

Review of Cleanroom Theory

The theoretical aspects of particle dynamics, electrostatics, filtration mechanisms, and flow velocity modeling are extremely important components of cleanroom design, maintenance, and improvement. However, the technical details involved are beyond the scope of this report. The interested reader is referred to recent publications including *Handbook of Contamination Control in Microelectronics* edited by Tolliver (1988), *Particle Control for Semiconductor Manufacturing* edited by Donovan (1990), *The Future Practice of Contamination Control* (Proceedings of the 11th International Symposium on Contamination Control, London, 1992), and publications presented in the *Journal of the IES*. The review presented below is intended to provide a basic level of understanding required for discussion of cleanroom design options. It discusses sources of particulate contamination in general, and the basic theory of air filtration, flow patterns and velocities.

Sources of Contamination

The ultimate goal of any cleanroom is to prevent contamination of samples or products by airborne particulates. Ideally, the designer has knowledge of the source or sources of the particles, as well as their relative number and size, and can thus be sure that the design will achieve the cleanliness objectives. In some cases it is necessary to know the chemical composition of particles contributed by the source(s). However, even without a detailed knowledge of the number and type of particles present in a given laboratory, the designer can proceed using some general estimates. For example, a typical non-smoking office environment has 100,000 to 200,000 particles that are 0.5 micron or larger per cubic foot of air (Matthews, 1994). Similarly, ordinary activities performed by people, such as sitting or walking, generate millions of particles every minute. Table 1 lists typical air particle emission rates from people engaged in various activities. The values are presented as general guides only; individual circumstances may differ significantly.

In order to determine the source of particulate contamination, some knowledge of other operations performed in the building or in adjacent buildings is helpful. In general, there are at least three major sources of airborne particulate contamination in any laboratory:

1. Airborne particles are brought into the laboratory from sources exterior to the laboratory. Outdoor air with a high particulate concentration is brought in through the air handling system, and indoor air from corridors and other laboratories is brought in through doorways and inter-laboratory air passages.
2. Particles are generated within the laboratory by personnel, equipment, and processes. Movement of personnel within the laboratory not only generates

Table 1. Typical Air Particle Emission Rates from People

Activity	Number of Particles 0.3 Micron or Larger Emitted from Each Person/Minute
Standing or sitting; no movement	100,000
Standing or sitting, average body movement, toe tapping	1,000,000
Changing positions, sitting to standing	2,500,000
Average walking (3.57 mph)	7,500,000
Fast walking (5 mph)	10,000,000
Calisthenics	15,000,000 to 30,000,000

particles, but also generates air currents and eddies that transport particles from one region to another.

3. Particles are brought into the laboratory on workers' apparel and supplies. Clothing fibers abrade and shed particles; shoes transport dirt and smaller particles; books, papers, reagent bottles all transport particles into a laboratory.

Proper design of the cleanroom can minimize the first two of these sources, i.e., it can reduce the importation of particles from sources external to the laboratory and it can reduce the impact of particles generated within the laboratory. This requires judicious choice of room size and shape, mechanism of air filtration, cleanliness level, volume and flow rate of air through the room, and direction of air movement in all regions of the room. A general discussion of these factors is presented below.

In order to minimize the contamination due to the third source of airborne particulates, i.e., workers' apparel, supplies, and operations, it is necessary to implement stringent cleanroom procedures for personnel and restrict apparel to special cleanroom garb. These procedures are highly specific to the operations performed in the cleanroom, and are discussed in Section 7, Cleanroom Use.

Air Filtration and Recirculation

Contaminated air can be filtered to remove most of the particles prior to entry into the cleanroom. A typical cleanroom has pre-filters to remove large particles, temperature and humidity controls for personnel comfort and equipment function, and high performance filters to remove submicron sized particles. Table 2 presents a list of common particle types and their size range. There are several definitions of particle size used for different purposes, but this report will use the definition provided by the Institute of Environmental Sciences [IES-CC-011-85-T, 1985] which defines particle size as "the maximum linear dimension of a particle as observed with an optical microscope or the equivalent diameter of a particle detected by an instrument. The equivalent diameter is the diameter of a reference sphere having known properties and producing the same response in the sensing instrument as the particle being measured."

The high efficiency filters used to remove the submicron particles are typically High Efficiency Particulate Air (HEPA) filters or Ultra Low Penetration Air (ULPA) filters. Specifications for HEPA filters require that they have a minimum particle-collection efficiency of 99.97% for 0.3 μm particles, and ULPA filters have a minimum particle-collection efficiency of 99.999% for particles in the size range of 0.1 to 0.2 μm [IES-RP-CC001.3, 1993]. Efficiency is defined as the ratio of the difference in concentrations (upstream - downstream) to the upstream concentration:

$$\text{Efficiency (\%)} = \frac{U - D}{U} \times 100$$

where **U** = upstream particle concentration (particles/unit volume)
D = downstream particle concentration (particles/unit volume).

This means that if the particle count upstream of a HEPA filter is 100,000 particles per cubic foot (0.3 μm) then the count downstream of the filter will be a maximum of 30 particles per cubic foot. Similarly for the ULPA filters, if there are 100,000 particles per cubic foot (0.12 μm) upstream of the filters, then the count downstream will be a maximum of 1 particle per cubic foot.

While it might naively be assumed that filters remove all, or nearly all, particles larger than a specified size and are penetrated by particles smaller than that size, that is not the case for HEPA or ULPA filters due to the effect of diffusion of particles in the submicron size range [Ensor and Donovan, 1988]. HEPA and ULPA filters exhibit a maximum penetration by particles of approximately 120 nm size (0.12 μm). Alternately stated, the filters have higher collection efficiencies for particles both larger and smaller than 0.12 μm and have poorest performance for particles approximately 0.12 μm.

After air has passed through the HEPA or ULPA filters it enters the cleanroom as highly purified air. Once inside, the purified air can become contaminated with particles from personnel, containers, implements, etc., but is generally still cleaner than air external to the cleanroom.

Table 2. Typical Particle Sizes

Particle Description	Approximate Particle Size Range(μm)[a]
Gas molecules	0.0005-0.01
Metallurgical dusts and fumes	0.001-100
Atmospheric dust	0.001-30
Viruses	0.003-0.06
Rosin smoke	0.01-1.0
Tobacco smoke	0.01-1.0
Oil smoke	0.03-1.0
Zinc oxide fumes	0.01-0.3
Combustion nuclei	0.01-0.1
Sea salt nuclei	0.03-0.6
Carbon black	0.01-0.3
Colloidal silica	0.02-0.05
Paint pigments	0.1-5
Alkali fumes	0.1-5
Ammonium chloride fumes	0.1-3
Bacteria	0.3-30
Insecticide dusts	0.5-10
Ground talc	0.5-50
Sulfuric concentrator mist	1-20
Coal dust	1-100
Fly ash	1-200
Cement dust	3-100
Red blood cell (adult human)	7.5 ± 0.3
ant spores	10-50
Pollens	10-100
Beach sand	90-3000

[a]Values taken from Austin (1970).

For that reason, the air is recirculated back through the filters and again enters the cleanroom as high purity air. Recirculation has several important technical and economic ramifications:

1. Recirculation increases the efficiency and longevity of the filters. Because the recirculated air upstream of the filters has a relatively low particle concentration, it presents a minimum burden to the filter substrate and prevents premature clogging of the pores. The cost of replacing HEPA or ULPA filters is substantial and thus maximizing filter life is an important economic goal. Recirculation also produces cleaner air with each successive pass through the filters, so that the cleanroom is made "cleaner" with each iterative cycle.
2. Recirculation increases the energy efficiency. Because outdoor air generally needs to be conditioned with respect to temperature and humidity, while recirculated air requires little to no additional conditioning, energy costs are much less for conditioning recirculated air than they are for outdoor air.
3. In a laboratory environment, recirculated air may be hazardous and/or toxic. Neither HEPA nor ULPA filters remove vapors from the air. Thus if hazardous or toxic vapors are generated in a laboratory cleanroom, they must be neutralized, captured, or vented to the outside in order to protect the health and safety of personnel in the cleanroom. Careful attention must be paid during the cleanroom design phase so that adequate space and facilities are designed for removal of hazardous or toxic vapors from the recirculating air. During subsequent laboratory usage, it is essential that personnel are trained in the use of the facility so that it is properly and safely used.

Air Flow Patterns

Conventional rooms and laboratories have air inlets located in one or a few regions of the room and the air disperses to other regions of the room in a turbulent flow pattern, as depicted in Figure 1. One consequence of turbulent flow is that it resuspends settled particles and transports them to different regions of the room. This represents a major source of contamination in many laboratories.

In order to prevent this contamination, unidirectional laminar air flow is used. Laminar flow means that the air moves in parallel planes from its plane of entry into the room to its plane of exit. Air flowing in laminar planes is stratified such that there is very little transfer of particles between plane boundaries. Thus, particles in one plane of air will remain in that plane until they are removed from the room.

In practice, it is impossible to achieve 100% laminar flow with personnel and equipment in the room because their presence is an obstruction to the air flow and turbulence is generated in the region of any obstruction. Thus some compromises to truly laminar flow are always necessary. Other compromises to laminar flow are dictated by safety, economics, or other practical factors, and have resulted in use of several different cleanroom designs with limited regions of laminar air flow. Figures 2 to 4 illustrate air flow patterns and particle movement for the cases of totally horizontal laminar flow and totally vertical laminar flow. A more thorough discussion of cleanroom design options is presented in Section 6.

Figure 2 depicts a horizontal laminar flow cleanroom in which HEPA filters are located in one wall of the room. Clean air sweeps through the room in horizontal sheets, entraining any particles enroute, and carrying them out a perforated side wall opposite the HEPA filters. The advantages of this design are that truly laminar flow is achieved in all work areas, i.e., bench top level and higher, and that the cost is relatively low. However, if people and/or equipment are present, laminar flow is disrupted and particles and vapors are transported horizontally. This results in contamination of samples and products downstream, closer to the exit wall. In practice, this design is useful for long, narrow cleanrooms with only one work bench, or cleanrooms with only one person present, but not in the configuration shown in Figure 2. A design similar to Figure 2 with multiple work benches and chemists was used during the 1970s at the National Institute of Standards and Technology (NIST) as a trace element cleanroom and was found to work poorly because of cross-contamination [Moody, 1982]. In some laboratories that generate toxic vapors or biological agents, the horizontal flow design would present a health and safety hazard because personnel would always be in the path of the generated species. Thus, the design has significant limitations, but in some cases may be useful.

A modification to the design in Figure 2 is to include one or more vertical flow, HEPA filtered fume hoods on the wall opposite the HEPA banks, as shown in Figure 3. This provides clean air in the region that previously was turbulent, and permits operations such as hot acid digestions to be performed. None of the cleanrooms visited uses this design, but it could potentially be useful.

Figure 4 shows a cleanroom with totally vertical laminar flow. In this design, the HEPA filters are located in a dropped ceiling and clean air sweeps downward through the laboratory, transporting any particles down through a perforated floor grid. Air is returned to the HEPA filters through a plenum wall space, refiltered, and recirculated through the cleanroom. The advantages of this design are that the cross-contamination of samples or products is minimized, particles swept off of personnel are transported down through the floor rather than onto samples, and nearly laminar flow is maintained throughout the room. Totally vertical laminar flow is generally recognized as the most effective design for achieving cleanliness classes 100 or lower, and has been successfully employed in ultra-trace element laboratories such as the lead isotope geochronology lab at the University of North Carolina [Su et al., 1994] as well as at other installations. The disadvantages of this design are the construction costs and engineering complications involved with the raised, chemical resistant, high strength, perforated floor grids and supports.

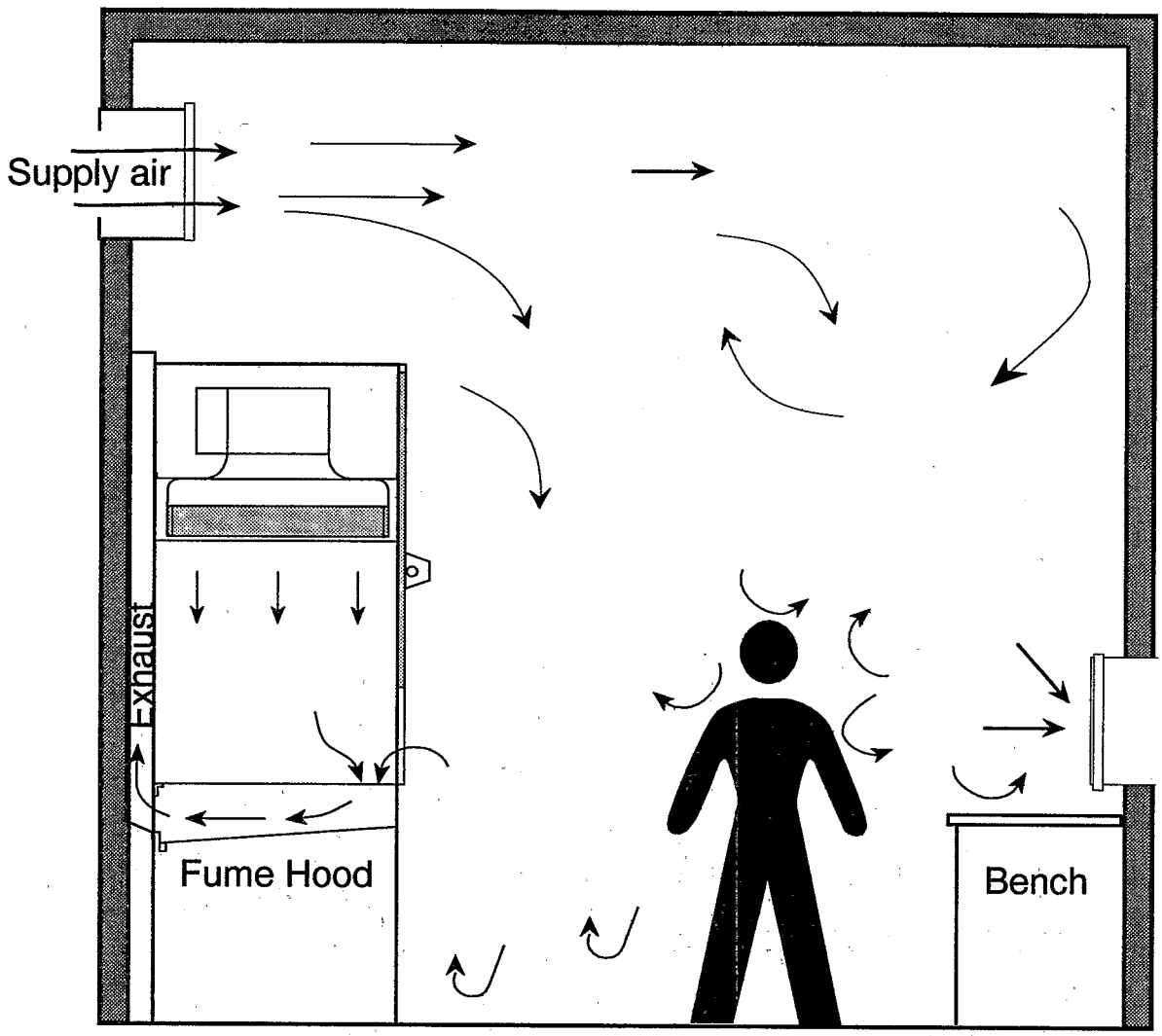


Figure 1. Conventional room with single air inlet and outlet and turbulent air flow patterns.

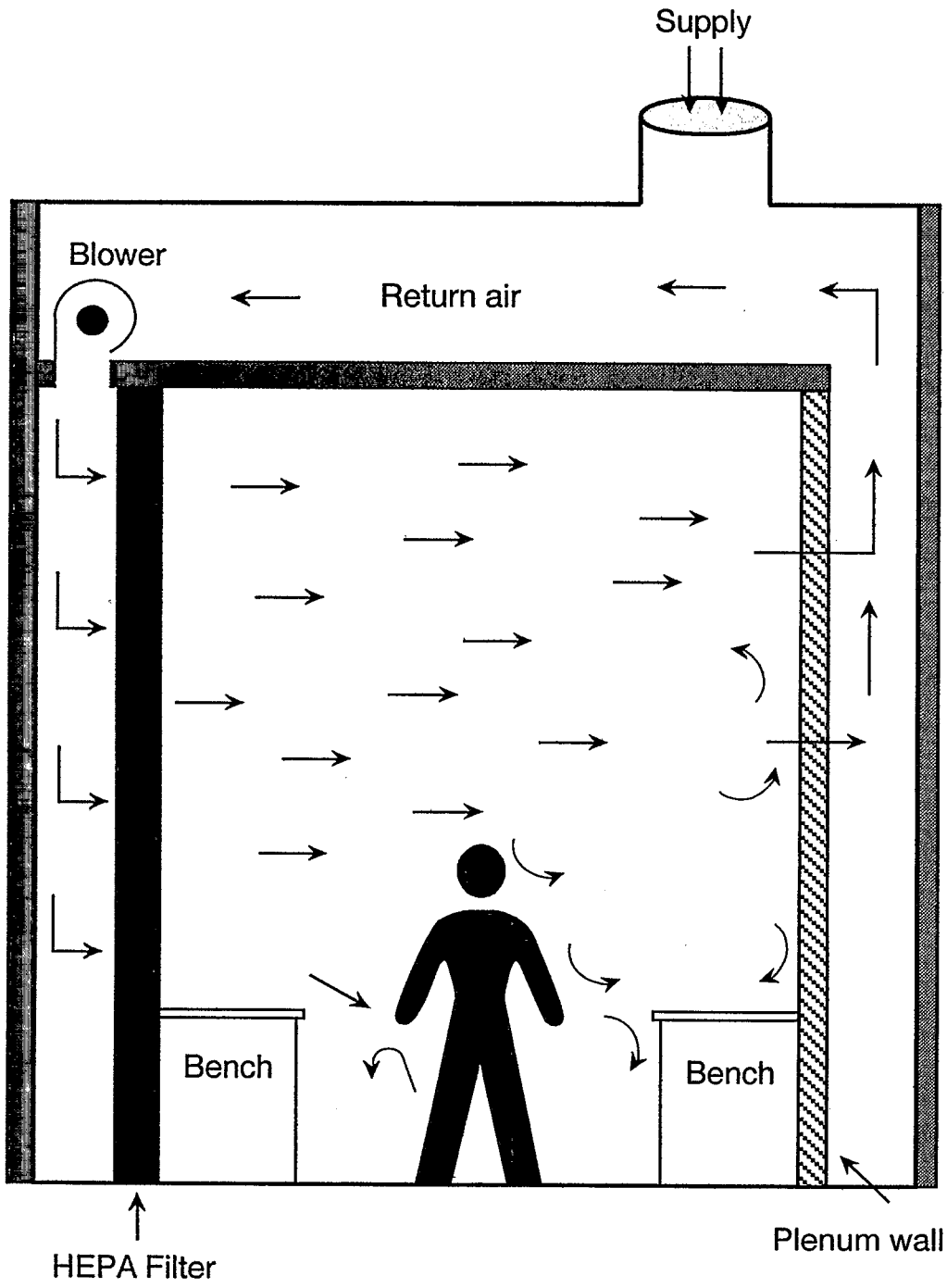


Figure 2. Horizontal laminar flow cleanroom.

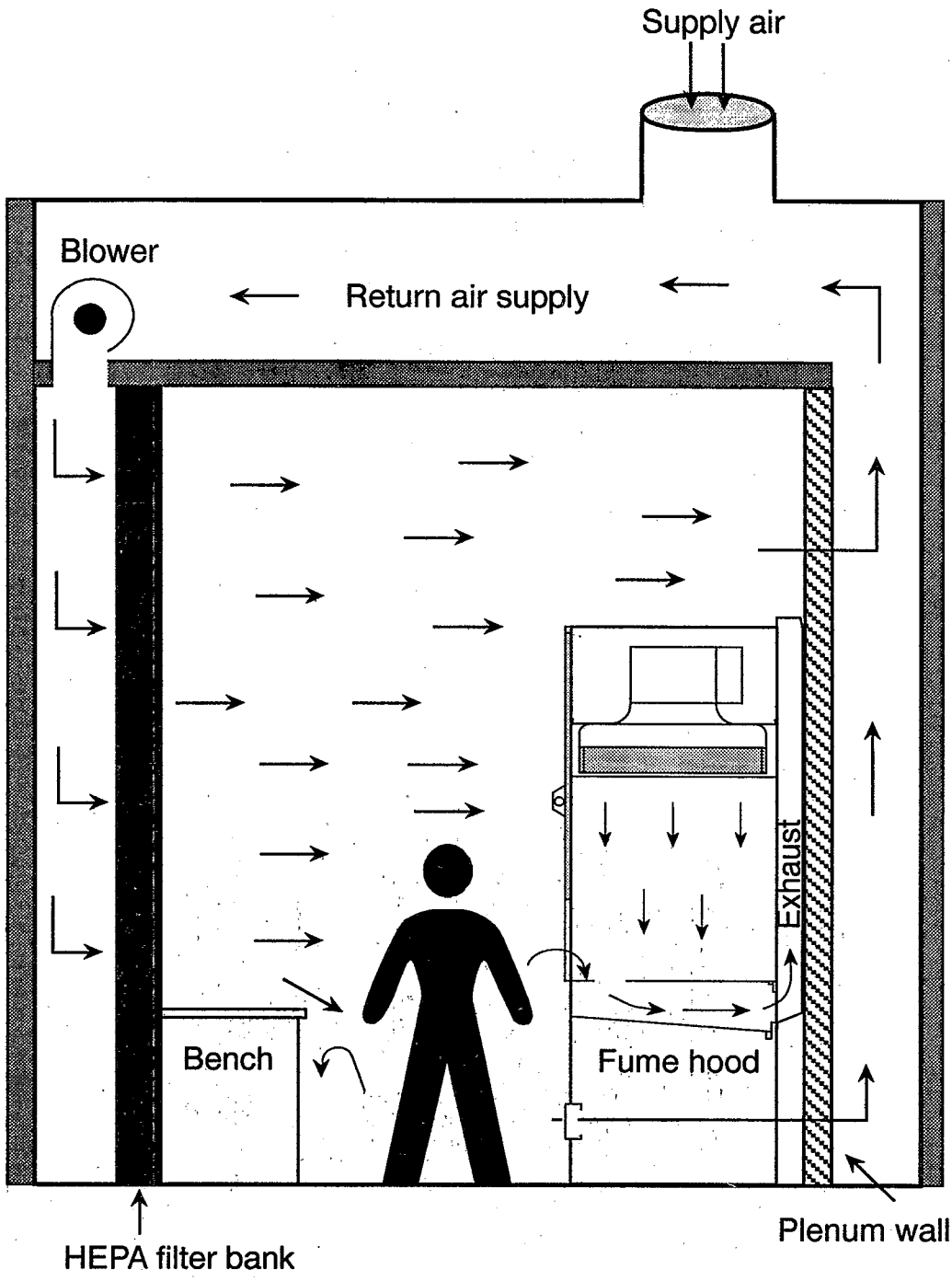


Figure 3. Horizontal laminar flow cleanroom with HEPA fune hood.

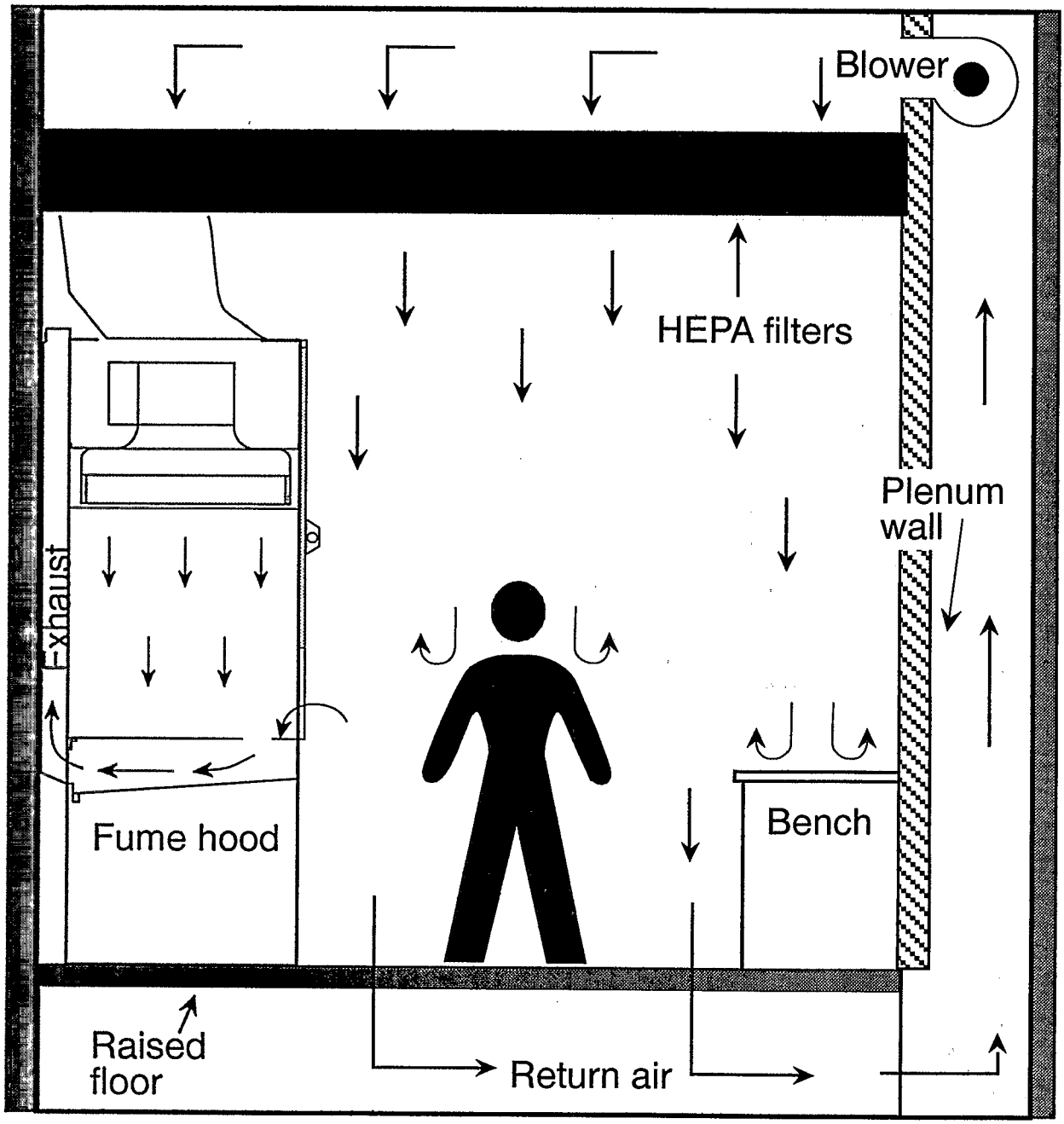


Figure 4. Vertical laminar flow cleanroom.

A modified vertical laminar flow design is used in the Class 100 cleanroom at RTI and is shown in Figure 5. It contains ULPA filters in a ceiling grid that disperse clean air downward in vertical laminar flow lines. One wall contains a row of polypropylene benches for sample and reagent preparation procedures that do not involve hot or concentrated acids. A small return air vent is located at hand level for recirculating air back through a plenum wall to the ULPA filters. This helps to remove any particles generated by turbulence at the bench top. Larger air return vents are located approximately 6 inches above floor level. Air travels through perforated grills at the front of the polypropylene benches, sweeps under the benches, and enters the return air vent at the wall. This helps to remove particles brought in on cleanroom foot covers as well as any settled particles generated in the laboratory. The flooring in the Class 100 laboratory is chemical resistant PVC (MIPOLAM; Huls America Inc.) with heat-welded seams, 6 inch covered edges at walls to prevent seepage of chemicals underneath the flooring, and a PVC floor drain leading to an emergency chemical spill tank outdoors. Thus, the flooring should not generate acid degradation products (volatile or particulate) even in the event of a chemical spill. Two fume hoods (one ULPA-filtered; one not filtered) are located on the opposite wall and provide space to perform acid digestions and other work with corrosive agents. Vapors and particles generated within the fume hoods are exhausted to the outdoors and not recirculated.

Air Flow Velocity

Clean, filtered air is the agent that transports particles out of a cleanroom and envelops samples in a contaminant-free environment. In achieving and maintaining a high level of cleanliness, two general rules are "the more clean air the better", and "the higher the flow rate the better." However, there are limits to those rules. If flow rate becomes too rapid, the airflow becomes turbulent rather than laminar, the environment is no longer comfortable to workers, operations become inefficient, and the cleanliness level decreases due to scouring of particles off of personnel or equipment. A minimum air flow velocity of 70 ft/min is required to maintain laminar flow. Most cleanrooms have air flow velocities between 70 and 110 ft/min.

Review of Existing and Proposed Standards

Existing cleanroom standards provide not only cleanliness class designations, but also guidelines and procedures used to monitor airborne particles, operate particle counting equipment, calculate the particle concentration, and verify the cleanroom class designation. However, because there are so many diverse applications of cleanrooms (pharmaceuticals, semiconductors, textiles, hospital operating rooms, chemical analysis), the authors of existing guidelines have chosen to provide general guides that are appropriate for any cleanroom application and have avoided making recommendations to any specific industry group. For example, they do not provide any information regarding cleanroom design, materials, construction, or use. However, the Institute of Environmental Sciences publishes a series of "Recommended Practices" that provide significant guidance to users of cleanrooms. While they are not federal standards, they have been thoughtfully compiled by experts in the contamination control industry. A listing of publications is available in the "Compendium of Standards, Practices, Methods, and Similar Documents Relating to Contamination Control" [IES-RD-CC009.2, 1993].

U.S. Federal Standard 209E

The U.S. Federal Standard 209E (dated September 11, 1992) consists of eight sections: (1) Scope and limitations; (2) Referenced documents; (3) Definitions; (4) Airborne particulate cleanliness classes and U descriptors; (5) Verification and monitoring of airborne particulate cleanliness; (6) Recommendation for change; (7) Conflict with referenced documents; and (8) Federal agency interests. The document provides a basis for establishing a common understanding of air cleanliness based on the number concentration of particles of a defined size in a defined volume of air over a defined length of time, as determined using approved particle counting techniques.

The intent of the document is to establish standard classes of air cleanliness for cleanrooms and clean zones and to prescribe methods for monitoring and verification. Tables and equations are provided to enable calculation of particle concentrations, the number of sampling locations required, the volume of air to be sampled, the cleanliness class, as well as other parameters. The document is used by cleanroom designers, builders, and users as a common set of definitions and procedures for determination and verification of air cleanliness class.

The particle concentration limits specified by the standard are a function of both the particle size and the cleanroom class. Particle concentration limits are summarized in Table 3 in both the U.S. standard units (particles per cubic foot) and the European (metric) standard units (particles per cubic meter).

CEN/TC243

The European committee charged with establishing cleanroom standards is the Comite' European de Normalisation (CEN). The committee drafted a document in 1992, CEN/TC243, which is similar in scope and intent to Federal Standard 209E. Equations and tables were included to enable determination of cleanroom cleanliness classes. However, in anticipation of the international standard, ISO 209, the document was not actively put into use. The class designations are presented in Table 4 for comparison with those in Federal Standard 209E.

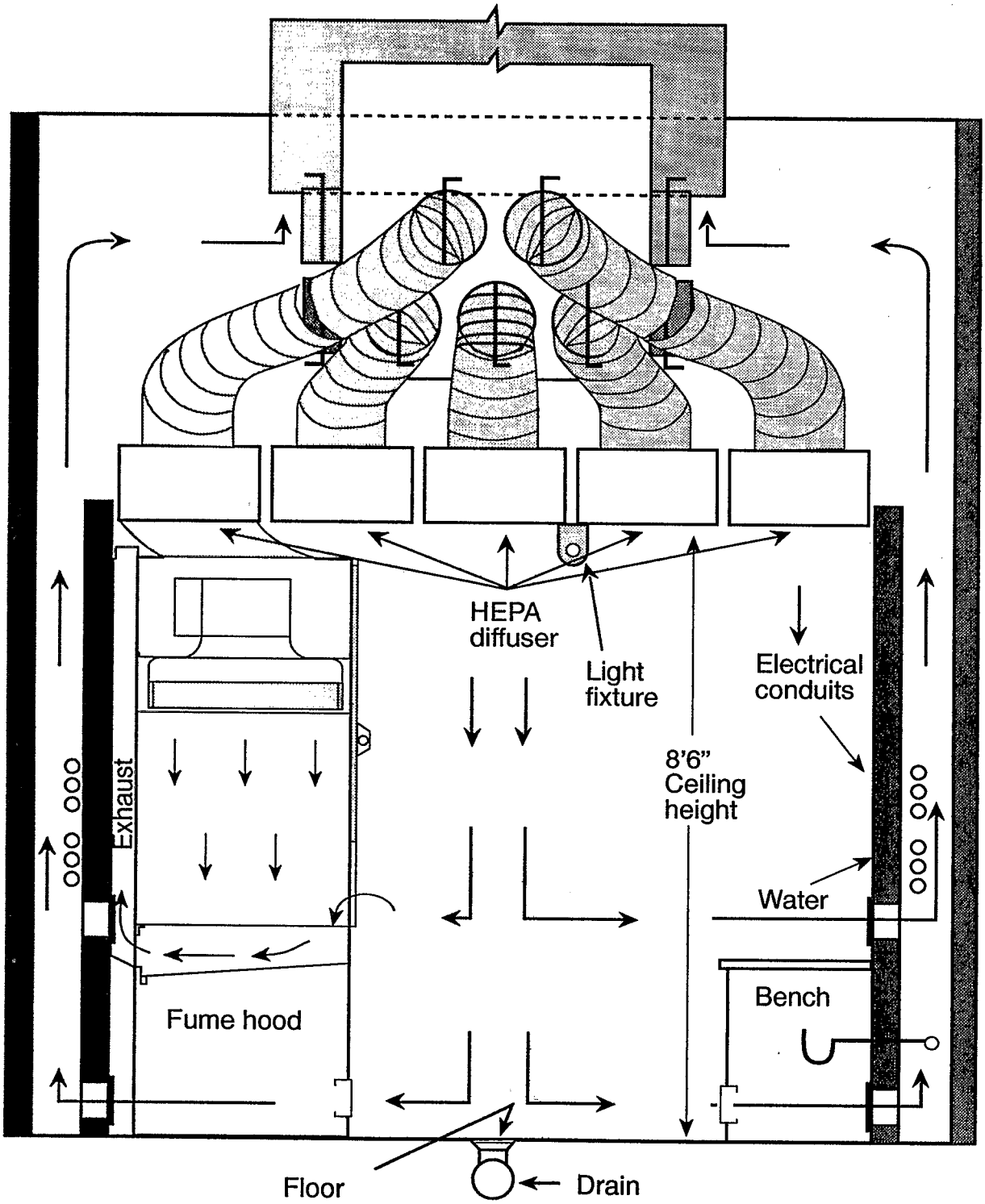


Figure 5. Vertical laminar flow cleanroom design used at RTI.

Table 3. Airborne Particulate Cleanliness Classes

Class limits are given for each class name. The limits designate specific concentrations (particles per unit volume) of airborne particles with sizes equal to and larger than the particle sizes shown.[a]

Class Name[b]		Class Limits									
		0.1μm		0.2μm		0.3μm		0.5μm		5μm	
SI	English[c]	Volume (m^3)	Units (ft^3)	Volume (m^3)	Units (ft^3)	Volume (m^3)	Units (ft^3)	Volume (m^3)	Units (ft^3)	Volume (m^3)	Units (ft^3)
M1		350	9.91	75.7	2.14	30.9	0.875	10.0	0.283	-	-
M1.5	1	1240	35.0	265	7.50	106	3.00	35.3	1.00	-	-
M2		3500	99.1	757	21.4	309	8.75	100	2.83	-	-
M2.5	10	12400	350	2650	75.0	1060	30.0	353	10.0	-	-
M3		35000	991	7570	214	3090	87.5	1000	28.3	-	-
M3.5	100	-	-	26500	750	10600	300	3530	100	-	-
M4		-	-	75700	2140	30900	875	10000	283	-	-
M4.5	1000	-	-	-	-	-	-	35300	1000	247	7.00
M5		-	-	-	-	-	-	100000	2830	618	17.5
M5.5	10000	-	-	-	-	-	-	353000	10000	2470	70.0
M6		-	-	-	-	-	-	1000000	28300	6180	175
M6.5	100000	-	-	-	-	-	-	3530000	100000	24700	700
M7		-	-	-	-	-	-	10000000	283000	61800	1750

[a] The class limits shown in Table 3 are defined for classification purposes only and do not necessarily represent the size distribution to be found in any particular situation.

[b] Concentration limits for intermediate classes can be calculated, approximately, from the following equations:
particles/$m^3 = 10^M (0.5/d)^{2.2}$
where M is the numerical designation of the class based on SI units, and d is the particle size in micrometers, or particles/$ft^3 = N_c(0.5/d)^{2.2}$
where N_c is the numerical designation of the class based on English (U.S. customary) units, and d is the particle size in micrometers.

[c] For naming and describing the classes, SI name sand units are preferred; however, English (U.S. customary) units may be used.

Table 4. Typical Airborne Particulate Cen Cleanliness Classes for Clean Rooms and Clean Air Controlled Spaces (Final Draft)

CEN Class	Maximum permitted number of particles/m^3 of a size equal to, or greater than, the considered size.						
	0.1 μm	0.2 μm	0.3 μm	0.5 μm	1 μm	5 μm	10 μm
0	25	6*	NA	(1)	NA	NA	NA
1	250	63*	28*	10	NA	NA	NA
2	2,500	625	278*	100	25	NA	NA
3	25,000	6,250	2,778*	1,000	250	10	NA
4	NS	62,500	27,778*	10,000	2,500	100	25
5	NS	NS	NS	100,000	25,000	1,000	250
6	NS	NS	NS	1,000,000	250,000	10,000	2,500
7	NS	NS	NS	(10,000,000)	2,500,000	100,000	25,000

NS - Not specified
NA - Not applicable
( ) - For reference only
* - Rounded values

ISO/TC209

An international standard, ISO/TC209, is currently being prepared that will become the cleanroom standard for European, Asian, and other countries. While it is anticipated that the United States will also agree to adopt the standard, it is not certain at this time that it will replace Fed. Std. 209E. According to Richard Matthews, Chairman of the ISO/TC209 committee, the ISO standard will redefine cleanliness classes using a formula that is slightly different than that in Fed. Std. 209E and it will present acceptable measurement and verification methods in a manner very similar to Fed. Std. 209E. However, the ISO standard will be much broader in scope, and will also provide guidance and specifications in areas of biocontamination control, design and con-

struction, personnel behaviors, support services, and other topics. While the intent is to provide a general document suitable for all cleanroom users and not specific to any one industry segment, the standard will venture into the area of biocontamination, which will be used to regulate the food, pharmaceutical, and medical industries. On the other hand, the guidance it will provide in cleanroom construction materials and protocols will be very general, and will not include issues specific to trace element laboratories, for example.

It is anticipated that the standard will be released in segments, with the cleanroom class designations and measurement protocols included in the first release in early 1996. The other segments are scheduled for release later that year.

Institute of Environmental Sciences Recommended Practices

The Institute of Environmental Sciences (IES) has published a series of "Recommended Practices" that provide guidance to cleanroom users in many areas of contamination control. The documents are not federal standards, but are fully compatible with Federal Standard 209E. One function of the documents is to provide definitions and cleanroom product and performance specifications that are used for a common basis of agreement among cleanroom designers, builders, and users. In addition, the documents provide recommendations for cleanroom garments (designs and fabrics) and gowning procedures, glove and wipe materials, cleanroom cleaning procedures and schedules, and other practices intended to maintain the cleanliness of the facility. An abbreviated listing of IES documents is presented in Table 5.

Table 5. IES Recommended Practice Documents

IES Document Number	Title	Date
IES-RP-CC-001.3	HEPA and ULPA Filters	1993
IES-RP-CC-002-86	Recommended Practice for Laminar Flow Clean Air Devices	1986
IES-RP-CC-003.2	Garments Required in Cleanrooms and Controlled Environments	1993
IES-RP-CC-004.2	Evaluating Wiping Materials Used in Cleanrooms and Other Controlled Environments	1992
IES-RP-CC-005.2	Cleanroom Gloves and Finger Cots	1994
IES-RP-CC-006.2	Testing Cleanrooms	1993
IES-RP-CC-007.1	Testing ULPA Filters	1992
IES-RP-CC-008-84	Recommended Practice for Gas-Phase Adsorber Cells	1984
IES-CC-009.2	Compendium of Standards, Practices, Methods, and Similar Documents Relating to Contamination Control	1993
IES-RP-CC-011-85-T	A Glossary of Terms and Definitions Related to Contamination Control	1985
IES-RP-CC-012.1	Considerations in Cleanroom Design	1993
IES-RP-CC-016.1	Recommended Practice for the Rate of Deposition of Nonvolatile Residue in Cleanrooms	1992
IES-RP-CC-018.2	Recommended Practice for Cleanroom Housekeeping-Operating and Monitoring Procedures	1992
IES-RP-CC-020-88-T	Recommended Practice for Substrates and Forms for Documentation in Cleanrooms	1988

Section 6.0

Design Specifications

General Considerations

The purpose of a trace metal cleanroom is to protect samples and materials from airborne contamination during sample preparation and analysis. The design of the cleanroom must obviously be compatible with this purpose, but it must also be compatible with the specific operations performed in the laboratory, the safety needs of the personnel, the chemical nature of the reagents and samples, and the economic constraints of the user. This chapter discusses the design specifications inherent in those needs and the options available to meet them. Several cleanroom designs that are currently used are presented along with the options and compromises required in each case.

Initially, the designer must determine the intended uses of the trace element laboratory including the specific elements to be determined, operations to be performed, reagents to be used, and waste handling requirements. Design options are significantly different for laboratories that analyze vapor phase metals such as mercury than for those that analyze metals that are subject to contamination primarily from air particulates, such as lead and zinc. Both types require high purity air that is free of the analyte of interest, but analysis of vapor phase metals does not necessarily require the particulate cleanliness level that analysis of other metals does. Instead, it may require the use of vapor sorbent traps or other forms of gas scrubbers. Similarly, laboratories that specialize in the analysis of water samples or perform sample digestions only in sealed ampules will not have the same restrictions on construction materials, safety requirements, or waste disposal that most trace element laboratories have due to the use of high quantities of hot, corrosive acids and oxidizing agents. The primary mandates for design of a trace element laboratory that uses hot acids are to eliminate the use of metal in construction materials to the extent possible, and to exhaust all corrosive vapors to the outdoors. Thus, if the intended use of the laboratory is analysis of mercury, then a relatively simple room design and traditional cleanroom construction materials can be used, perhaps in combination with vapor sorbent traps. However, if the intended use of the laboratory is analysis of other metals, then the room design must include laminar flow exhausting hoods and must eliminate, or at least minimize, metallic construction materials.

Amplification and re-emphasis are provided here regarding the need for non-metallic construction materials in trace element laboratories. These labs use large quantities of hot mineral acids and oxidizing agents for sample dissolution, generate corrosive vapors that need to be vented, and generate toxic vapors that need to be contained or vented. The acids and oxidizers (primarily HNO_3, H_2SO_4, aqua regia, H_2O_2, NaOCl, and alkaline fluxes) wreak havoc with most construction materials, such as steel, aluminum, cement, and paint. In fact, the method of choice for dissolving most of those materials is to subject them to hot mineral acids and oxidizing agents. The rust, corrosion, and dust level observed in most traditional trace element laboratories are testament to the effectiveness of this process and to the need to use different construction materials in a laboratory where particle counts are to be minimized. It is held as axiomatic that any metals present in a trace element laboratory will eventually be corroded by the acids and oxidizers, and that any corrosion-particle sources present in the laboratory or air system will eventually contaminate samples. Thus, use of non-metallic construction materials is essential for long-term viability of the trace element cleanroom.

The most acid-resistant materials are plastics, silicates, and precious metals, and of these, plastics are the obvious choice for an affordable construction material. For that reason, plastics are used wherever possible in trace element cleanrooms and great pains are taken to totally exclude metallic components. However, because of limited demand, plastic construction components are generally more expensive than their metallic counterparts, and the cost of building a plastic cleanroom laboratory can become extremely high. This fact complicates the design choices for trace element cleanrooms and has prompted most designers to opt for some forms of compromise between ideal cleanroom conditions, affordable materials, and designs and materials that can be maintained for an extended period of time without corrosion or contamination.

After the designer has established the intended analytical uses of the cleanroom and compatible construction materials, the designer must then determine the level of cleanliness required and the engineering and construction requirements necessary to meet that cleanliness class. This includes the number, type, and arrangement of filters and air returns, the air flow rate, the volume and rate capacity of the air handling system, the heating and cooling requirements, etc. The level of cleanliness typically sought for trace element laboratories is Class 100, which means that there are no more than 100 particles of 0.5 μm diameter or larger per cubic foot of air. This level of air cleanliness is arbitrary because the true relationship between airborne particle concentration and airborne metal concentration has not been established, and indeed, will certainly vary from location to location. Nevertheless, Class 100 has become the unofficial standard for trace metal cleanrooms. This view is likely to persist due to the fact that in well maintained Class 100 laboratories where analytical blanks are carefully tracked and evaluated, the cause of high blanks is nearly universally attributable to contaminated reagents, insufficiently clean labware, or analyst error, and not attributable to air contamination.

The next section of this chapter presents cleanroom design options suitable for Class 100 cleanrooms or clean zones that are compatible with the analytical requirements for ultratrace element analysis.

Cleanroom Laboratory Designs

There are five categories of Class 100 cleanroom laboratory designs that can be used for trace metal analysis. From the simplest and least expensive to the most complex and expensive, they are: clean air cabinets or benches, transportable enclosures, class 100 nonmetallic fume hoods, class 100 non-metallic laboratories, and cleanroom suites. The choice of laboratory design is dictated by several technical factors, including the metals being analyzed, the types of laboratory operations required, and the instrumental analysis methods used. Nontechnical factors include cost and size of the operation (number of samples, number of analysts, etc).

Clean Air Cabinets

The simplest, least expensive, and most limited purpose category of clean air enclosure is a clean air cabinet. Clean air cabinets typically consist of bench top boxes that contain a prefilter, HEPA filter, and blower motor. They have sufficient space to perform limited operations such as cleaning or preparation of labware or sample containers, or some sample preparation procedures. If constructed of non-metallic materials, they can be suitable for use with any metal. Commercially available units are non-exhausting, hence they are not suitable for operations that generate toxic or hazardous vapors, such as acid digestion, solvent extraction, or generation of Hg(0) or AsH_3 vapors. For reasons of size, economy, and historical use, most commercially available cabinets provide horizontal laminar flow of clean air. As discussed previously, horizontal laminar flow is less desirable than vertical laminar flow for trace metal analysis because of sample and material cross-contamination.

An alternate form of clean air cabinet is a glove box which is designed to flush high purity gases through the enclosure and out through a vented exit. In some situations, glove boxes are an economical approach to contamination prevention. If the goal of the glove box is to prevent samples from becoming contaminated with dirty air, and if limited preparation of samples is required for analysis, then placing samples in a glove box is much more economical than treating and recirculating a room full of air. However, the glove box approach to sample handling has very limited application in most laboratories because of several factors:

1. Gas flow is typically horizontal and rarely, if ever laminar. Thus chances for cross-contamination of open samples or reagents are very high.

2. Additional apparatus is required to verify and maintain the purity of the gas with respect to the analyte. This can be expensive and time-consuming, which defeats the original purpose of the glove box approach.

3. Because access to samples and equipment inside the glove box is slower and more difficult than in other laboratory settings, the types of operations that can be performed need to be considered carefully to avoid unsafe operating conditions. Thus many operations that might be performed in a fume hood, for example, would pose a safety hazard if performed in a glove box.

Clean air cabinets can be used either as stand-alone enclosures within an ordinary trace element laboratory or as a clean zone within an enclosed cleanroom. A caution is included here regarding use of clean air cabinets as stand-alone enclosures. While some models have sufficient motor size to maintain laminar air flow, others are marginally sufficient and the air flow lines degrade into turbulent flow when the front sash is opened to allow the analyst to work in the enclosure. Use of a clean air cabinet in an otherwise unclean laboratory should be carefully evaluated before the cabinet is commissioned with samples.

Figure 6 illustrates the use of clean air cabinets within an enclosed cleanroom. A small cleanroom was built within an existing laboratory for the purpose of Ultratrace mercury analysis. The cleanroom contains two commercial horizontal laminar flow benches where all high cleanliness tasks are performed, and a workbench opposite them where other tasks with lesser requirements are performed. Air intake for the HEPA filters is at the top of the laminar flow benches. A sorbent trap for mercury vapors is located between the prefilter and HEPA filter; it consists of gold-coated activated carbon held stationary between layers of air conditioning felt. Room air is recirculated from within the room rather than through

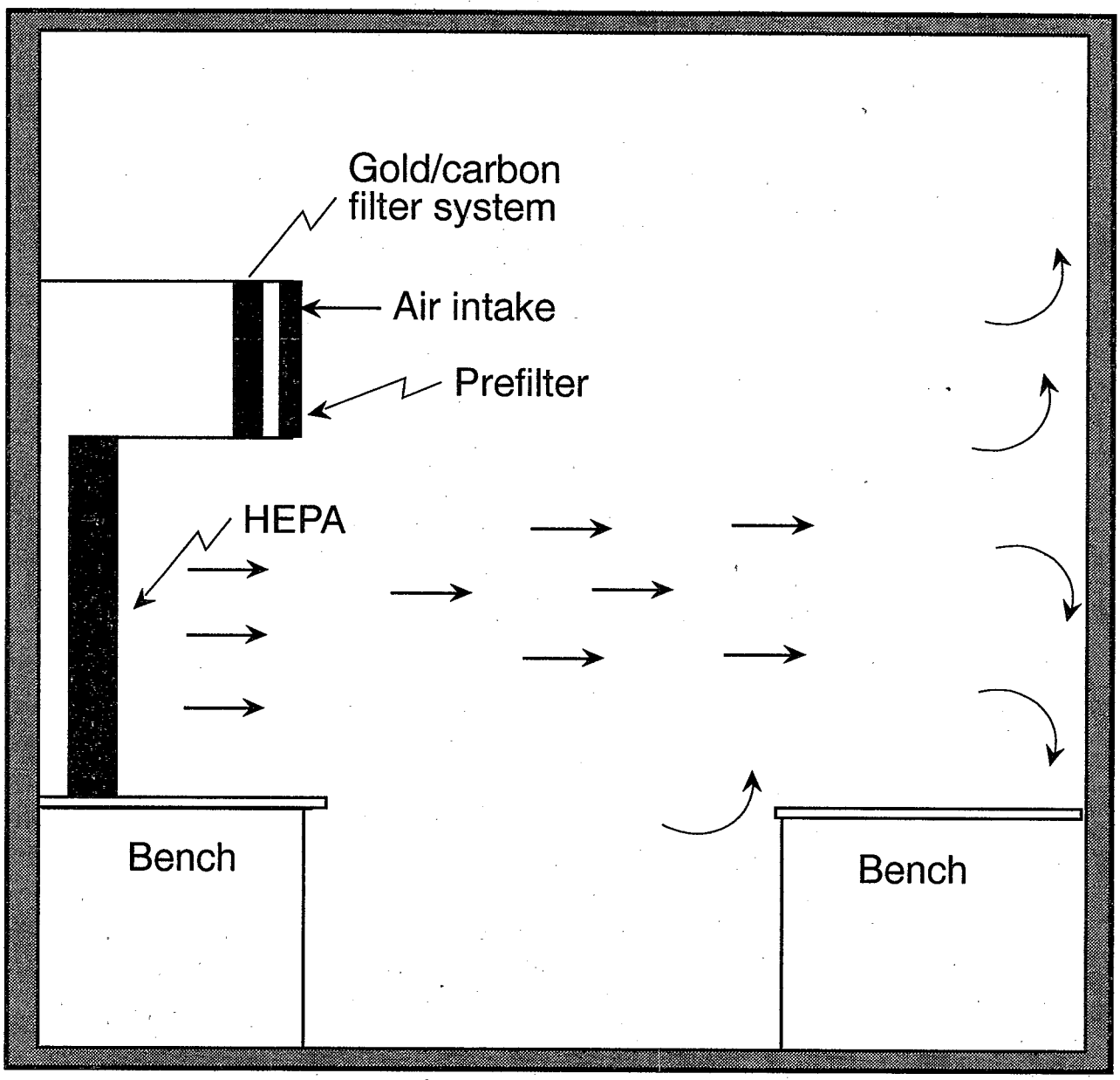


Figure 6. Horizontal laminar flow clean air cabinets within an enclosed laboratory.

plenum spaces, and samples and sensitive operations are protected from the turbulent flow by keeping them inside the laminar flow benches. The advantages of this design are that it is an inexpensive laboratory to build and maintain, and that it is very convenient to use. The disadvantages of the design are in the small size, limited region of laminar flow air, and potential cross-contamination inherent to horizontal flow designs. It should be pointed out that although this design would not achieve the same cleanliness class rating as a totally vertical laminar flow design and could not be used for open vessel acid digestion procedures, the horizontal design was adequate for the ultra low mercury analysis.

Transportable Enclosures

Larger clean air enclosures can be purchased that consist of two or more HEPA filters arranged in a rigid, overhead, aluminum grid which is supported on four aluminum legs. This design provides a vertical laminar flow column of clean air which is enclosed either by rigid walls (typically Plexiglass® or polypropylene) or by soft walls (typically thick sheets of clear polymeric materials). Prefilters and blower motors are compactly attached to the filter housing. The entire enclosure is transportable and can be used in different laboratories as needed or even shipboard on research vessels. The design is especially well suited to some types of analytical instrumentation. If the entire instrument, or at least the sample introduction region of the instrument, can be enclosed in a soft-walled, clean air environment, then sample contamination can be prevented at a critical point of analysis.

There are two significant limitations to this design: (1) it is non-exhausting, and so only operations that do not generate toxic or hazardous vapors can be performed; and (2) all commercially available units use aluminum or other metals for structural support and filter and motor housing, and so are not suitable for analysis of various metals. Theoretically, they could be constructed of polypropylene and thus eliminate the second limitation, but no polypropylene units are commercially available yet.

Class 100 Non-Metallic Fume Hoods

For laboratory operations that require use of acids or solvents, exhausting fume hoods are necessary to protect laboratory personnel and prevent corrosion within the laboratory. Class 100 HEPA-filtered hoods are commercially available in vertical laminar flow designs, and in non-metallic construction materials. Typically either polypropylene or fiberglass is used, with optional containment wells of heat-resistant or solvent-tolerant polymeric materials. These hoods are relatively expensive ($6,000 to $25,000 typically, depending on size and options), are not transportable, and require training for proper use and maintenance, but are suitable for all types of sample and labware preparative activities. Commercial models are available from several manufacturers in lengths of 4 to 8 feet. These units can be used as stand-alone clean zones or included as components of a larger laboratory design.

A typical hood design is shown in Figure 7 illustrating air intake through prefilters and HEPA filters, vertical laminar flow through the work surface, and venting of fumes. Room air intake is minimized by maintaining the sash at a height of 4 to 6 inches above the work surface and ducting room air through a perforated grill that extends to the front of the hood. In this way, samples and materials inside the hood are exposed to clean, HEPA filtered air only.

Class 100 Non-Metallic Laboratories

Currently available options for design and construction materials for Class 100 nonmetallic laboratories are presented below along with the compromises that each choice implies.

One of the first choices for the designer of a trace element cleanroom laboratory is whether to use a totally vertical laminar flow design or a design that includes regions of vertical laminar flow and regions of horizontal or turbulent flow. In most cases, the totally vertical laminar flow design will provide a better cleanliness rating, but it is substantially more expensive and carries a long-term risk associated with maintenance of the floor material. As discussed previously, a floor grid is used in combination with a raised floor to enable return air to pass under the floor grid. However, structural supports for the floor grid are necessarily composed of metal (typically, steel), and although these can be epoxy painted to make them resistant to acid vapors, they are not resistant to spills of acids or oxidants. Thus a major risk with this design is that the floor supports will become sites of corrosion and particle generation.

In the case of the cleanroom with only regions of vertical laminar flow, all exposed surfaces within the room can be non-metallic. However, there will be regions of turbulent flow, and hence particle resuspension, within the room. The vertical laminar flow regions are the cleanest regions, and so the most sensitive operations are performed in them. The turbulent regions must be carefully considered and designated a less critical function. An example of such a design is shown in Figure 8, and is the design currently used at NIST for trace element sample preparation. The laboratory is a hybrid design that combines the effectiveness of a vertical laminar flow design and the economy of a horizontal laminar flow design. Modular HEPA filters and blower units are located in or just below the ceiling and clean air is passed downward in a vertical laminar flow over a workbench area used for tasks that require the highest cleanliness level. The air then passes across an aisle and a second workbench where tasks of lesser cleanliness requirements are performed. The air is returned through a plenum wall at foot, hand, and ceiling levels and recirculated through the HEPA filters. Clear Plexiglass screens are hung from the HEPA frame to provide isolation of the clean, filtered air from that of the turbulent, less clean air

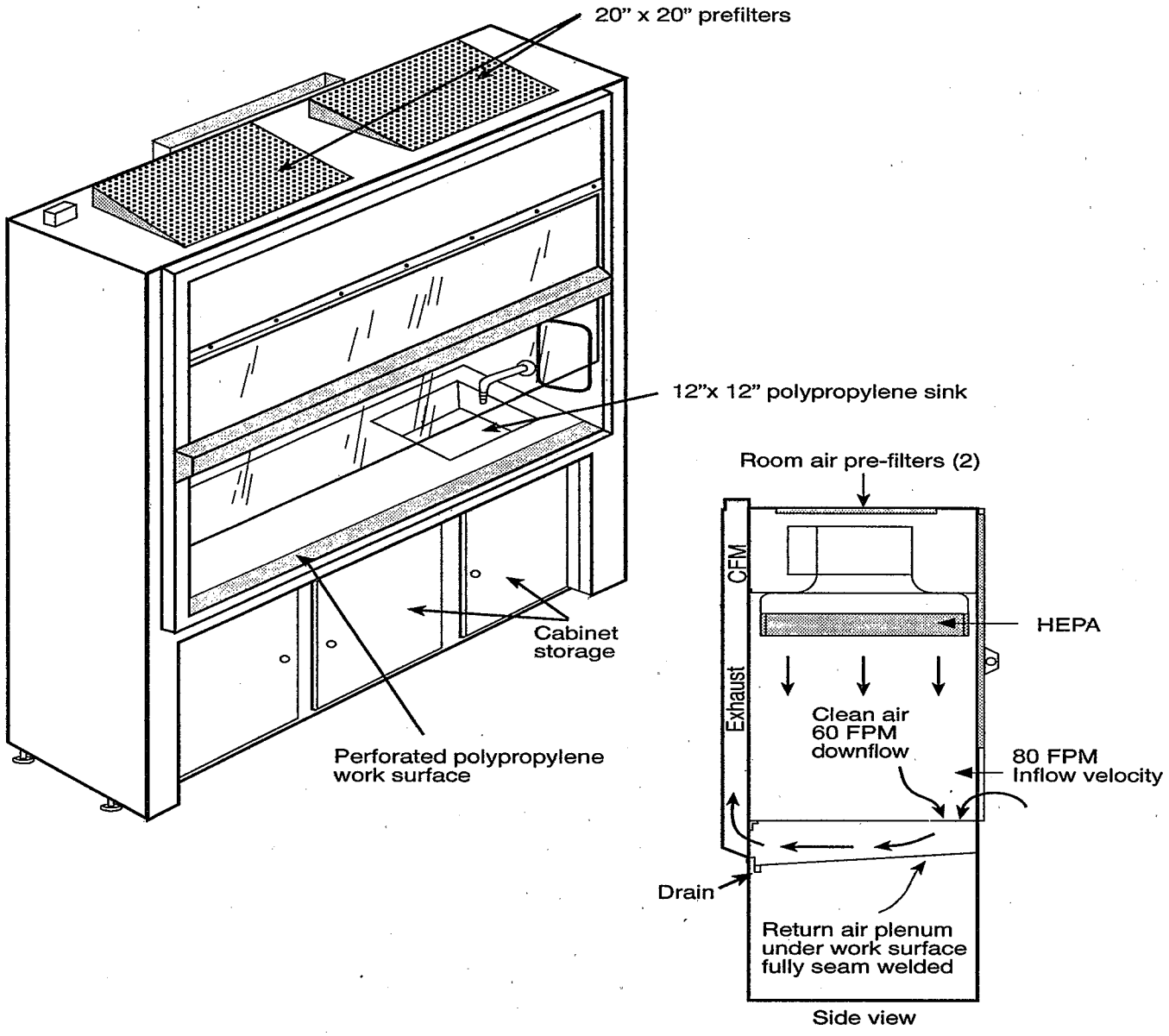


Figure 7. Vertical laminar flow fume hood.

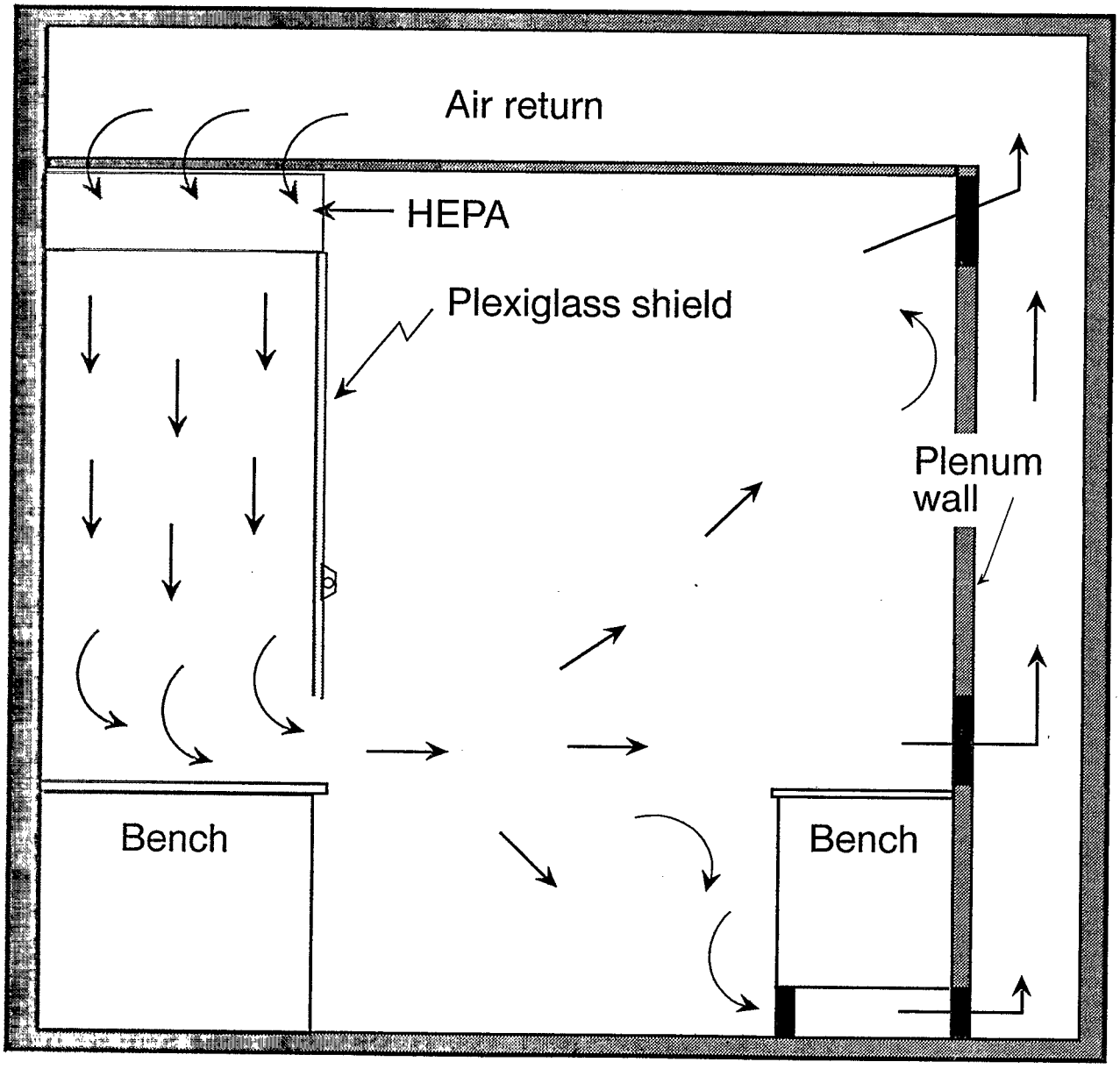


Figure 8. Mixed vertical laminar flow and turbulent flow cleanroom.

in the aisle and elsewhere in the room. The screens also protect samples from particles shed by personnel. Only a minimum of open space is left between the bench top and the Plexiglass shield for the chemists' hands to manipulate the samples. In this way, the samples are maintained in a clean air stream and shielded from contamination. The advantage of this design is that a large cost savings is realized in using only zones of HEPA filtered air rather than an entire room. The disadvantage is that only one workbench is maintained as a clean zone. The other is suitable only for tasks with less stringent cleanliness requirements such as notebook recording, logging unopened samples and containers, performing "rough cleaning" procedures, etc.

Fume hoods are located along the less clean wall and may be HEPA-filtered or non-filtered designs.

Cleanroom Suites

For laboratories that analyze a variety of metals at ultra-trace concentrations, the best choice of design is undoubtedly a cleanroom suite. The suite consists of several rooms including one or more of each of the following:

1. A non-metallic Class 100 or lower cleanroom laboratory where sample preparations and digestions are performed;
2. A Class 100 anteroom for gowning and isolation;
3. A Class 10,000 or lower instrument room where instrumental analysis is performed;
4. A service room for supplies, gas cylinders, refrigerators and freezers, etc.; and
5. Office areas adjacent to the laboratories.

An example design of a cleanroom suite is shown schematically in Figure 9. The five areas listed above are shown with their relationship to each other. The Class 100 cleanroom is the inner-most room of the suite so that it is the most isolated from the air and activities elsewhere in the building. The design of the Class 100 cleanroom was shown in Figure 5. In order to enter the Class 100 cleanroom, the chemist must first enter the service room, the Class 10,000 Instrument Room, the anteroom, and finally the Class 100 cleanroom. Entrance to each room requires additional layers of protective garb, and is explained in more detail below. Three emergency exit doors are shown; they are not used for access into or out of the suite except in the case of an emergency.

The philosophy underlying the design of the cleanroom suite is that the operations required for sample receipt, preparation, storage, instrumental analysis, and data analysis can be separated and performed in designated areas. In that way, operations that require high purity air and a metal-free environment can be performed in the Class 100 region, those that will generate significant particulate matter can be performed in the Service Room or Office, etc. Similarly, personnel and chemicals associated with those tasks are permitted to enter only the rooms associated with their tasks.

Class 100 Cleanroom Laboratory

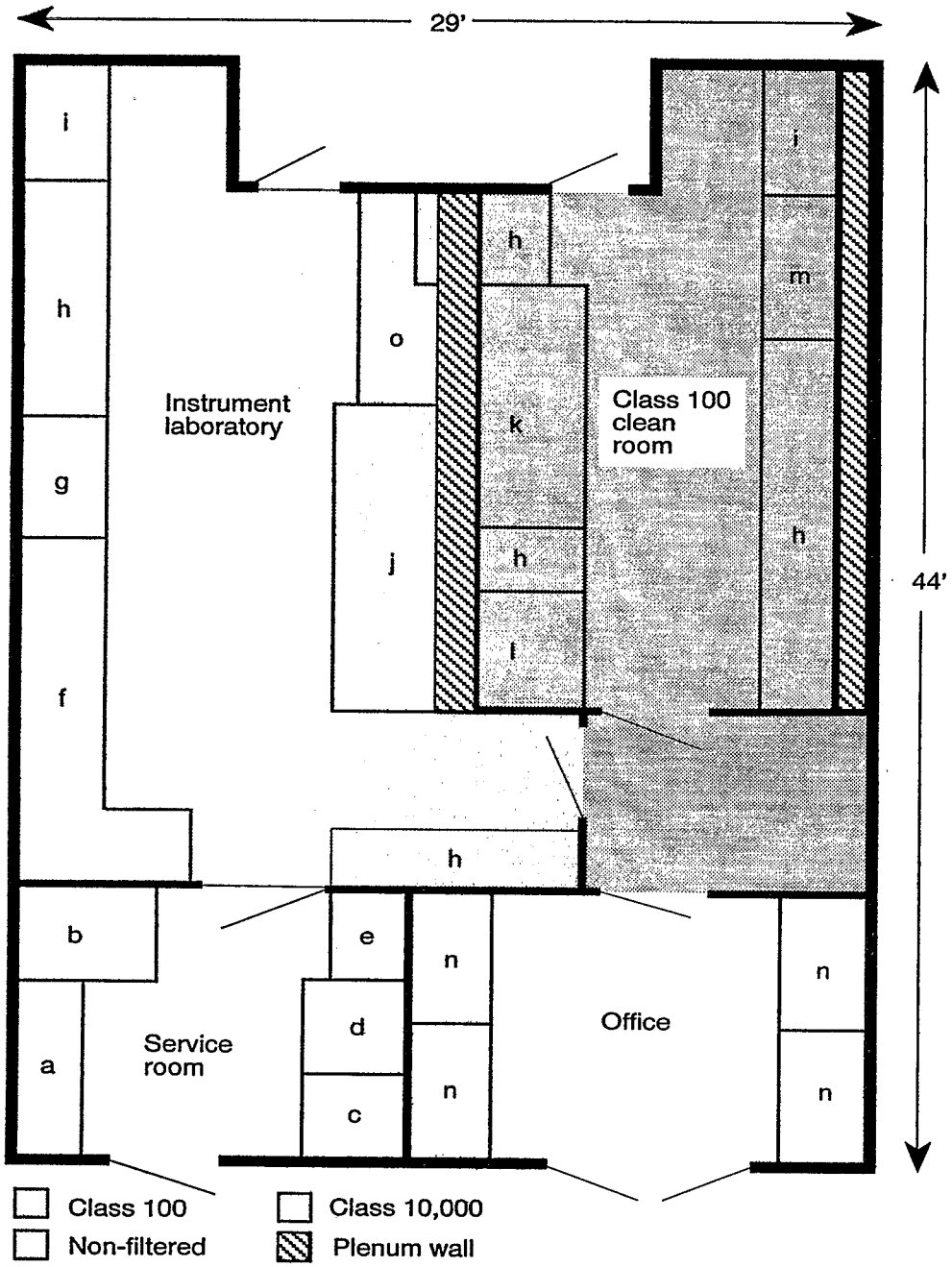
The Class 100 laboratory is used for sample and labware preparation and was designed to tolerate large quantities of acids and oxidants. Ultra-clean air is supplied through ULPA filters located in the ceiling grid; over 90% of the ceiling is covered with 99.99975% ULPA filters. High purity air is forced downward in a laminar flow and bathes work benches with particle-free air. Air is then returned to a recirculation unit at two heights along a plenum wall: the primary air return is a baseboard return extending from the floor up to a height of 18 inches; the secondary return is at hand level extending from the benchtop surface to a height of six inches above the benchtop. This air is recirculated through the ceiling ULPA filters to achieve continuous removal of particles.

Metal-free construction materials were used to the extent possible to build the Class 100 laboratory. The walls, floors, benches, fume hoods, acid bath cabinet, drawers, and sinks are made of polypropylene, and all plumbing materials are either polypropylene or PVC. All other service lines (electrical, gas, etc.) are encased in PVC tubing.

The Class 100 laboratory contains several features in addition to the clean air supply that facilitate ultra-trace level metal analysis. The laboratory is equipped with three areas of one-pass, air exhaust: an 8-foot fume hood with a vented base cabinet; a 4-foot fume hood with vented base cabinet; and a 6-foot vented acid bath cabinet. The fume hoods are used for acid digestion, evaporation, or extraction of samples and prevent exposure of personnel or equipment to acid vapors. The acid bath cabinet houses multiple plastic tubs for acid leaching of glassware and plasticware. The interior of the cabinet is flushed horizontally with Class 100 air and vented to the outside of the building in order to minimize exposure of personnel to acid vapors even when the baths are open.

Class 100 Anteroom

The Class 100 anteroom is a small room located between the Class 100 laboratory and the Class 10,000 instrument room. The purpose of the anteroom is to provide a space for gowning in cleanroom garb and enveloping personnel, samples, and materials in a clean atmosphere prior to admittance to the Class 100 laboratory. The anteroom is maintained as a Class 100 cleanroom by a ceiling ULPA filter, but at an air pressure intermediate between that of the Class 100 laboratory (highest pressure) and the Class 10,000 instrument room. In that way, air moves from the Class 100 laboratory to the anteroom to the instrument room, and thus particles and contaminants always move from a more clean region to a less clean one and contamination of clean regions is prevented. Some cleanroom suites use an air shower as a passageway into the Class 100 room.



a. Gas Cylinders
b. D. I. Water
c. Refrigerator
d. Freezer
e. Clean Room Supplies
f. PE 5100 ZL
g. 4' HEPA Hood
h. Bench
i. Sink
j. ICP
k. 8' HEPA Hood
l. Non-HEPA 4' Exhausting Hood
m. 6' Acid Baths
n. Desk
o. Questron V6 AFS

Figure 9. ACS Inorganics Class 100/10,000 Clean Lab Facility.

Their use, however, is controversial, as some experts believe that the air shower scours additional particles off of personnel and materials and suspends them, causing higher airborne particle concentrations.

Class 10,000 Instrument Room

The instrument room is a class 10,000 area, with partially ULPA-filtered air supply. The filters are located in areas of sample handling and sample introduction to instruments. The design philosophy of this room has three principal tenets:

1. Prevent the corrosion of metal-based instruments and construction components by minimizing the presence of acid in the room. A low concentration of acid is typically required in samples and standards to enhance analyte stability prior to analysis, but the acid concentration should be kept low and confined to closed bottles or flasks prior to analysis.
2. Locate HEPA/ULPA filters where clean air is most needed in this room. Complete ceiling coverage is not required in the instrument room, but filters should be located in the ceiling above autosamplers, locations where samples are opened, and other sensitive areas.
3. Include one small HEPA-filtered, polypropylene, exhausting fume hood in the instrument room so that simple manipulations can be performed on samples and standards in that room. In that way, the analyst can quickly and efficiently prepare dilutions or spikes, for example, without being required to take the samples to the anteroom, dress in cleanroom garb, perform the manipulations in the Class 100 Lab, remove the cleanroom garb, and return to the instrument room. No digestions or hot acids are permitted in the instrument room fume hood, but room temperature dilute acids are permitted.

Minimal particulate control measures are used: authorized personnel only are permitted to enter, cleanroom booties are required, and a tacky mat is located near the entrance.

Service Room

A service room is an essential part of the cleanroom suite because there are many items necessary for operation of the laboratories that cannot be made clean enough to bring them directly into the laboratories. For example, many analytical instruments require cylinders of compressed gas for their operation. These cylinders are dirty, many have corrosion products or peeling paint on their surfaces, and cannot be allowed to enter the Class 100 or 10,000 laboratories. Thus a service room in close proximity to the laboratories is required to house the gas cylinders. Gas plumbing lines should be brought through the walls to all necessary locations with small, final stage regulator valves located near the instruments.

Similarly, water deionizing equipment, air driers, refrigerators, freezers, cleanroom supplies, HVAC controls, and other miscellaneous items necessary for proper function of the laboratories or instruments should be located in the service room.

Construction Materials

There are two fundamental properties that construction materials must have in any cleanroom: they must provide the structural support necessary, and they must not contribute particles to the environment. In addition, materials used in a trace element cleanroom must be compatible with the chemicals, operations, and analytes used in the laboratory. That is, they must not degrade, corrode, or alter their surface properties as a result of contact with chemicals (acids, oxidants, alkaline fluxes, solvents) used in the laboratory or as a result of the operations (digestions, extractions, refluxing) performed there, and they must not contribute analyte(s) to the environment by outgassing or extraction. This list of requirements is highly restrictive and in practice necessitates the use of plastics whenever possible. However, even plastics should not be used indiscriminately. Polyurethane may contain high concentrations of mercury, fiberglass may contain significant cobalt, and PVC may contain lead and other metals. Many plastics are not compatible with all mineral acids. Thus the choice of plastic must be made with an understanding of the limitations of the material not only for strength, but also for durability and contamination of the cleanroom environment. A discussion of materials available for each section of a trace element cleanroom is presented in the following sections.

Ceilings

In a Class 100 cleanroom using vertical laminar flow, the HEPA/ULPA filters are located in a ceiling grid. The filters are the most important component of the ceiling, and so other components of the ceiling should be selected after determining the availability and limitations of the filters. While the most commonly used HEPA and ULPA filters are not suitable for a trace element laboratory, other designs of the filters are available and are suitable. For example, the pleated filter material is typically supported with aluminum separators between the folded pleats, and the filters are framed in an aluminum support. For a trace element cleanroom, the use of exposed aluminum is not desirable because it's surface can be readily oxidized and generate corrosion particles. However, HEPA and ULPA filters can be purchased without aluminum if the purchaser specifies separatorless filters housed in wooden supports. As an extra precaution against particles shed from wood, the supports should be painted with a chemical resistant epoxy paint. A list of chemical spot test results for an epoxy paint from one manufacturer is presented in Table 6.

Commercially available ceiling grids used to support the HEPA/ULPA filters consist of a T-grid system typically made of aluminum. A plastic T-grid system is available, but at a very high price. One option used in Class 100

Table 6. Chemical Resistance of Glid-Guard (Glidden Xo.) Epoxy Paint[a]

Chemical	Concentration (%)	Result of 48 Hour Spot Contact
Hydrochloric acid	5	No change
Sulfuric acid	5 and 50	No change
Phosphoric acid	10	No change
Nitric acid	5	No change
Hydrogen peroxide	30	No change
Acetic acid	5 and 50	No change
Oleic acid	NL[b]	No change
Lactic acid	NL	Slight softening after 48 hours
Sodium hypochlorite	6	Discoloration after 24-48 hours
Chromic acid	20	Discoloration after 24-48 hours
Ammonium hydroxide	30	No change
Sodium hydroxide	5 and 50	No change
Xylene	neat	No change
Ethyl alcohol	NL	No change
Trichloroethylene	neat	No change
Methyl ethyl ketone	neat	No change
Gasoline	neat	No change
Sodium chloride	5 and 50	No change
Copper chloride	20	No change
Mineral oil	neat	No change
Formaldehyde	37	No change
Phenol	saturated	Slight discoloration and softening

[a] Information from The Glidden Company, GLID-GUARD product information sheet.
[b] NL = not listed by paint manufacturer.

trace element cleanrooms is to paint the aluminum with an epoxy paint that is acid resistant. The epoxy paints typically perform well in the presence of acid vapors, but are not able to withstand contact with concentrated (liquid) acids. Thus, for the purpose of coating a ceiling grid, epoxy paints are a suitable option. Great care must be taken however to insure that all surfaces are covered. If there are holes in the coverage, the acid vapors can readily attack the aluminum and start to corrode the ceiling grid. In a Class 10000 instrument room, the aluminum grid can be used without epoxy painting, but it is prudent to paint the aluminum ceiling grid.

Two notes of caution are included here regarding paints. First, the paints recommended for use in a cleanroom are epoxy paints rather than latex paints because epoxy provides a better sealant that prevents emission of particles from the underlying material into the air. Secondly, while none of the epoxy paints tested during construction of the RTI cleanroom contained significant mercury or lead, and paint manufacturers said that those elements have not been used historically in epoxy paints, it is prudent to analyze all paints before application in a cleanroom. Older latex paints may contain both mercury and lead and represent a continual source of elemental contamination. Cleanrooms located in previously-built laboratories may require paint removal before cleanroom construction can proceed.

Cleanroom lighting fixtures are available that are compatible with the air streamlines in a vertical laminar flow cleanroom and with the requirements of no exposed metal surfaces. Teardrop shaped lights that attach to the T-grid system are available with plastic exteriors and no exposed metal. In a mixed vertical laminar flow and turbulent flow cleanroom such as the one used at NIST, traditional light fixtures are located above the ceiling, and plastic light diffusers are used flush with the ceiling. No metal is exposed in the path of the air.

Walls and Supports

Wood, steel, and cement structures are needed in any facility to provide strength and structural support, and laboratory walls are typically made of gypsum, wood board, or cinder blocks. Unfortunately, none of these materials is suitable for an exposed surface in a trace element cleanroom. Most shed particles even when newly installed, and all shed particles after extended interaction with acid vapors. Therefore, in order to use these construction materials in a trace element cleanroom, even inside a plenum wall, they must be covered in such a way as to prevent generation and transport of particles. The best solution is to cover all walls and supports with a chemical-resistant plastic, such as some polypropylene or PVC products. The chemical resistance for one such product, MIPOLAM (Huls America Inc.), is summarized in Table 7. The nature of these plastics requires that all seams be welded to provide complete coverage of the underlying materials. This is an expensive option in the short term, but will provide long term protection against acid-generated particles.

A less expensive, but less permanent, option is to epoxy paint all surfaces to a "no pinholes evident" finish. That is, the paint should be applied in enough coats that a complete barrier is provided and no gaps, or pinholes, are evident in the epoxy surface. This surface is susceptible to chipping and chemical dissolution, and is thus not likely to provide the length of service that a plastic laminate will. Some form of acid-resistant splash or spill guard will always be necessary with this option. A painted surface inside a cleanroom will require continual monitoring, "touch up" painting, and eventually may require complete resurfacing.

This option is presented because of the use of seam-welded plastic laminate is so expensive. However, it can only be recommended if the cleanroom manager is extremely diligent and the labor for painting (and re-painting) is inexpensive.

Floors

The choice for flooring materials depends on the design of the cleanroom and the operations performed in it. For a totally vertical laminar flow Class 100 or lower cleanroom, a plastic or fiberglass grid is used for the raised flooring. In general, plastic is preferred over fiberglass because fiberglass abrades more easily and generates particles after a period of time. The flooring in the

Table 7. Typical Chemical Resistance for Mipolam (HULS America Inc.) Floor Covering[a]

Chemical	Concentration (%)	Temperature °F	Resistance[b]
Acetic acid, aqueous	6	70	resistant
Ammonia, aqueous	saturated	100	resistant
Boric acid, aqueous	any	140	resistant
Carbon dioxide	any	100	resistant
Caustic soda, aqueous	4	100	resistant
Chromic acid, aqueous	0.5...10	70	resistant
Copper sulfate, aqueous	any	140	resistant
Exhaust gas, carbon dioxide	any	140	resistant
Exhaust gas, hydrochloric acid	any	140	resistant
Ferric chloride, aqueous	any	140	resistant
Glucose, aqueous	saturated	70	resistant
Hydrochloric acid	any	140	resistant
Hydrogen bromide, aqueous	any	100	resistant
Hydrogen peroxide	up to 80	70	resistant
Nitric acid, aqueous	15	70	resistant
Oxygen	any	140	resistant
Phosphoric acid, aqueous	any	140	resistant
Photo fixing baths	common concentration	100	resistant
Potassium hydroxide solution	15	70	resistant
Potassium salts, aqueous	any	140	resistant
Sea water	normal	100	resistant
Silver nitrate	10	140	resistant
Sodium chloride	any	140	resistant
Sulfuric acid	up to 60	140	resistant
Urea, aqueous	any	140	resistant
Urine		70	resistant
Acetic acid	50	70	limited resistance
Acetylene	100	70	limited resistance
Butyric acid, aqueous	20	70	limited resistance
Diesel oils, pressure oil	100	100	limited resistance
Ethylene glycol	100	100	limited resistance
Glycol	100	100	limited resistance
Lead acetate, aqueous		70	limited resistance
Potassium hydroxide solution	concentrated	70	limited resistance
Acetone	any	70	unstable
Carbon disulfide	100	70	unstable
Ethyl acetate	100	70	unstable
Methylene chloride	100	70	unstable

[a] Information from the Huls America Inc., MIPOLAM product information sheet.
[b] Results after 1 month of storage and subsequent drying during not less than 18 days.

air return beneath the raised flooring should be a seam-welded, acid resistant plastic, such as MIPOLAM (Huls America Inc.). For other styles of Class 100 or lower cleanrooms, a seam-welded acid resistant plastic should also be used.

The flooring materials used in an instrument room, anteroom, or service room do not generally need to conform to the same chemical resistance requirements that those in the Class 100 laboratory do, because wet chemical operations are not performed in those areas. However, the floor must still be easily cleaned and not generate particles, and thus vinyl flooring is recommended.

Exhausting Fume Hoods and Acid Baths

Large quantities of acids are used in fume hoods for sample digestions, and in acid baths for leaching metals out of labware. Any metal in these areas would be rapidly corroded and contribute metal contamination and particles to the laboratory environment. Thus, only plastic, fiberglass, or glass are permissible in those areas. Both polypropylene and fiberglass fume hoods are commercially available with several different design options. The polypropylene hoods can be purchased in ULPA- or HEPA-filtered options that provide Class 10 or 100 vertical laminar flow environments, respectively. It is essential that air be continuously exhausted from fume hoods and acid baths so that the acid vapors do not enter the recirculation path where they could damage filters or pose a health threat to personnel.

A note of caution is presented regarding commercially available fume hoods. Some manufacturers use polypropylene only in parts of the hood that are visibly in the path of acid vapors, and thus the purchaser must be careful to specify that all surfaces, sash guides and runners, pulley weight enclosures, fan blades, exhaust ducts, etc. be composed of plastic or fiberglass. Any metal present in the path of hot acid fumes will eventually corrode and/or dissolve.

Storage Cabinets

Storage cabinets for labware and supplies are commercially available in polypropylene, fiberglass, and wood. In a Class 10,000 laboratory, wooden cabinets are suitable after they are painted to minimize particle generation. In a Class 100 or lower laboratory, polypropylene or fiberglass should be used. As is the case for floor grids, polypropylene is preferred over fiberglass because it has less potential to generate particles. Some cleanroom laboratories use epoxy-painted wooden cabinets and drawers, but these should be used with the greatest of caution and the understanding that they will need to be repainted frequently.

Utilities

Electrical and plumbing utilities present a special challenge to the designer of a trace element cleanroom because the customary construction materials include extensive use of metal components and solders. However, non-metallic substitutes are available for most materials. Some examples for plumbing and electrical materials are presented in the following sections.

Plumbing Materials

Table 8 presents both customary plumbing materials and substitute materials that are suitable for use in trace element cleanroom laboratories. All of the materials are commonly available and meet plumbing code requirements.

The use of copper, stainless steel, or other metal pipes for water or gas lines should be prohibited to the extent possible. For tap water and aspirators, PVC pipes are available in both ambient and high temperature varieties. Deionized water should be transported in polypropylene or teflon tubing if a recirculating system is used. For gas lines, it is possible to substitute PVC, teflon, or polypropylene for some inert gases; for other reactive gases, such as acetylene, it is necessary to use stainless steel tubing inside PVC conduit. The use of metallic solder can also be avoided with plastic tubing. Polypropylene joints can be heat welded and PVC joints are generally solvent welded. Safety equipment, such as emergency showers and eye wash stations, are available in plastic versions. Any metallic parts should be coated with epoxy paints.

Electrical

In the trace element laboratory, the corrosive effect of acids and their vapors on electrical components is a serious concern, but use of plastic components where possible reduces problems greatly. Electrical wiring can easily be made safe from acid vapors and spills by encasing it in PVC conduit. If one or both ends of the conduit is exposed to acid vapors, rubber grommets or other acid-resistant fittings can be used for isolation. Similarly, plastic junction boxes can be substituted for metal ones and plastic outlet covers can be used to protect duplexes and quadriplexes when not in use. A note of warning is included here regarding local electrical code requirements. The substitution of plastic for metal components may be a problem in some regions due to local electrical code requirements. For example, during the construction of the RTI cleanroom, it was learned that one style of plastic junction box that was permissible in North Carolina was not permissible in Minnesota. In some cases, substituting components of difference size from the original design was all that was necessary to meet local code requirements. Thus, it is necessary to consider local codes and make design changes where necessary.

Table 8. Plumbing Materials for Trace Element Cleanrooms

Application	Customary Material	Substitute Material for Trace Element Cleanroom
Cold tap water	copper pipe	PVC pipe
Hot tap water	copper pipe	high temperature PVC
Deionized water	high density polyethylene (HDPE) or polypropylene	polypropylene
Aspirators	copper pipe; brass, copper of stainless steel nozzle and handle	PVC pipe; plastic nozzle and handle
Safety shower and eye wash	assorted metals	assorted plastics; epoxy-coated metal pull chain
Plumbing	metallic solder	heat welded polypropylene; solvent welded PVC

Section 7.0

Cleanroom Use

Laboratory Operations

In any trace element laboratory, there are a great variety of operations that must be performed from receipt of samples and supplies, to preparation of labware and samples, instrumental analysis, data reduction, report preparation, cleanup, and archiving activities. Each operation has its own requirements for the level of cleanliness, chemical resistance, vapor exhaust, etc., and thus each operation should be performed in a designated room or zone to best meet those requirements. In a trace element cleanroom setting, the segregation of "dirty" from "clean" operations is even more important than in non-cleanroom laboratories because the analyte concentrations are significantly lower and hence the air cleanliness requirements are significantly greater in the cleanroom laboratories. Performance of "dirty" operations in a cleanroom environment would immediately contaminate any open samples or reagents in the region, and would eventually overwhelm the ability of the air filtration system to remove the particle load. Thus laboratory operations are strictly segregated in trace element cleanrooms and personnel need to be instructed in the theory and practice of maintaining the cleanroom environment.

In general, laboratory operations that involve acids should be performed in clean rooms or clean zones that are constructed of acid-resistant materials and that efficiently vent vapors. Laboratory operations that require analytical instrumentation should be performed in regions or rooms that have clean, purified air, but are removed from all acid vapors.

IMPORTANT - Operations that are inherently dirty, particulate-generating, or metal vapor-generating should be performed in a service room or hazardous materials laboratory separate from the cleanroom laboratories. Tables 9-12 list some of the commonly performed laboratory operations, a suggested location for the operation, and the facility considerations required. Examples of materials handling and operation segregation procedures are presented in Appendix A, Standard Operating Procedures for the RTI-ACS Inorganic Class 100/10,000 Clean Lab Facility.

Procedures are also required for transferring samples and supplies from one region or room to another. In general, decontamination procedures are required for transfer of materials from a less clean region to a more clean one. The procedures typically involve removing

Table 9. Operations Performed in Class 100 Laboratory

Operation	Location	Special Considerations
Acid digestions	Polypropylene, HEPA exhaust hood	No metal: corrosion from hot acid. Air: Once through. Special plumbing and air
Microwave digestions	Polypropylene exhaust hood	No metal in hood. Vent exhaust from microwave directly to hood exhaust. Air: Once through.
Glassware soak	Acid bath cabinet	No metal. Air: Once through; intake at hand level; vented at top back.
Weighing	Semi-micro balance on bench top	Vibration damping needed. Air: Laminar downflow; recirculating
Filtration	Bench top aspirators	No metal. Air: Laminar downflow; recirculating. Water: tap with splash guards
Centrifugation	Polypropylene exhaust hood	Air: Laminar downflow; once through.
Sample preparation	Bench top;	No metal
Glassware rinsing and drying	Sink	Air: Laminar downflow; recirculating. Access to deionized water.
Micro-ware rinsing and drying		All plumbing (in and out) through plastic pipes: No glass; No metal.
Ion exchange	Bench top	Air: Laminar downflow; recirculating
Solvent extraction	HEPA exhaust hood	Chemical resistant materials: solvent and acid tolerant; Air: Laminar downflow; exhausting.

external cartons or shipping materials in the service room, wiping all surfaces of bottles and containers with deionized water on cleanroom wipers under HEPA-filtered air in the Class 10,000 room or anteroom as appropriate, allowing sufficient time in the anteroom for the container to be well flushed with Class 100 air, and finally entry into the Class 100 cleanroom laboratory. These procedures are very important because particles and analytes are readily transported on shipping cartons, exteriors of containers and sample vials, paperwork, etc., and can contaminate materials in the cleanroom.

Table 10. Operations Performed in Class 10,000 Laboratory

Operation	Location	Special Considerations
Instrumental Analysis	GFAA; ICP; ASV	Air: HEPA over critical regions.
Sample Dilutions	HEPA exhaust hood	Air: Laminar downflow; once through.
Rough cleanup	Sink	Plastic plumbing

Table 11. Operations Performed in the Anteroom

Operation	Location	Special Considerations
Storage of cleanroom garb and special supplies	Perforated plastic shelves; Plastic clips	New garb is stored on perforated shelves inside cleanroom plastic wrapping until ready for use. Used frocks are stored upright, attached by plastic clips. Plastic shelves are perforated to allow air to flush vertically and prevent settling of particles.
Gowning cleanroom	Under ULPA filter	Reusable, low particulate, garb; Talc-free plastic gloves; Plastic trash can with lid for disposing of garb after use.
Final cleaning of supplies.	Under ULPA filter	Cleanroom wipers; deionized water.

Table 12. Operations Performed in Service Room

Operation	Location	Special Considerations
Gas cylinder storage		Metal gas lines encased in PVC conduit are routed through walls to specified locations in labs.
Supply storage		None
Water deionization		Polypropylene plumbing; Recirculating system; 18 MΩ
Sample storage	Refrigerator, freezer	None
HVAC controls		Continuous readout of air temp., pressure, humidity, alarm status for each room.

Although a list of possible routes or mechanisms of sample contamination is nearly infinite, a short list of the most common causes would include analyte-bearing particles emitted by personnel, clothing, and supplies, and contaminated labware and reagents. The procedures used by cleanroom personnel to minimize those sources of contamination are discussed included in Appendices A through D.

Personnel

Authorization for Entry

One of the most significant sources of particles and contamination in a cleanroom laboratory is personnel. Particles are transported on shoes, clothing, supplies, and hair; and as illustrated earlier, millions of particles are generated every minute by people performing ordinary movements. Thus, one of the simplest methods used to reduce airborne particle concentrations in the laboratory is to restrict entrance to the laboratory to only those personnel who need to work there. Cleanrooms are restricted access areas; appropriate signs should be posted outside the rooms so that unauthorized personnel do not enter.

Laboratory Behaviors and Training Programs

All personnel who work in a cleanroom need to be trained in the theoretical and practical aspects of cleanroom procedures in order to ensure a high level of cleanliness and minimization of contamination. Cleanroom training courses are commercially available both as formal instruction sessions and as video taped lectures. However, this form of training is designed for the microelectronics and pharmaceutical industries. While the general concepts are the same, specific training for trace element laboratory use is not presented. The types of behaviors that require instruction are listed below and discussed more fully in the following sections:

1. Movement inside the cleanroom facility. All personnel must understand the location of the clean air source, the direction of air flow, return air locations, and relative air cleanliness in all regions of the facility in order to properly use the facility. In addition, they need to be made aware of the relative number of particles generated and the direction of their movement as a result of walking, reaching, manipulating samples, etc. Special features of the laboratory, such as those designed to prevent work bench contamination or degradation of materials, need to be thoroughly understood so that they can be properly used.
2. Gowning procedures. The policy adopted for cleanroom garb, use of tacky mats, use of cosmetics, etc. needs to be understood and followed by all who enter the cleanroom areas.
3. Sample and reagent handling procedures. In vertical laminar flow regions, it is imperative that nothing be placed upstream of an open sample or reagent

because contamination will be swept directly into the container. This is a difficult requirement for chemists who have become accustomed to manipulating several samples on a hot plate or bench in a non-cleanroom setting. Their hands, arms, faces, pipettes, etc., can no longer pass over any container or apparatus, but must always approach/observe from the side where any particles shed will be carried away in a separate air flow stream.

4. Labware use and cleaning procedures. In general, labware used in the cleanroom for ultra-trace analysis has a designated function and is used solely for that purpose.

5. Housekeeping procedures. The type of cleaning activities, frequency of performance, and personnel responsible for cleanroom cleaning need to be clearly stated and understood in order to maintain the cleanliness level desired.

6. Facility maintenance procedures. Parameters that should be monitored to ensure that the air filtration, recirculation, and exhaust systems are functioning properly might include pressure drop across HEPA filters, relative static pressures in different rooms of the facility, air flow rates, particle counts, temperature, and humidity. In addition, analyte concentrations should be measured in air, particle traps, acid baths, deionized water, etc., to ensure that the facility has adequate cleanliness for ultra-trace analysis procedures. The types of measurements, frequency of performance, and personnel responsible need to be clearly stated and understood.

Cleanroom Garb and Gowning Procedures

Probably no topic in trace element cleanroom use is more controversial than that of garb requirements. While it is generally recognized that protection of samples from personnel generated particulates is required and that appropriate cleanroom garb can effectively minimize emission of particles from personnel, it is also recognized that excessively elaborate cleanroom gowning procedures can be counter-productive in terms of lost productivity, safety hazards in a chemical laboratory, and labor and supply monies associated with cleanroom gowning. The IES recommended practices for personnel garments recommend that personnel wear the following garments: a cleanroom frock, shoe covers, and hair cover in Class 10,000 rooms; cleanroom coverall, boots, hair cover, hood, and facial cover in Class 100 rooms; and cleanroom coverall, boots, hair cover, hood, facial cover, barrier gloves, and inner suit in Class 10 rooms. Of the operating trace element cleanrooms visited during the course of this project, none adheres completely to the IES recommendations, four require some level of cleanroom gowning, and three (mercury laboratories) do not require any cleanroom attire.

Each laboratory has its own set of needs, personnel abilities, facility design, and philosophy of achieving required cleanliness. Laboratories that analyze mercury only are not greatly concerned about airborne particulate contamination even though that has been shown to be a significant route of global mercury transport through the environment. They typically require either no special gowning or simply foot covers. Mercury-bearing particulates from clothing and personnel have not historically been a problem in these laboratories even though they are determining mercury at sub parts per trillion levels.

Laboratories that analyze other metals are more cautious about gowning prior to entering a cleanroom. All require foot cover at a minimum, and most also require cleanroom frocks, head covers, and barrier gloves. The general belief among trace element cleanroom managers, however, is that contamination from personnel who follow proper behavioral procedures in the cleanroom is minor relative to contamination from labware and reagents.

Labware Use and Cleaning Procedures

If a cleanroom facility is well designed and functioning properly, and if laboratory personnel wear protective cleanroom garments and follow proper behavioral procedures, then the major sources of contamination remaining are labware and reagents. Both of these sources can be effectively minimized, but frequent analysis of labware and reagent blanks is mandatory to ensure that contamination levels are not rising.

Routine Labware

The types of labware used in the Class 100 cleanroom include typical glass, quartz, teflon, polyethylene, polystyrene, and polypropylene containers and pipettes. With the exception of micropipettors, metal implements are rarely used in the trace element cleanroom. The first major difference between cleanroom labware and non-cleanroom labware is that cleanroom labware is divided into "low level" and "high level" labware. "Low level" labware is used only with solutions that have metal ions at concentrations below 1 ppm. In this way, the surface sites of the labware should never be exposed to high concentrations of analytes. "High level" labware is used for solutions that have analyte concentrations greater than 1 ppm, such as stock reagents, matrix modifiers, or concentrated metal standards used for preparing more dilute standards. Because glassware tends to exhibit "memory" effects (i.e., strong, slowly reversible adsorption) from previous solutions, it is very important to maintain the separation between the "low level" and "high level" glassware. Further segregation of labware is strongly advised for specific metals and ultra-low concentration ranges. For example, lead, chromium, and boron are highly sorbed by silicate surfaces of glass or quartz, and to a lesser extent by plastics. When possible, labware that is used for those metals should be dedicated to those metals only. Also, analytes that are present in the parts per trillion concentration range should have dedicated ultra-low level labware and acid soaking baths.

If the uptake of analyte by the labware is not permanent, the labware is subjected to rigorous cleaning procedures and stored in a clean manner appropriate to the analytical use. Labware cleaning procedures are included in Appendix B, "Standard Operating Procedures for Cleaning Labware in the ACS Inorganic Class 100/10,000 Clean Lab Facility." In brief, the procedures involve the following steps:

- separation of labware into "low level" and "high level" basins
- pre-treatment (removing labels, rinsing with tap water)
- detergent washing
- rinsing with tap water followed by deionized water
- acid leaching
- final rinse with deionized water
- drying in clean air stream
- storage

Teflon labware used for mercury analysis require an additional cleaning step to oxidize and bind any residual mercury. One cleaning recipe is to fill cleaned teflon containers with 1% HNO_3 plus a brominating solution ($KBr + KBrO_3$), store the containers until ready for use, discard the solution just prior to reuse, and rinse with low mercury deionized water (see Appendix E: Standard Operating Procedures for Mercury Analysis in Water, Sediment and Tissue). Another cleaning procedure involves boiling cleaned containers in concentrated HNO_3 for 24 hours in a large teflon vat, rinsing with low mercury water, filling with 1% HCl and warming for 24 hours, discarding the HCl and refilling with fresh 1% HCl for storage until use (see Appendix F: Standard Operating Procedure for Total Mercury in Aqueous Samples by Cold Vapor Atomic Fluorescence, and Appendix G: Total and "Acid-Labile" Mercury in Aqueous Media). Glassware used for mercury analysis can be heated in an oven prior to use to remove any residual reduced forms of mercury.

Microwave Vessels

Teflon microwave vessels are treated differently for some analytes. While teflon is generally an extremely inert material with minimal adsorptive or surface-active properties, it has been found to exhibit a memory effort for some metals, such as indium. In those cases, routine cleaning and acid leaching procedures are not adequate to quantitatively remove the metal. A high pressure, acid "steam cleaning" procedure is used for these vessels in which high purity acids are added to the pre-cleaned teflon vessels, and the vessels are carried through a microwave cycle to extract the teflon with acid at high temperatures and pressures. The extract is analyzed for the metal of interest and the vessels are reused only if the extract is sufficiently clean.

Deionized Water

A high quality deionization system is essential for performing ultra-trace analysis of metals. In general, the existing literature for water deionization for the microelectronics industry is appropriate for trace metal analysis as well. Both groups require 18 MΩ resistivity with ultra-low concentrations of metal ions. However, the metal ions of concern for the two groups are significantly different, and while vendors of water deionization systems can produce ICPMS analyses of metals of interest to semiconductor manufacturers, they are generally ill-prepared for the needs of the trace element chemistry laboratory. For example, ultra-low sodium and potassium ion concentrations are required for semiconductor manufacture, but are generally of little concern for ultra-trace level environmental analyses. However, sub-parts per billion levels of lead and arsenic are of great importance for environmental analysis, but relatively little for the semiconductor industry.

Water deionization systems are not generally optimized for ultra-trace mercury analysis and in several cases actually contribute mercury to the water. Deionization resins that are recycled using a caustic treatment are particularly susceptible to mercury contamination. Deionized water should be routinely analyzed for contamination of metals of interest. Water that is 18 MΩ resistivity is not necessarily sufficiently pure in any one metal.

Section 8.0

Cleanroom Maintenance

Initial Certification

Once the cleanroom has been completed and is ready for occupancy, testing should be performed to verify its cleanliness and performance. The Institute for Environmental Sciences has published guidelines for testing cleanrooms and made recommendations for the tests to be performed [IES-RP-CC-006-84-T, 1984]. The document includes tests for airflow velocity and uniformity, HEPA filter leaks, airflow parallelism, room recovery, airborne particle count, particle fallout count, room pressurization, noise, vibration, and other tests.

Monitored Parameters

Following initial certification of the cleanroom, a schedule should be established for continued monitoring of air quality parameters. In the RTI cleanroom, various parameters are monitored on a continuous, weekly, quarterly, or semi-annually basis as explained in Appendix C (Standard Operating Procedure for Monitoring and Maintaining Cleanliness of the ACS Inorganic Clean Lab Facility).

The parameters that are monitored most frequently are those that indicate failure of the air handling equipment or emergency situations (smoke, excess heat). Continuous display, real time monitors display static pressure, temperature, and relative humidity in each room of the cleanroom suite, and a computer interface to the display updates a record of these parameters every 12 hours. In a properly functioning cleanroom, the cleanest room should have the highest static pressure, the next most clean room should have the next highest pressure, and so on, so that air will travel from the more clean rooms to the less clean rooms. If pressures are inverted between rooms, the direction of air travel will be reversed and contaminated air will enter the cleaner rooms.

Parameters that are monitored on a daily basis include deionized water resistivity, HEPA filter pressure drop, and refrigerator and freezer temperatures. Less frequently monitored parameters include particle counts (every three to six months), air flow rates (annually), acid bath concentrations of analytes of interest (semi-annually or as needed), and "room blank" concentrations of analytes. The "room blanks" are non-volatile particles that settle in open teflon beakers placed in specific regions of the laboratory. Control teflon beakers with lids in place accompany the "room blank" beakers to enable subtraction of the teflon contribution to determined background analyte concentrations.

Maintenance Activities

Housekeeping and maintenance activities in the cleanroom include wiping work surfaces, mopping floors, replacing sticky mats, and removing trash. These activities are performed by the technical staff rather than the janitorial staff because entrance is restricted to cleanroom trained personnel only. Monthly charts are used to record performance of each activity and note any problems. In the Class 100 room, work surfaces (benches and hoods) are wiped daily with deionized water on cleanroom wipes. The floor is mopped with tap water weekly. A metal-free detergent may be used if needed, but is generally avoided because of the potential of a particle-generating residue on the floor. Sticky mats at the entrance to the Class 100 and 10,000 rooms are changed weekly or more often if needed. Trash is removed from the cleanroom daily.

Non-routine maintenance activities include semi-annual replacement of bag filters and pre-filters, checks on motor drive belts, and examination of HVAC equipment for signs of wear or stress.

Section 9.0

Reagent Purification

Procedures

Reagents commonly used in trace element analysis include deionized water, acids, bases, oxidizing agents, reducing agents, buffers, complexing agents, and to a lesser extent, solvents. The demand for high purity reagents has been met by commercial chemical manufacturers in some cases. However for many others, specific purification procedures need to be developed to adequately control contamination.

Acids are prepared by triple distillation in sub-boiling teflon stills. Commercially available high purity mineral acids are generally excellent quality (ULTREX, SUPRAPUR, OPTIMA), but at a relatively high price. Equal or better quality acids can be prepared in a cleanroom setting using custom-designed teflon stills such as those at NIST or the University of North Carolina Geochronology Lab. These stills require daily attention and maintenance, but if properly tended yield a more economical source of high purity acids.

In general, reagents are purified by repetitive distillation, repetitive crystallization, ion exchange, or complexation followed by solvent extraction. Volatile impurities, such as mercury, can often be eliminated by heating or inert gas purge. Many examples and recipes are available in publications on trace element contamination control [Zief, 1976; Moody and Beary, 1982; Mitchell, 1982]. Standard Operating Procedures used for purification of graphite furnace matrix modifiers used at RTI are presented in Appendix D (Standard Operating Procedures for Purification of Reagents in the ACS Inorganic Clean Lab Facility for Trace/Ultratrace Metal Analysis). Procedures for purification of reagents used for mercury analysis are presented in Appendices E, F, and G.

Storage

Storage of high purity reagents and standards requires careful consideration. Ultra-low concentration standards and samples need to be contained in materials that do not adsorb the analyte and hence cause loss of analyte over time, and the container materials must not leach or otherwise contaminate the solution with the analyte and thus cause elevated analyte concentrations. In general, teflon is the material of choice due to its highly inert nature, but other plastics, such as polyethylene, are well suited for storage of most trace element solutions. Glass is generally avoided because of its high affinity for adsorption and because it contains a variety of metallic impurities in its matrix which can slowly leach into solution. For volatile analytes such as reduced forms of mercury, arsenic, selenium, etc., the containers must be dense enough that vapors do not permeate through the container walls causing loss of analyte from the sample or contamination by analyte in the atmosphere. Similarly, the caps must be able to be closed very tightly to prevent exchange of vapors in or out of the bottles. Nicolas Bloom recommends teflon bottles that can be torqued closed with a wrench for aqueous mercury samples. As an additional precaution against atmospheric contamination of samples, mercury analysts typically include a mercury vapor phase sorbent trap, such as gold coated activated carbon or gold impregnated fabric, inside the cleanroom and also inside refrigerators and storage areas.

Storage of high concentration standards or samples represents a different problem. In the case of volatile analytes, loss of analyte from the solution to the atmosphere and eventually to other samples is a major concern. One mercury analyst reported that storage of a 1000 ppm mercury standard in a refrigerator contaminated the refrigerator so badly that it had to be removed from service. High concentration standards and samples need to be stored in vaportight containers in regions that are remote from low level analytes. Several of the cleanroom designers/operators interviewed stated that they do not permit high concentrations of volatile analytes to enter the cleanroom areas. The maximum permissible mercury concentration that is stored in the cleanroom is typically 1 ppm for laboratories that conduct analyses in the ppt range.

Vapor exchange is not a problem for storage of non-volatile reagents and standards, but spills of high concentration solutions are still a problem. Some cleanrooms do not allow storage of any standard at a concentration greater than 1 ppm. Others allow storage of higher concentration standards and reagents but in designated regions of the cleanroom only. For example, in the RTI cleanroom, all work performed with solutions containing analytes at concentrations greater than 1 ppm must be performed on specified "high level" laboratory benches using designated "high level" labware. Those regions and labware are never used for "low level" work and thus chances of cross-contamination are kept low.

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Appendix A

RTI/ACS/SOP-174-001

Revision 0

Standard Operating Procedures for the ACS Inorganic
Class 100/10,000 Clean Lab Facility

Research Triangle Institute
Analytical and Chemical Sciences
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May 1994

Table of Contents

Standard Operating Procedures for the ACS Inorganic Class 100/10,000 Clean Lab Facility RTI/ACS-SOP-174-001

1.0 Introduction

The ACS Inorganic Class 100/10,000 Clean Lab Facility is a controlled access laboratory designed for minimizing particulate contaminants for the purposes of ultra-trace metal analysis. The facility is restricted to Inorganics Research personnel and escorted visitors trained in the protocols necessary to maintain Class 100 conditions. Appropriate garments must be worn and protocols followed for admittance into the Class 100 Clean Lab area. This document outlines the proper procedures and equipment for use and maintenance of the ACS Inorganic Class 100/10,000 Clean Lab Facility.

2.0 Laboratory Description

The ACS Inorganic Class 100/10,000 Clean Lab Facility is a suite of rooms within Dreyfus 193. Figure 1 shows the layout of each room; a description of each is provided in the following sections.

2.1 Office Area

The 193 Office is accessed from the Dreyfus hallway and has no entrance restrictions. There is a door from the Office into the Inorganic Class 100/10,000 Clean Lab Anteroom; this door remains locked from the Office side, but is unlocked from the laboratory side. This arrangement permits emergency egress from the Inorganic Class 100/10,000 Clean Lab and anteroom, but prohibits routine entrance from the office. The key to this door is kept in the Office.

2.2 Service Room

The Service Room is a small, general access room that contains supplies, gas cylinders, water deionizer, air purification system, refrigerator, and freezer. It is accessible from the Dreyfus hallway and has no entrance restrictions. The door from the Service Room into the Instrument Lab is a restricted passage. Only authorized personnel may enter the Instrument Lab. Both the Office and Service Room are supplied with air filtered through standard 95% efficiency filters, but not HEPA filters.

2.3 Instrument Lab

The Instrument Lab is a Class 10,000 area, with a partially HEPA-filtered air supply (HEPA-filters are located in areas of sample handling). Minimal particulate control measures are used ("tacky-mat" flooring at the entrance). The lab contains instruments for trace and ultra-trace metal analysis (GFAA, ICP, HGAF, etc.). The Instrument Lab is a restricted area accessible only to authorized personnel. It is entered via the 193 Service Room and may be exited to the Service Room, Anteroom, or through an emergency exit door to the outside of the building.

2.4 Anteroom

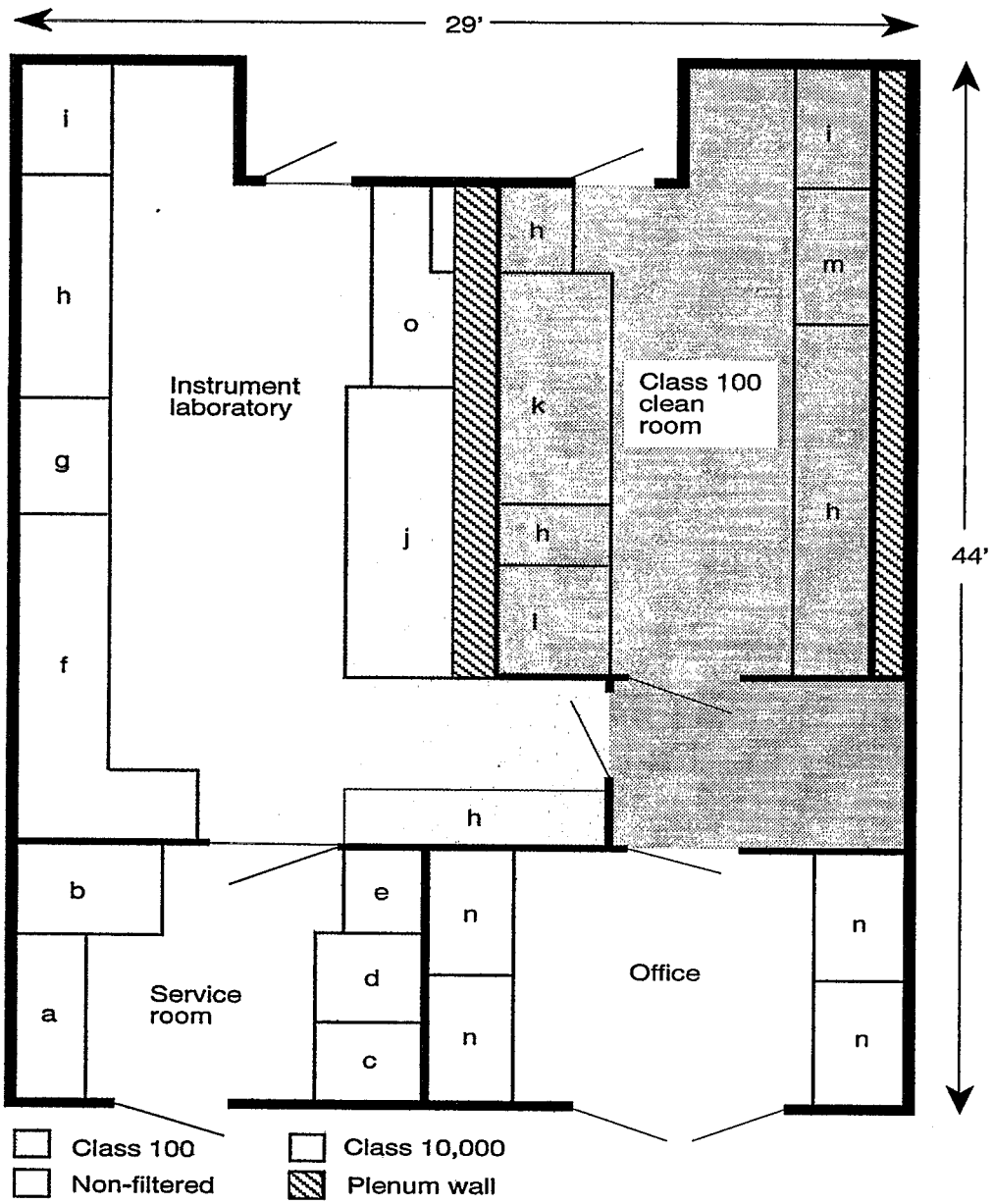
The Anteroom is the transition zone from the Instrument Lab to the Class 100 Clean Lab and is a restricted area, limited to trained personnel only. The Anteroom is supplied with HEPA-filtered air and provided with "tacky mat" flooring to reduce particulates carried into the Class 100 area. The Anteroom is used to put on Clean Lab garments before entering the Class 100 area (clean-lab coats, lab shoes or shoe covers, head covers) and to store Clean Lab cleaning supplies (lab cart, mop, bucket, clean wipes).

The Anteroom is the sole access to the Class 100 Clean Lab. The Anteroom has a door which opens to the Office area, which is to be used as an emergency exit only.

2.5 Class 100 Clean Lab

The Class 100 Clean Lab is an inorganic analysis laboratory designed to enable contaminant-free preparation of samples for metal analysis at the parts-per-trillion level. The two most unique features of the room are the ultra-clean air supply and the metal-free construction.

Ultra-clean air is supplied through HEPA filters located in a ceiling grid; over 90% of the ceiling is covered with 99.9975% efficient HEPA filters. As depicted in Figure 2, Class 100 air is forced downward in a laminar flow and bathes work benches with particle-free air. Air is re-



a. Gas Cylinders
b. D. I. Water
c. Refrigerator
d. Freezer
e. Cleanroom Supplies
f. PE 5100 ZL
g. 4' HEPA Hood
h. Bench
i. Sink
j. ICP
k. 8' HEPA Hood
l. Non-HEPA 4' Exhausting Hood
m. 6' Acid Baths
n. Desk
o. Questron V6 AFS

Figure 1. ACS Inorganics Class 100/10,000 Clean Lab Facility.

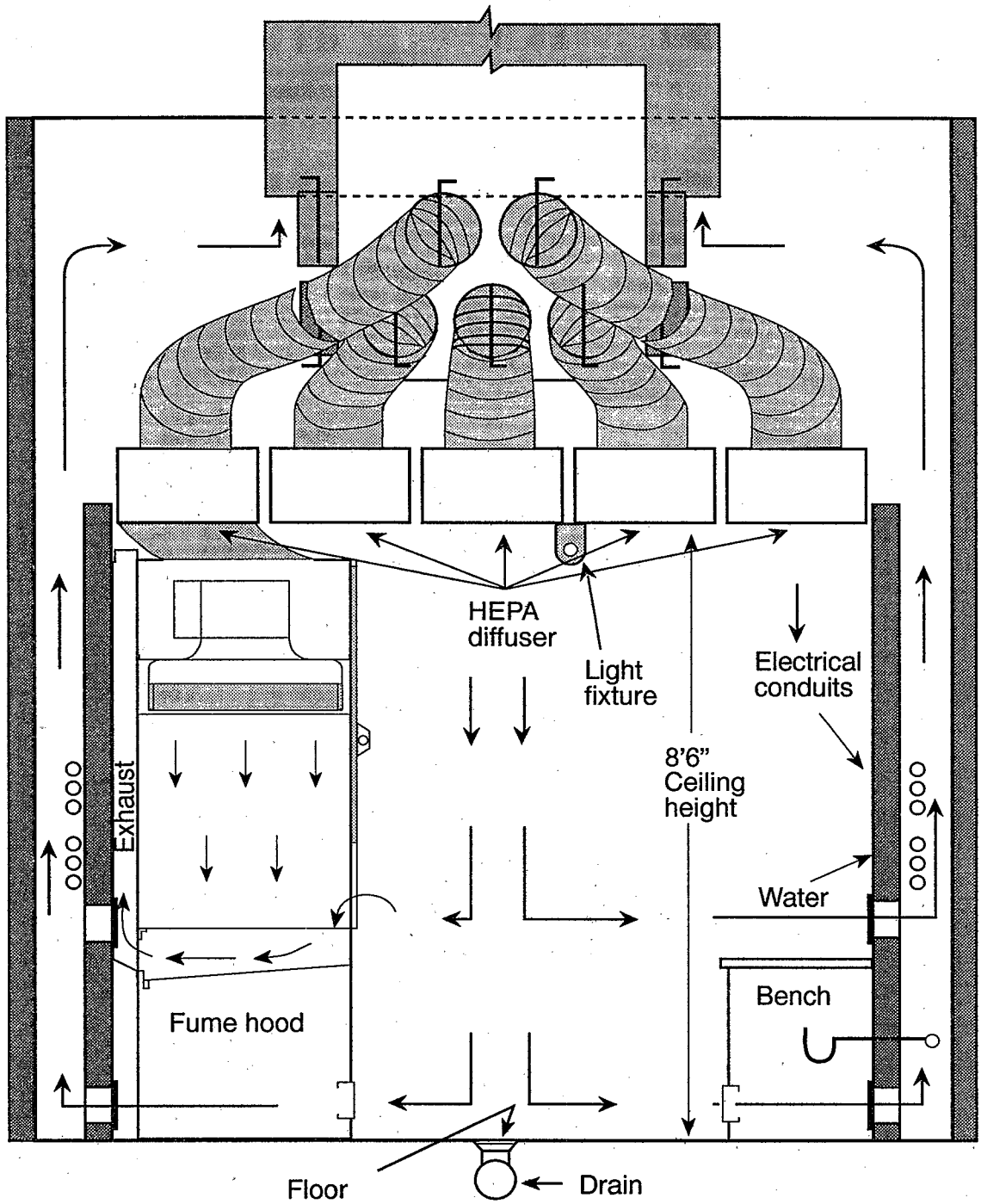


Figure 2. Vertical laminar flow cleanroom design used at RTI.

turned to a recirculation unit at two heights along a plenum wall: the primary air return is a baseboard return extending from the floor up to a height of 18 inches; the secondary return is at hand level extending from the benchtop surface to a height of six inches above the benchtop. This air is recirculated through the ceiling HEPA filters to achieve continuous removal of particles.

Metal-free construction materials were used to the extent possible to build the Class 100 Clean Lab. This was done to prevent acid corrosion and resulting metal contamination. The walls, floors, benches, fume hoods, acid bath cabinet, drawers, and sinks are made of polypropylene, and all plumbing materials are either polypropylene or PVC. All other service lines (electrical, gas, etc.) are encased in PVC tubing.

The Class 100 Clean Lab contains several features in addition to the clean air supply that facilitate ultra-trace level analysis. The lab is equipped with three areas of one-pass, air exhaust: (1) an 8-foot wide Class 100 fume hood with vented base cabinet; (2) a 4-foot wide fume hood with vented base cabinet; and (3) a 6-foot wide vented acid bath cabinet. The fume hoods are used for acid digestion, evaporation, or extraction of samples and prevent exposure of personnel or equipment to acid vapors. Diagrams showing the design and air flow patterns in the 8-foot hood are presented in Figure 3. A microwave digestion system is located in the 4-foot hood, and two remote control ceramic hot plates are located in the 8-foot hood. The acid bath cabinet houses multiple tubs for acid leaching of glassware and plasticware. The interior of the cabinet is flushed horizontally with Class 100 air and vented to the outside of the building in order to minimize exposure of personnel to acid vapors even when the baths are open.

The Class 100 Clean Lab is the most restricted area of the Inorganic Class 100/10,000 Clean Lab facility, requiring appropriate apparel for entry (cleanroom shoes or shoe covers, cleanroom lab coats, etc.). Access to the Class 100 Clean Lab is through the anteroom via the Instrument Lab. The Class 100 Clean Lab also has an emergency door escape route leading to the outside of the building.

3.0 Laboratory Apparel

3.1 Office Area

The Dreyfus 193 Office area is non-restricted, non-laboratory area with no dress restrictions.

3.2 Service Room

The Dreyfus 193 Service Room is a non-restricted area with no dress restrictions.

3.3 Instrument Lab

The Inorganic Class 100/10,000 Clean Lab Instrument Lab is designated as a Class 10,000 environment; lab apparel requirements are set up to maintain this environment and for the safety of laboratory workers; requirements are described in the following sections.

3.3.1 Clean Garments

Cleanroom shoes or shoe covers are required for entrance to the Instrument Lab. Laboratory coats and powder-free gloves are available. A "tacky-mat" at the entrance door will reduce footborne contamination. The lab personnel should always be conscious of minimizing contamination when entering the Instrument Lab, and when passing from the Instrument Lab into the Class 100 Clean Lab.

3.3.2 Safety Garments

Working in the Instrument Lab will entail exposure to a variety of chemicals including dilute acids, sample digests, and standard metal solutions. The required protective measures are safety glasses and closed-toe shoes. Lab coats and acid-resistant gloves are recommended when handling samples, standards, reagents and wastes.

Visitors to the Instrument Lab will be required to wear appropriate shoes and safety glasses.

3.4 Class 100 Clean Lab

The Class 100 Clean Lab is designated as a Class 100 facility; the procedures and lab apparel required are designed to maintain the Class 100 conditions and for the safety of the lab personnel.

3.4.1 Clean Garments

There are strict requirements for garments to minimize particulates and contaminants carried into the Clean lab. Entry to the Class 100 Clean Lab is through the Anteroom, where cleanroom garments will be kept for laboratory personnel. Extra cleanroom garments will be available for visitors on the Service Room storage shelves.

- All personnel will remove or cover street shoes (laboratory workers will have shoes designated for cleanroom use only, visitors will use disposable shoe covers).
- All personnel will wear low-particulate, Class 100 lab jackets in the Class 100 Clean Lab.
- All personnel will wear disposable head covers.

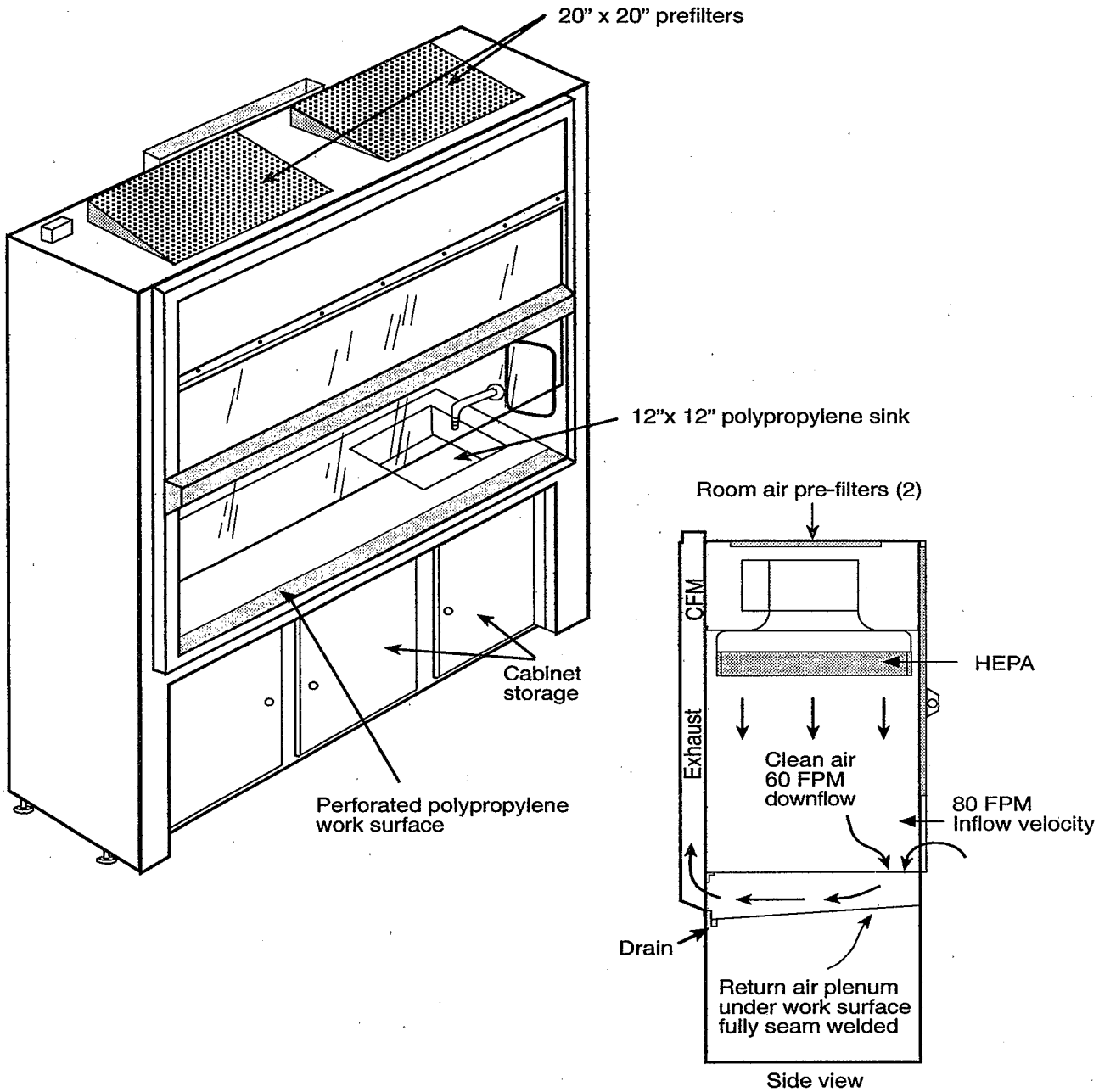


Figure 3. Vertical laminar flow fume hood.

- Class 100 powder-free gloves are available for working in the Class 100 Clean Lab. Gloves worn in the Instrument Lab should be rinsed or discarded before entry into the Class 100 Clean Lab.

3.4.2 Safety Garments

Safety garments are required in the Class 100 Clean Lab to protect the lab workers from exposure to concentrated acids and oxidizers used in sample digestion and from exposure to sample materials which may be partly or totally uncharacterized. Safety glasses, lab coat and appropriate shoes are minimally required. Gloves are recommended for handling toxic chemicals, acids or unknowns.

Additionally, lab personnel may use a face shield, lab apron, dust mask or respirator or other safety equipment for certain tasks. Routine safety equipment must meet Class 100 standards of cleanliness before being brought into the Class 100 Clean Lab, either purchased prepackaged for Class 100 use from a cleanroom supplier, or cleaned and packaged in the Class 100 hood in the Instrument Lab. However, personnel safety is always the primary consideration and in some cases it may be necessary to use non-Clean Lab equipment or supplies.

4.0 Materials Handling and Exchange

The working areas of Dreyfus 193 are laid out so that personnel pass from the "dirty" areas (i.e., the hallway, the office, the Service Room) to increasingly cleaner areas (the Instrument Room, the Anteroom, the Cleanroom). Appropriate measures are taken to reduce contamination in each progressively cleaner area (see Sections 3.3-3.4.)

To minimize contamination in the cleaner areas, each area will be provided with its own supplies according to the tasks performed there. To this end, procedures are in place regarding supplies, cleaning procedures, sample storage and waste disposal for the Inorganic Class 100/10,000 Clean Lab Facility.

4.1 Office Materials Handling Protocols

No reagents or samples will be permitted in the Office area. Any documents, notes, notebooks or raw data brought into the Office area from the Instrument Lab or Class 100 Clean Lab will be as contamination free as possible.

4.2 Service Room Materials Handling Protocols

The Service Room acts as a storage area for the Instrument Lab and the Class 100 Clean Lab. Materials must pass from the Service Room to the Instrument Lab, then through the Anteroom into the Class 100 Clean Lab, with appropriate decontamination procedures in the Instrument Lab and Anteroom.

4.3 Instrument Lab Materials Handling Protocols

The Instrument Lab is designed for instrumental analysis and will have minimal reagent handling procedures. Reagents and samples will be brought into the Instrument Lab only for analysis purposes (samples, calibration standards, matrix modifiers, acids, blanks, etc.) or for cleaning procedures before transfer to the Class 100 Clean Lab.

4.3.1 Acid exposure in the Instrument Lab will be limited. No concentrated acids will be stored in the Instrument Lab.

4.3.2 Acid solutions used for ICP or AA flushing solutions will generally be <25% concentration and <1.0 L volume. Instrument reservoirs for acid solutions will be closed or covered with parafilm or similar sealant to minimize acid vapors in the Instrument Lab.

4.4 Class 100 Clean Lab Materials Handling Protocols

4.4.1 High Level Materials Handling Protocols

Specific lab bench space and lab supplies will be designated for High Level Operations (> 1 ppm metals concentration) within the Class 100 Clean Lab. High Level Operations are defined as the handling and dilution of concentrated metal standard solutions and for preparing reagents for sample preparation or analytical applications.

4.4.2 New supplies and equipment for High Level Operations will be initially washed and/or rinsed with deionized water and dried in a Class 100 environment.

4.4.3 High Level Operations labware will be washed and acid leached according to ACS-SOP-174-002 "Standard Operating Procedure for Cleaning Labware in the ACS Inorganic Class 100/10,000 Clean Lab Facility" then stored in cabinets designated for High Level Operations equipment. Washing basins, baskets and acid leaching baths will be designated for High Level Operations.

4.4.4 In general, High Level Operations equipment and supplies will not be used for other applications. Exceptions may be made on the judgement of the Laboratory Supervisor, or Project Officer.

4.4.5 The High Level Operations bench space will be cleaned and equipment and chemicals put away after all operations.

4.5 Low Level Operations Materials Handling Protocols

The Class 100 Clean Lab is supplied with designated labware (glassware, teflon and plasticware, pipettors and tips, volumetric pipets and bulbs) for exclusive use in Low Level Operations.

4.5.1 Supplies will be initially cleaned and decontaminated with stringent measures (see SOP 174-002, "SOP for Cleaning Labware in the ACS Inorganic Class 100/ 10,000 Clean Lab Facility") before being brought into the Class 100 Clean Lab.

4.5.2 Low Level Operation labware will be rinsed in the Class 100 Clean Lab or, if necessary, washed in the Instrument Lab, then acid leached in acid baths designated for Low Level Operations.

4.5.3 Under no circumstances will Low Level Operations equipment or supplies be removed from the Class 100 Clean Lab Facility or used in High Level operations.

4.5.4 Exposure of Low Level Operations labware to metal concentrations >1ppm will be strictly avoided. Metal solutions or reagents brought into the Low Level Operations working areas will be of the lowest working concentrations possible.

4.5.5 New or replacement supplies (including reagents, acids, and samples) brought into the Class 100 Clean Lab will be in Class 100 packaging, or cleaned by laboratory personnel in the Class 100 hood in the Instrument Lab (see SOP 174-002, "SOP for Cleaning Labware for the Class 100 Clean Lab Facility") before being transferred into the Class 100 Clean Lab.

Supplies packed in Class 100 packaging will be brought into the Anteroom, where external packaging can be discarded, then brought into the Class 100 Clean Lab, where internal packaging can be opened and discarded.

4.6 Waste Disposal

4.6.1 Office Area

Trash from the Office area will be recycled or disposed of by Housekeeping personnel according to RTI guidelines.

4.6.2 Service Room

Non-chemical trash from the Service Room will be disposed of by Housekeeping personnel according to RTI guidelines.

4.6.3 Instrument Lab

Non-contaminated trash from the Instrument Lab will be disposed of by Housekeeping personnel according to RTI guidelines. The trash can will be placed in the Service Room or the hallway for pickup.

Chemical wastes from the Instrument Lab will be collected in plastic or glass containers. When possible, wastes will be neutralized and flushed down the drain. Otherwise, wastes will be labelled as to metals and matrix, and approximate concentrations (if >5ppm) and collected by RTI Safety personnel.

If necessary, chemical wastes will be transferred to Lab 169 for characterization, then collected by RTI Safety personnel. Lab personnel will be responsible for disposal of waste generated by their projects.

4.6.4 Class 100 Clean Lab

RTI Maintenance and Safety personnel will not need access to the Class 100 Clean Lab or Anteroom. Laboratory personnel will be responsible for all cleaning and waste collection in these restricted areas.

Non-contaminated trash from the Class 100 Clean Lab (and Anteroom) will be collected by the lab worker from lined, plastic trash cans in the Class 100 Clean Lab. The trash bag will be collected daily and disposed of with trash from other lab areas.

Chemical wastes from the Class 100 Clean Lab will be collected in plastic containers and transferred to the Instrument Lab for treatment and disposal.

Chemical wastes will not be generated or stored in the Anteroom.

Appendix B

RTI/ACS/SOP-174-002

Revision 0

Standard Operating Procedures for Cleaning Labware in the
ACS Inorganic Class 100/10,000 Clean Lab Facility

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May 1994

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Standard Operating Procedures for Cleaning Labware in the ACS Inorganic Class 100/10,000 Clean Lab Facility

1.0 Introduction

This document describes the cleaning procedures necessary for labware used exclusively in the ACS Inorganic Class 100/10,000 Clean Lab Facility. The ACS Inorganic Class 100/10,000 Clean Lab Facility (Dreyfus 193, A-D) is supplied with separate, dedicated sets of labware for High Level Operations (> 1 ppm) and Low Level ultra-trace metal applications.

Cleaning procedures will be done by ACS technical support personnel or laboratory personnel within the ACS Inorganics Class 100/10,000 Clean Lab Facility (when possible), using the sinks and designated basins, deionized water lines and the acid-leaching baths.

2.0 Summary of Procedure

A general, routine cleaning procedure for Clean Lab labware can be summarized in the following steps:

- separation of High Level and Low Level labware
- pre-treatment (removing labels, rinsing with tap water)
- detergent wash (soaking or scrubbing)
- thorough rinse (tap water and deionized water)
- acid leaching
- final rinse in deionized water
- drying (in Clean Lab hood)
- storage (if required)

The steps outlined above will be adequate for most Clean Lab applications. For some sensitive, non-routine applications, additional hot acid leaching may be required. All procedures are detailed below.

3.0 Routine Labware Cleaning RTI/ACS/ Procedure

The routine labware cleaning procedure is applicable to most High Level or Low Level Operations. For some applications an alternate cleaning procedure may be used (i.e. eliminate the detergent soak in order to reduce contamination risks from the detergent or employ other soaking agents).

3.1 Laboratory technical personnel will be responsible for preparing the labware for cleaning:

- empty the dirty labware of any chemical residue and rinse well with tap water.
- remove label tape or lab marker labelling (acetone or methanol is usually suitable for removing lab marker or tape residues from teflon, glass and most plastics.)
- segregate labware as to High Level or Low Level applications.

3.2 ACS support personnel (dishwashers) or laboratory personnel will be responsible for washing and rinsing procedures:

- prepare a solution of a suitable laboratory detergent (Alconox, Liquinox etc.) in the designated basin (Low Level or High Level) for soaking the labware.
- submerge the labware, careful to wet all surfaces.
- soak for as long as necessary (to remove any organic residue).
- rinse the labware with tap water until all detergent residue is gone.
- rinse with deionized water three times.
- place the labware onto the appropriate lab cart (High Level or Low Level Operations) for return to the lab.

3.3 Laboratory technical personnel are responsible for all acid leaching procedures: submerge rinsed labware into an appropriate acid leaching bath set up in the acid bath cabinet, or in the Clean Lab hood. All surfaces (interior and exterior) must be exposed to the leaching solution. Labware will be soaked for at least 8 hours.

3.4 For some Low Level ultra-trace applications, only the interior of the labware will be leached in order to

prevent contamination from the printed labels on the exterior. This labware will be filled with the leaching solution and allowed to sit at room temperature for an appropriate length of time (2-4 hours). Alternately, the acid-filled labware will be soaked in a warm water bath.

CAUTION

1. Laboratory personnel must take safety precautions when working with acid leaching baths: lab coat, safety glasses or face shield, Playtex-type latex gloves, sleeve covers, use of plastic tongs etc. Gloves should be used exclusively for acid-bath tasks.

2. All laboratory surfaces exposed to acid solutions should be rinsed and if necessary neutralized.

3.5 Labware will be retrieved from the acid bath, drained of excess leaching solution, and rinse with copious deionized water (5-10 rinses.)

3.6 The labware will be dried on a drying rack or on Class-100 lab wipes on the lab bench or in the hood (if necessary).

3.7 Labware not used immediately will be stored in the acid baths until needed, or placed in plastic ziplock bags and stored in appropriate cabinets designated for High Level or Low Level Operations.

3.8 Volumetric Pipets

3.8.1 High Level Operations

High Level Operations include diluting calibration standards for use in the Instrument Lab, and diluting or transferring reagent solutions for sample preparation or analytical uses.

Volumetric pipets used in High Level Operations will be copiously rinsed with an appropriate rinse solution after use, then rinsed with at least 5 volumes of deionized water before being returned to the pipet acid bath designated for High Level Operations labware. Pipets should be cleaned with detergent (or alcoholic KOH) only when poor wetting and drainage characteristics compromise volumetric performance.

3.8.2 Low Level Operations

Pipets used for low level operations will be etched with an "L" to designate low level applications only. Low Level Operations include transferring or diluting sample preparations and dilute, decontaminated reagent solutions. Volumetric pipets used in Low Level Operations will be copiously rinsed with an appropriate rinse solution after use, then rinsed with at least 5 volumes of deionized water before being returned to the pipet acid bath designated for Low Level Operations labware. Pipets should be cleaned with detergent (or alcoholic KOH) only when poor wetting and drainage characteristics compromise volumetric performance.

3.9 Microwave Digestion Vessels

Microwave digestion vessels will be numbered and segregated according to the application and analyte. Between uses the vessels will be washed or soaked in detergent, rinsed and acid-extracted according to the needs of the project and analyte (see Section 6.0)

4.0 Maintenance of Acid Leaching Baths

The Clean Lab acid baths will be prepared as 20% HNO_3 (reagent grade concentrated nitric acid) in deionized water. Separate acid baths will be prepared and designated for High Level Operations labware and Low Level Operations labware.

Contamination levels of the baths will be monitored by sampling the leaching solution from each bath for analysis. The acid leaching solutions will be sampled and analyzed when the baths are first prepared and once every month thereafter (see ACS/SOP-174-005 "Monitoring and Maintenance of the Cleanliness of the Clean Lab Facilities"). The samples will be analyzed for cadmium, lead and arsenic (Cd, Pb, As) or for the current analyte of interest.

The acid leaching baths will be changed when any of the analytes of interest reach a concentration > 1 ppm or in the event of known accidental contamination. If any analyte exceeds the 1 ppm threshold but is not currently being determined for a project, it is not necessary to change the acid bath until that analyte is being determined for a project.

To prevent accidental contamination, laboratory personnel will check that High Level and Low Level labware are kept separate and are leached in the designated baths. All labware will be rinsed with deionized water before submerging in the acid leaching baths.

Lab personnel will have Playtex (or similar) gloves specifically designated for use in the acid leaching baths. These gloves will be rinsed with deionized water before each use in the acid leaching baths. After use in the acid baths, the gloves will be rinsed copiously with tap water and hung to dry. These gloves will not be used for handling samples, reagents or waste.

5.0 Non-Routine Cleaning Procedure

For sensitive Low Level applications (ultra-trace Pb, Hg etc.) an alternate hot acid leaching procedure may be used for Low Level labware.

5.1 A leaching bath will be prepared in a 1.0 or 4.0 L beaker dedicated for use as an ultra-trace level leaching bath. Usually a 20% HCL or 20% HCl + 20% HNO_3 solution is adequate, though specific methods may require other leaching treatments. Acids of trace-metal grade or better will be used to prepare the leaching solution. The minimum volume of leaching solution sufficient for the labware being treated will be prepared. The leaching bath will be kept in a Class 100 hood during preparation and during the leaching process.

5.2 Labware will be submerged into the ultra-trace leaching bath, taking care to expose all interior and exterior surfaces to the leaching solution. The bath will be heated on a hotplate for at least two hours.

NOTE: Some plastics will discolor, soften or become brittle with exposure to hot, oxidizing acids. Lab personnel must make sure labware is compatible with hot acid leaching.

5.3 The ultra-trace leaching bath will be allowed to cool and the labware will be retrieved and rinse with copious volumes of deionized water.

5.4 The labware will be allowed to dry in the hood or on the benchtop on Class 100 lab wipes. The volumetric flasks will be stored with deionized water in them until use, other labware will be stored dry in plastic bags.

6.0 Microwave Digestion Vessels

6.1 Microwave vessels will be labelled with unique numbers and segregated for use with trace or ultra-trace level analyses. Databases will be maintained to keep records for the vessels, including sample types, acids and oxidants, acid-extraction and any contamination of analytes for each vessel.

6.2 For sensitive analyses, the high-pressure acid-extraction will be performed and the extract analyzed for analyte carry-over. (Specific acids, extraction times and programs, analytes and analysis methods will vary according to the needs of the project.)

An example of a general-use high-pressure extraction is as follows:

- add 10-mL of 10% HNO_3 to each detergent-cleaned microwave vessel, cap and seal.
- microwave for a total 30 minutes at 100% power (alternating 3 minutes at 100% power, 2 minutes at 0% power.)
- cool and vent the vessel.
- dilute the extract 1-to-2 with deionized water.
- analyze for the analyte of interest by GFAA, FAA, HGAF, ICP, etc.

For GFAA analyses, a signal of <0.002 absorbance units will be acceptable for the extracts and the vessel will be considered suitable for use. Suitability thresholds will be established for analyses as needed.

6.3 The extract solutions will be neutralized and discarded. The microwave vessels will be rinsed copiously with deionized water and dried in a Class 100 environment (benchtop or hood.) If not used immediately, the microwave vessels will be capped and stored in sealed plastic bags until needed.

6.4 Vessels that do not meet the suitability criterion must be re-extracted.

7.0 Contamination

If any Low Level Operations labware is inadvertently exposed to high levels of metals of interest (e.g. from highly contaminated samples, or high metals in a sample matrix) the labware will be removed from Low Level applications and replaced, or decontaminated by non-routine methods until its suitability for Low Level applications is verified.

8.0 Cleaning Expendable Supplies

Accurate analysis of trace and ultra-trace metals requires minimizing all contamination sources, including disposable and expendable lab supplies. Pasteur pipets, pipettor tips, disposable microbeakers, plastic weigh boats and other supplies with which samples or reagents will come into contact will be rinsed with deionized water and (if necessary) dried in Class 100 air.

For sensitive Low Level methods requiring non-routine cleaning of labware, these expendables will be soaked in or rinsed with dilute acid (approximately 10% HNO_3 or HCl) before the deionized water rinse.

Appendix C

RTI/ACS/SOP-174-005

Revision 0

Standard Operating Procedures for Monitoring and Maintaining Cleanliness of the ACS Inorganic Clean Lab Facility

Research Triangle Institute
Analytical and Chemical Sciences
Post Office Box 12194
Research Triangle Park, NC 27709

August 1994

Standard Operating Procedures for Monitoring and Maintaining Cleanliness of the ACS Inorganic Clean Lab Facility

1.0 Introduction

Cleanrooms are designed to control and limit the airborne particles in the working environment. The level of cleanliness is specified by the maximum allowable particles per cubic foot of air as determined by statistical methods (Table 1). The class designation is taken from the maximum allowable number of particles, 0.5 μm or larger, per cubic foot. This document concerns the Class 100 and Class 10,000 clean labs located in room 193 in the Dreyfus building. The floor diagram of the facility is given in Figure 1. This Clean Lab Facility is specially designed to eliminate the contamination problems in trace and ultra trace metal analysis.

The purpose of this SOP is to provide guidelines to all laboratory personnel for proper maintenance of the facility to preserve its cleanliness over time.

2.0 Initial Testing and Certification of the Clean Lab Facility

The certification of the Clean Lab Facility was performed by an independent company (Contamination Control Technologies, Inc.) certified by the National Environmental Balancing Bureau. A copy of the certified document is attached (Appendix I). The certification procedure involved the measurement of particle counts that would reflect the performance of the air handling system of the Clean Lab Facility. Particle counts will be monitored periodically to ensure that the initial standards are maintained.

Table 1. Airborne Particulate Cleanliness Classes

Class Name[a]	Class Limits[b] 0.5 μm (particles/ft³)
1	1.00
10	10.0
100	100
1,000	1,000
10,000	10,000
100,000	100,000

[a] Concentration limits for intermediate classes can be calculated, approximately, from the following equation:

$$\text{Particles/m}^3 = N_c(0.5/d)^{2.2}$$

Where "N_c" is the numerical designation of the class based on English (U.S. customary) units, and "d" is the particle size in μm.

[b] Class limits designate specific concentrations (particles per unit volume) of airborne particles with sizes equal to and larger than 0.5 μm diameter.

3.0 Monitoring the Cleanliness of the Clean Lab Facility

3.1 More Frequently Monitored Parameters

The parameters that indicate the proper operation of the air handling system will be monitored on a weekly basis. A proper air handling system will ensure that no flow of air occurs from a less clean area to a more clean area, for example, the flow of air should not proceed from the anteroom to the Class 100 room or from the instrument room to the anteroom. It also helps in rapid exhaust of any particulate matter that is created by the movement of people, operation of equipment, etc., so that they do not contribute to sample contamination.

3.1.1 The air pressure of each room in the Clean Lab Facility will be monitored on a weekly basis by lab personnel to ensure that the following gradient exists. From most positive pressure to least positive pressure the order should be:

Class 100 > Anteroom > Class 10,000 > Service Room = Office Room.

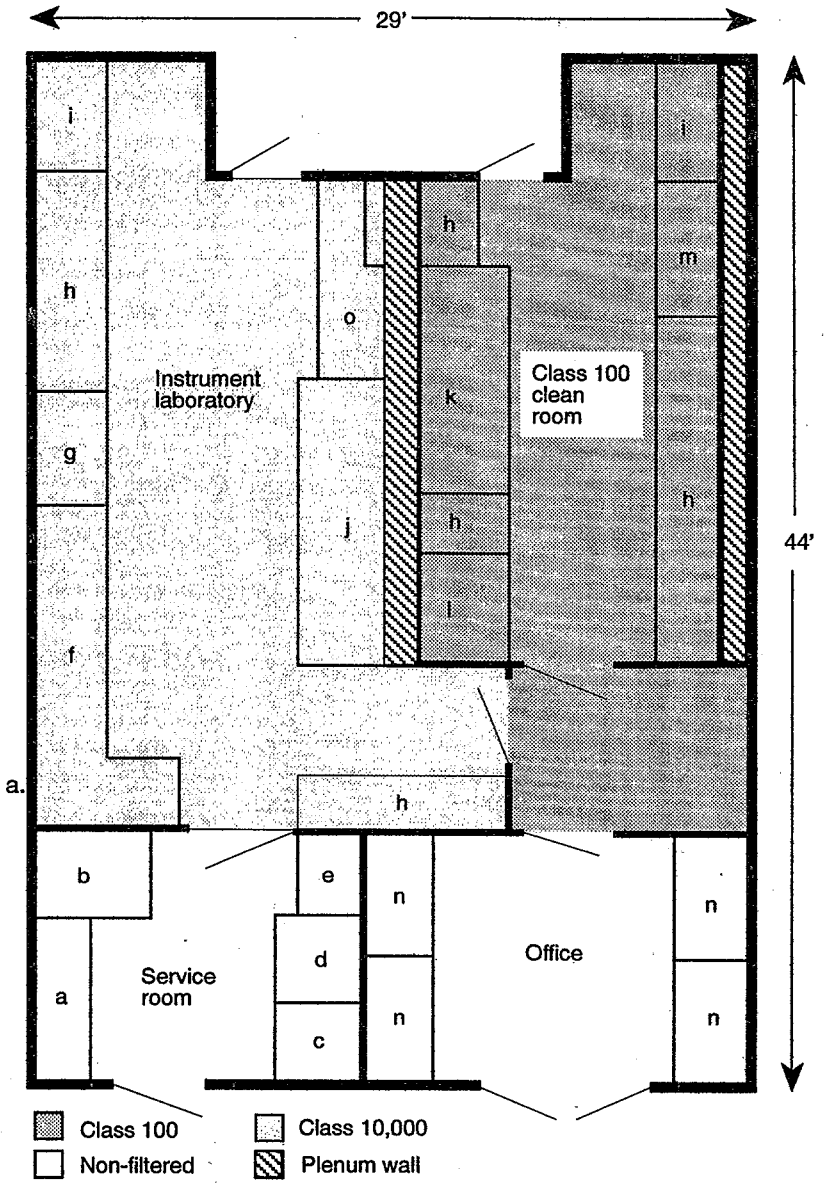
In the event the measured gradient is different from the above gradient, the laboratory manager will contact John Berkley or the person in-charge at RTI Heating, Ventilation and Air Conditioning.

3.1.2 The temperature in the Clean Lab Facility will be monitored by lab personnel continuously through an electronic monitoring system installed in the lab. Data will be printed out on a daily basis. In the event the temperature makes the facility uncomfortable to the working personnel, HVAC will be contacted by the laboratory manager for corrective actions (refer to section 3.1.1 for a contact person).

3.2 Less Frequently Monitored Parameters

These include physical and chemical properties that are measured less frequently.

3.2.1 Particle measurements will be made in both Class 100 and Class 10,000 areas once every three months and will be compared to those obtained at the initial certification. The laboratory manager is responsible for making necessary arrangements to have the



a. Gas Cylinders
b. D. I. Water
c. Refrigerator
d. Freezer
e. Clean Room Supplies
f. PE 5100 ZL
g. 4' HEPA Hood
h. Bench
i. Sink
j. ICP
k. 8' HEPA Hood
l. Non-HEPA 4' Exhausting Hood
m. 6' Acid Baths
n. Desk
o. Questron V6 AVS

Figure 1. ACS Inorganic Class 100/10,000 Clean Lab Facility.

particle measurements made as specified. The measurements must be made at locations where an open sample would be located, and will include, but not limited to, inside the 8' hood and on top of the 11' bench in the Class 100 room.

3.2.2 All of the parameters mentioned above (under sections 3.1 and 3.2.1) are good parameters for monitoring the air handling efficiency of the Clean Lab Facility. However, they do not provide direct information on contamination levels of various metals of interest within the laboratory. Lead (Pb), arsenic (As) and cadmium (Cd) are chosen as the contamination monitoring agents. These three elements will be measured in laboratory blanks and controls on a monthly basis to establish the cleanliness of the Clean Lab Facility with respect to trace metals. Suitable sample locations will be chosen and two samples will be taken from each location along with blanks. One sample will be tested for As by hydride generation atomic fluorescence spectrometry and the other will be tested for Pb and Cd by GFAAS. Sampling will be done by placing four clean Teflon beakers, two open (samples) and two covered (blanks) at each sampling location. The sampling locations and acceptable limits are given in Tables 2 and 3.

Table 2. Sampling Locations

Sampling Locations	No. Samples
Class 100 Room	
11' Bench	2
8' Hood	2
Class 10,000 Room	
4' Hood	1
On top of the GFAA Autosampler	1

Table 3. Acceptable Contaminant Limits

Element	Limit[a] (ng/cm²/24 h)
Pb	1.0
As	0.1
Cd	0.1

[a] Concentrations are given in mg of analyte per cm² of exposed area per 24 hours of sampling.

4.0 Maintenance of the Clean Lab Facility

All routine maintenance performed will be recorded in clean lab maintenance log books. All periodic maintenance schedules are prepared as checklists and are given in Appendix II.

4.1 All exposed bench surfaces and hood surfaces in the Class 100 room will be wiped with a lint free, static free wipe wetted with deionized water on a daily basis prior to any laboratory activities. (Appendix II, RTI/ACS/174-005/94-01.)

4.2 The floor in the Class 100 room will be moped with tap water on the first working day of every week. A suitable metal free detergent may be used when necessary. (Appendix II, RTI/ACS/174-005/94-01.)

4.3 The cleaning procedure described in section 4.1 will be carried out in the Class 10,000 area as well, but with the exception that it will be performed every 4-5 weeks. (Appendix II, RTI/ACS/174-005/94-02.)

4.4 The cleaning procedure described in section 4.2 will be carried out in the Class 10,000 area as well, but with the exception that it will be performed only once each month. (Appendix II, RTI/ACS/174-005/94-02.)

4.5 Sticky mats that are kept at entry doors of Class 100 and 10,000 areas must be replaced when necessary. Proper maintenance of these sticky mats will minimize the entry of dirt into the clean area from chemist's feet. (Appendix II, RTI/ACS/174-005/94-03.)

4.6 All fume hoods will be inspected by the custodian for their proper operation regularly. The static pressure through the filter(s) in the fume hood will be monitored once a week and recorded in the log book (Appendix II, RTI/ACS/174-00/94-04, 05, 06.)

4.7 The entrance and exit procedures and the transfer of items (samples, reagents, labware, apparatus, etc.) from one area to another also play an important role in maintaining the cleanliness of the Cleanroom Facility. These issues will be addressed by separate documents.

5.0 Corrective Actions

If any of the measured physical or chemical parameters indicate any sign of degradation of the cleanliness of the Clean Lab Facility it should be reported to the laboratory manager immediately. Once any measured physical or chemical parameters exceed the recommended level, necessary steps will be taken to ascertain the source and appropriate corrective actions will be taken to reestablish the cleanliness of the Clean Lab Facility.

Appendix I

Airborne Particle Count
Laser Particle Counter Method

Purpose:

This test is performed to measure the airborne particulate levels within the Class 100 and 10,000 Cleanrooms, the Anteroom, the two HEPA filtered workstations and the one exhaust hood and to identify potential problem areas.

Instrumentation:

Laser Particle counter with Built-in Recorder

Manufacturer: Particle Measuring Systems, Inc.
Model: LPC-525A

Calibration: November 15, 1993
SN: 12005-1287-14

Procedure:

Sampling duration for each count is one minute, with a total sample volume of 1.0 cubic foot. Sampling height is approximately 46 inches above the floor. Three counts are taken to each sampling location. Counts are recorded for all particles greater than or equal to 0.5 micrometers and for all particles greater than or equal to 5.0 micrometers. A 95% upper confidence levels (UCL) is calculated for each room for counts greater than or equal to 0.5 micrometers and for all particles greater than or equal to 5.0 micrometers as described in Federal Standard 209E.

Results:

The rooms were tested in an "as built" condition, i.e., only testing personnel were present during the particle count sampling.

The Class 100 Cleanroom meets requirements for an English (U.S. customary) Class 100 (at 0.5 µm and 5.0 µm) or a SI (metric) class M3.5 (at 0.5 µm and 5.0 µm) classification under "as built" conditions.

The Class 10,000 Cleanroom meets requirements for an English (U.S. Customary) Class 10,000 (at 0.5 µm and 5.0 µm) or a SI (metric) Class M5.5 (at 0.5 µm and 5.0 µm) classification under "as built" conditions.

Particle count locations, particle counts, and the statistical analyses of the counts are provided on the following pages.

Appendix II
RTI/ACS/174-005/94-01

Routine Maintenance of the Clean Lab

1. Wiping of all surfaces (bench tops and hood areas) with a wetted (with deionized water) cleanroom wipe must be done at the beginning of each working day. .
2. Mopping of the clean lab floor with tap water must be done on the first working day of every week.

Please sign and put check marks where appropriate after performing duties.

DATE	NAME	WIPE SURFACES	MOP THE FLOOR	INITIAL

Appendix II
RTI/ACS/174-005/94-01

Routine Maintenance of the Instrument Room

1. Wiping of all surfaces (bench tops and hood areas) with a wetted (with deionized water) wipe must be done on the first working day of every week.
2. Mopping of the clean lab floor with tap water must be done once every 5 weeks.

Please sign and put check marks where appropriate after performing duties.

DATE	NAME	WIPE SURFACES	MOP THE FLOOR	INITIAL

Appendix II
RTI/ACS/174-005/94-03

Routine Maintenance of the Clean Lab Facility

Sticky Mat Replacement

Please record the date when a fresh sticky mat is exposed.

Location: Inst. Room		Location: Anteroom	
Date	Initial/Comments	Date	Initial/Comments

Appendix II

RTI/ACS/174-005/94-04

Routine Maintenance Procedure for Hoods in the Cleanroom Facility

Monitoring of hoods must be done at the beginning of each week.

Please notify R. Fernando (Room 181, Ext. 6730) in the event of any problem or malfunction of the hood operation.

Hood Type: 8' hood in the Clean Lab.

Period: MM/YY - MM/YY

Date	Pressure (i.w.g.)	Indicator Light		Comments	Initial
		Normal	Caution/Alert		

Appendix II
RTI/ACS/174-005/94-05

Routine Maintenance Procedure for Hoods in the Cleanroom Facility

Monitoring of hoods must be done at the beginning of each week.

Please notify R. Fernando (Room 181, Ext. 6730) in the event of any problem or malfunction of the hood operation.

Hood Type: 4' hood in the Clean Lab. Period: MM/YY - MM/YY

Date	Pressure (i.w.g.)	Indicator Light		Comments	Initial
		Normal	Caution/Alert		

Appendix II
RTI/ACS/174-005/94-06

Routine Maintenance Procedure for Hoods in the Cleanroom Facility

Monitoring of hoods must be done at the beginning of each week.

Please notify R. Fernando (Room 181, Ext. 6730) in the event of any problem or malfunction of the hood operation.

Hood Type: 4' hood in the Instrument Lab.

Period: MM/YY - MM//YY

Date	Pressure (i.w.g.)	Indicator Light		Comments	Initial
		Normal	Caution/Alert		

Appendix D

RTI/ACS/SOP-174-007

Revision 0

Standard Operating Procedures for Purification of Reagents in the ACS Inorganic Clean Lab Facility for Trace/Ultratrace Metal Analysis

Research Triangle Institute
Analytical and Chemical Sciences
Post Office Box 12194
Research Triangle Park, NC 27709

September 1994

RTI/ACS-SOP-174-007

Standard Operating Procedures for Purification of Reagents in the ACS Inorganic Clean Lab Facility for Trace/Ultratrace Metal Analysis

1.0 Scope and Application

1.1 Analysis of trace element concentrations at and below 1 mg/mL (ppb) is often required. Contamination is the major problem in getting high quality data at these levels. Contamination in trace and ultratrace analysis is understood as the increase in the measured amount or concentration of a component, resulting from its introduction at various stages of the analytical procedure from sources other than the sample. Several independent sources, besides the sample itself, add to the final signal for a particular analyte. These are the laboratory atmosphere and working areas, tools and apparatus associated with sampling, sample preparation, laboratory ware, and reagents. Compared to other sources of contamination, contribution from reagents can often be measured quantitatively and can also be reduced effectively. Purification of reagents as a means to prevent contamination from reagents is discussed here along with the purification procedures.

1.2 A list of most commonly used reagents in trace/ultratrace metal analysis is given in Table 1. These include water, acids, bases, buffers, oxidants, reductants and other reagent chemicals. The demand for high purity reagents has been met by commercial chemical manufacturers in some cases. However for many others, specific purification procedures need to be developed to adequately control the contamination level.

Table 1. Reagents that are Commonly Used in Trace/Ultratrace Metal Analysis by Graphite Furnace Atomic Absorption Spectrometry and Hydride Generation Atomic Fluorescence Spectrometry

Acids and Bases Analysis	Reagents for As and Hg
Nitric acid	Potassium iodide
Sulfuric acid	Potassium bromide
Hydrochloric acid	Sodium borohydride
Perchloric acid	Potassium bromate
Sodium hydroxide	Tin (II) chloride
Matrix Modifiers for GFAA	**General Purpose Reagents**
Palladium	Hydrogen peroxide
Magnesium nitrate	
Ammonium dihydrogen phosphate	
Diammonium hydrogen phosphate	
Hydroxylamine hydrochloride	
Nickel nitrate	
Triton X-100	
Ascorbic acid	

2.0 Summary

The procedures given here are used to purify the reagents that are not available from the manufacturer at the required purity. These procedures are tested in the cleanroom environment for their performance in producing high purity reagents and will be modified as necessary.

3.0 Interferences

3.1 Extreme care must be taken to avoid the contamination during the purification process and also not to recontaminate the purified reagents. The primary sources of contamination are particulates in air, impurities in reagents that are used to purify the component of interest, trace elements from containers, sample handling by analysts, etc. With the use of Class 100 cleanroom environment and by following proper procedures for sample handling, it may be possible to keep the contaminants at a level below the instrument detection limit.

3.2 Storage vessels, storage conditions (temperature, humidity, etc.), and storage locations must be chosen appropriately to maintain the quality of the purified reagents.

4.0 Safety

Since the toxicity of the chemicals used in these procedures is not clearly defined, they should be treated as potential health hazards at all times and personal exposure to these chemicals should be minimized.

5.0 Equipment

5.1 Graphite Furnace Atomic Absorption Spectrometer (GFAA)

Perkin-Elmer 5100 atomic absorption spectrometer with a transversely heated graphite furnace and Zeeman background correction is used to analyze the purified reagents to established their purity. The procedures for the operation of PE 5100ZL are given in ACS/SOP-171-005.

5.2 Hydride Generation Atomic Fluorescence Spectrometer (HGAF)

PS Analytical hydride generation atomic fluorescence spectrometer (HGAF) is used to analyze the purified reagents for As, and thus establish their purity. The procedure for the operation of the HGAF is given in ACS/SOP-174-008.

6.0 Storage

Once purified, the purity of the reagent is critically dependent upon the storage conditions and the duration of storage, container material, storage location and temperature are particularly important.

Purified reagents are stored in thoroughly cleaned vessels (Sections 7.1.2.1 and 7.2.2.1) and prior to transfer of the purified reagent, the vessel must be rinsed with the reagent being stored. Storage vessels must be clearly labelled with the reagent name, concentration, date prepared and other relevant information. If the reagent requires specific storage conditions, it will be stored accordingly, otherwise it is placed inside an air-tight polyethylene bag and stored in metal free cabinet in the Clean Lab.

7.0 Reagent Purification Procedures

7.1 Ammonium Dihydrogen Phosphate (Ammonium Phosphate, Monobasic) - $NH_4H_2PO_4$

7.1.1 Reagents

All reagents must be of recognized analytical grade, unless specified otherwise.

- Ammonium dihydrogen phosphate - 1.667 g
- Ammonium hydroxide - about 100 mL
- Chelex-100 chelating ion exchange resin
- Ethylenediaminetetraacetic acid diammonium salt (EDTA) - 0.556 g.

7.1.2 Equipments

7.1.2.1 Laboratory Ware

All labware (glassware/plasticware) must be thoroughly cleaned by washing with detergent and water followed by rinsing with deionized water. Then they are acid leached in warm 20% nitric acid for at least 12 hrs, drained, thoroughly rinsed with deionized water and are dried under HEPA filtered air.

The storage bottles that will be used to store the purified reagent must be cleaned by warming in 50% HNO_3 for 12-24 hrs followed by rinsing with deionized water several times.

- 50 mL Teflon or polypropylene beakers (2)
- 600 mL Teflon or polypropylene beaker
- 500 mL Teflon or polypropylene storage bottle
- Plastic column

7.1.2.2 Apparatus

- Analytical balance

7.1.3 Procedure

- Weigh out 1.667 g of ammonium dihydrogen phosphate into a 600 mL Teflon or polypropylene beaker and add sufficient deionized water to dissolve the material.
- Add 5.0 mL of 14 M ammonium hydroxide solution to the beaker containing ammonium dihydrogen phosphate and dilute to 500 mL with deionized water.
- Prepare a column of chelex-100 and pass a solution of ammonium hydroxide through the column to convert the chelating resin to ammonium form.
- Pass the ammonium dihydrogen phosphate solution through the column at a flow rate of 0.5 mL/min.
- Add 0.556 g of ethylenediaminetetraacetic acid diammonium salt to the eluent and dilute to 500 mL with deionized water. Store the solution in an acid leached storage bottle. The concentration of $NH_4H_2PO_4$ in the final solution is 0.03 M.

7.2 Magnesium Nitrate - $Mg(NO_3)_2$

7.2.1 Reagents

All reagents must be of recognized analytical grade, unless specified otherwise.

- Magnesium nitrate - 4 g
- Ammonium pyrrolidinedithiocarbamate (APDC) 0.25g

- Nitric acid - several drops
- Methyl isobutyl ketone (MIBK) - 750 mL

7.2.2 Equipment

7.2.2.1 Laboratory Ware

All labware (glassware/plasticware) must be thoroughly cleaned by washing with detergent and water followed by rinsing with deionized water. Then they are acid leached in warm 20% nitric acid for at least 12 hrs, drained, rinsed thoroughly with deionized water and are dried under HEPA filtered air.

The storage bottles that will be used to store the purified reagent must be cleaned by warming in 50% HNO_3 for 12-24 hrs followed by rinsing with deionized water several times.

- 1 L Teflon or polypropylene volumetric flask
- 1 L Teflon or polypropylene beaker
- 2 L Teflon or polypropylene separatory funnel
- 100 mL graduated cylinder (glass or plastic)
- 1 L Teflon or polypropylene storage bottle

7.2.2.2 Apparatus

- Analytical balance
- pH meter

7.2.3 Procedure

- Dissolve 4 g of $Mg(NO_3)_2$ in 1 L of deionized water in 1 L volumetric flask.
- Transfer the solution to 1 L beaker and add 0.25 g of APDC.
- Adjust the pH of the solution to 5.8 with HNO_3.
- Transfer one half of the solution to a 1 L separatory funnel and add 125 mL of MIBK.
- Extract the metal complexes with MIBK.
- Repeat the extraction 2 more times with a fresh portion of MIBK each time.
- Repeat the extraction procedure for the second half of the solution.
- Combine the aqueous phases and boil for 20 mins. to remove any MIBK.
- Store in a clean Teflon or polypropylene storage bottle.

8.0 References

1. Purification of Matrix Modifiers Used in GFAA, Fishman, et al., J. Assoc. of Anal. Chem., *69*, 706 (1986).
2. Purification of Analytical Reagents, Mitchell,J.W. Talanta, *29*, 993 (1982).
3. Purified Reagents for Trace Metal Analysis, Moody, J.R. and Beary, E.S., Talanta, *29*, 1003 (1982).
4. Purification of Analytical Reagents and Other Liquids by Low Temperature Vacuum Sublimation, Mitchell, J.W., Anal. Chem., *50*, 194 (1978).

Appendix E

Standard Operating Procedures for Mercury Analysis in

Water, Sediment and Tissue

Southeast Environmental Research Program
Florida International University
University Park
Miami, Florida 33199

July 27, 1993

Revised:
September 13, 1993
September 21, 1993
November 4, 1993

Mercury

Collection and Storage

Field Collection of Samples

Water samples are collected in Teflon (FEP) bottles. Collection is done while wearing vinyl gloves (Polyethylene shoulder length PPE glove, OakTech). Samples are then placed in zip-lock polyethylene bags and placed in an additional bag in a plastic ice chest/cooler. In the laboratory 1 ml of trace metal grade HCl is added per 100 ml of sample. These additions are done in a "Hg-free" room (described below). Solid (soil) samples are collected in polyethylene specimen cups (Elkay non-sterile wide-mouth specimen cups with screw caps) - 128 ml volume) and placed in polyethylene zip-lock bags. All field samples are kept in a cooler until they are returned to the laboratory. These coolers are used exclusively for low level Hg samples.

Sample Storage

A number of storage tests have been done in this laboratory to determine the type(s) of bottles which are best suited for long term storage of low level samples. Acidified water samples (1 ml 12 N HCl/100 ml sample) may be stored in Teflon (Nalgene FEP) bottles in either a refrigerator or outside the mercury-free room with no effects on the mercury concentration of the sample. Refrigerated storage and FEP bottles are recommended. Polyethylene (Nalgene LDPE) bottles stored in the refrigerator show minor accumulation and cannot be used for storage of low level samples unless refrigerated. The plastic itself leaches mercury into the solution. This effect is facilitated by acid washing. Mercury accumulated in acid washed bottles to approximately 70-80 ppt with accumulation in non-washed bottles at 15 ppt in 30 days. For higher level samples the storage vessel type is not as critical.

Analytical Methods

Cleanroom

All glassware, acids, reagents, etc. Are stored in this room. It is equipped with a bank of laminar flow hoods, a separate water supply and gold-charcoal filter apparatus, refrigeration unit, oven, analytical balance, and a "flypaper" covered floor which is changed when needed.

Contamination is checked weekly by monitoring acidified (1% HCl) replicate water samples which are stored open in and outside the laminar flow hoods. Data on this quality control monitoring is stored in both as lotus file on computer and as hard copy in a data notebook. If significant levels of Hg are found (>20ppt) the source of this Hg will be located and if necessary, gold and charcoal filters will be reconditioned.

All new glassware and teflon used in analysis has been previously monitored for Hg content. In addition, acid-water blanks are run in glassware each analysis. No glassware is reused. All pipettes, reusable teflon beakers, and constantly used lab items are rinsed 0.5 N HCl, followed by DI water (described in sample preparation section) directly prior to use.

Teflon bottles which have been previously used for samples are rinsed twice with DI water and filled with a 1% HCl. After filling, 1 ml of mixed brominating agent (see reagent section for preparation) for every 50 ml of acid water is added and the bottle is shaken. This mixture remains in the bottles until it is used. The bottle is then rinsed 3X in DI water.

Sample Preparation

Water

Water samples may be analyzed for inorganic Hg and for total Hg. Inorganic samples are acidified as mentioned above. They are then ready for analysis.

Water Sample Preparation for Total Hg Analysis

Reagents/equipment list

Mercury-free Water:

Tap water is first filtered through a Culligan system consisting of activated charcoal and two mixed bed ion exchange cartridges and then piped to the mercury-free room. It is then passed through a Barnstead Mega-ohm B Pure System. This system is fitted with two filters (Thermolyne: colloid/organic-D0835, and ultrapure-D0809) in line with a 0.22 micron pleated particle filter. Mercury levels are not detectable by both our methods and independent lab analysis (<0.1 ppt). The only water available for use in the Hg laboratory is this Hg-free water. All reference to DI water in this "SOP" should be assumed to be Hg-free water as described above.

Bromination Reagents:

0.1 M Potassium Bromate:

Heat 8.385 g $KBrO_3$ overnight in a glass scintillation vial (Kimble 74511) at 250°C +/- 20°C in a furnace to remove mercury. After cooling dissolve the potassium bromate in 500 ml of deionized water and store in a glass bottle. Prepare weekly.

0.2 M Potassium Bromide:

Heat 11.9 g KBr overnight in a glass scintillation vial at 250°C +/- 20°C to remove mercury. After cooling dissolve the potassium bromide in 500 ml of deionized water and store in a glass bottle. Prepare weekly.

0.05 M Potassium Bromide (KBr): - 0.1 M Potassium Bromate ($KBrO_3$)

Mix equal volumes (100 ml) of bromate and bromide in a borosilicate 150 ml screw cap glass bottle. Prepare daily.

Hydroxylamine Hydrochloride:

Dissolve 2.4 g of $NH_2OH.HCl$ in 20 ml of deionized water in a glass scintillation vial. Prepare weekly.

Stannous Chloride:

To 20 grams of Stannous Chloride ($SnCl_2$) add 165 ml of 12 N HCl. Bring to 1000 ml using Hg-free deionized water in the borosilicate glass bottle used to hold the reagent during analysis. NOTE: no special treatment is needed for low level Hg (<10ppt). The instrument analysis compensates for background levels in the reagent.

12 N HCl:

Concentrated HCl (12 N HCl) is dispensed via a pipette or poured into a graduate cylinder either which has been previously acid washed and rinsed three times with DI water. 5% acid (0.6 N HCl) is contained in a Nalgene "low-boy" bottle and dispensed through a tube connected to the spigot.

Nitric Acid:

16 N Nitric acid is dispensed through a repipette which has been previously washed and rinsed three times with DI water.

Water sample digestion:

125 ml of acidified sample (1 ml 12 N HCl/100 ml sample) is brominated (2.5 ml $KBrO_3$/KBrO mixed reagent as described above). After that time, 500 µl of hydroxylamine hydrochloride is added to the solution to inhibit further reaction. Samples are permitted to settle for 30 min. before analysis.

Soils and Sediments (Carbonate and Clastic)

Sediment samples are homogenized and slurried using a glass bottled blender. 120 ml of sediment is slurried with 50 ml of distilled water. This mixture is then blended for 3 min. Using a syringe, 5 ml of slurry are removed, placed in a polyethylene specimen cup and diluted by adding 45 ml of 5% HCl. (The HCl acts to neutralize carbonate sediments prior to digestion. It is necessary to prevent a violent reaction when the vessel is subsequently sealed and autoclaved.) After mixing, 1 ml of this solution is transferred to a 10 ml ampule. Nitric acid (2 ml conc. HNO_3) is added to the ampule and it is left to stand for 20 min. The ampule is then sealed and autoclaved for 1 h at 150°C. Ampules must be cooled completely before further processing.

To process ampule contents pipette 0.5 ml of the digested solution into a 20 ml polyethylene scintillation vial (Kimble #58504) containing 19.5 ml of 0.12 N HCl solution.

Plant and Animal Tissue

Animal and plant tissue are treated similarly. Initial dilutions of homogenate vary with the type of tissue. In addition, the HCl step used to neutralize carbonates is not used for tissue analysis. Additional details of the tissue processing procedure will be added in future drafts.

Digestion of Standard Reference Material and Spiked Material

A series of method tests have been run both with spiked tissue, spiked sediment, and NBS or NIST certified samples to test for digestion efficiency. NBS oyster tissue (566a), NIST sediment nominal 50 µg/g (8407) and 60 µg/g (8406) were used in these tests. In addition, a sample of certified material is digested and run with each analysis of tissue or sediment. Digestion efficiency is 100% in all cases.

Sample Storage After Preparation

Samples may be stored, ampulated for an indefinite time until ready to be analyzed.

Standard Preparation

All preparation and storage of secondary standards is done in a Hg-free room. Primary working standard is prepared and stored outside the Hg-free room. The primary stock standard is made by addition of 1 ml of NBS certified primary Hg standard (SEPEX PLHG4-2X) 1000 µg/ml) to 1000 ml of filtered deionized water plus 5 ml of trace metal grade HCl. This standard is good for two days. Secondary standards are made in 500 ml nalgene FEP bottles. Concentrated HCl (5 ml) is added to 495 ml of filtered deionized water in a Polyethylene

bottle. When acids and brominating agents are added, the external laboratory hood is turned on creating a negative pressure in the area where acid addition is being done. The primary stock is then brought into the Hg-free room and 5 µl-25 µl (depending on final concentration, i.e. 10ppt-50ppt) is added to the bottles containing the water-acid mixture. Make up daily. All pipettes, micropipettes and pipette tips are calibrated before use using analytical balance. The temperature of the cleanroom is assumed to approximate 25°C and DI water is used as a standard to weigh.

Analytical Instrumental Technique

Cold Vapor Atomic Fluorescent Spectrometry (AFS) is the method used for Hg determination. The system used is a PSA Merlin plus supplied by Questron Corporation, Princeton, New Jersey 08543. This system contains an autosampler, vapor generator, fluorescence monitor and an IBM compatible computer system as the electronic data interface. In the AFS method, $SnCl_2$ is mixed with the liquid sample fed by the auto sampler, which then enters a gas liquid fritted separator (no maintenance has been necessary for this separator to date). The sample flows through peristaltic pump tubing (changed once every two weeks). As mercury enters the vapor phase it is stripped and carried along a gas stream (Argon- Zero grade) to the detector. The detection limit is better than 0.3 pg/1 (SD X 100/mean) of the baseline variation. Baseline noise translates into variation of between 0.087-0.185 ppt.

The only modifications we have made in the apparatus are:

1. Modification of the tubing in the hydride generator pump to prevent it from being pulled through the pump (a small rubber-banding is wrapped on approximately 5mm of the tubing at the left side of hydride generator pumping system).
2. Modification of the computer output using lotus to permit more accurate representation of the peak height data.
3. Placing the entire sampling system in a vented hooded area.

The procedure for running the instrument is as follows:

1. Tighten the three tubes in the hydride generator by clamping.
2. Tighten the peristaltic pump (pumps wash water and waste water).
3. Turn on the wash water to the system.
4. Turn on the computer.
5. Turn on the gas to the system. The argon (Zero grade) is flowed through two gas purifiers (charcoal and gold) before reaching the instrument.
6. Turn on the line stabilizer/conditioner.
7. Check to make sure no tubes are crimped, and that flow is smooth in all tubes before proceeding.
8. Allow the system to run on water for 15 minutes. NOTE: Depending on the age of the HG lamp, there may be considerable drift encountered in the first two hours of running. If drift problems arise, permit the lamp to stabilize before running samples.
9. Check gas flow at gas controller. The Argon is more precisely controlled through mass flow controllers. The optimum level for the carrier gas is 125 cc/min, with a shield gas level optimized at 140 cc/min. This flow controller is installed in front of the instrument, therefore, flow controllers on the instrument itself are open to full capacity.
10. After 15 min. switch the instrument on the $SnCl_2$.
11. NOTE: the sensitivity dial on the instrument is run at highest sensitivity for water but may be lowered for running of sediment, soil and tissue samples. This method is adequate for samples of the range we have run to date (final concentration 0.5-100ppt).
12. When the instrument is ready, zero the fluorescence detector and run D.I. acidified water to check baseline response of the instrument and guard against unexplained contamination from reagent preparation. When peak height of D.I. is 0.0-0.3 a standard may be run. Initially, one high standard is run to test for consistency of standard preparation and machine function. The range of standards will reflect the concentration of samples to analyze. Our water standards range from 0 ppt, to 50ppt while our soil sample standards will include a 100ppt standard. Eight standards (four concentrations, two replicates) are run for each standard curve. Standards run for low level samples are 10, 20, and 30 ppt. Standards, blanks, and high level samples (generally fish, sediments and soil) may be run in plastic scintillation vials. Water samples for total Hg are digested and run in glass scintillation vials. Digested acidified D.I. water samples are analyzed along with the samples as "digestion" blanks. This number is subtracted from sample values.
13. After running the standards, run four water blanks, and then run samples. All samples are run in replicate. One run of fifty samples plus standards takes 2 hours and uses 10ml of $SnCl_2$ per sample. A new standard curve is run when the $SnCl_2$ is replaced. In addition to running a full set of standards at the beginning of the analysis for each bottle of stannous chloride, replicate 50 ppt and zero ppt are

run after every 12 samples. NOTE: when running in 125 ml teflon bottles, remove the auto sampler and run three analyses from each bottle. After 5 bottles analyze the high (30ppt) standard and a D.I. Blank twice each. At the end of the run analyze 3 replicates of the 30ppt standard.

To turn off the instrument:

1. If you are using the results directly from the company supplied computer program, make sure you have printed and/or saved results. This program does not reliably transfer files to ascii or Lotus although it has functions for these tasks.
2. Replace the $SnCl_2$ solution with water and flush the instrument for 5 min.
3. Turn off the wash water.
4. Turn off the gas.
5. Run the pump until no more liquid is present in the pump tubing.
6. Turn off the line stabilizer and the computer.
7. Release tubing in the Hydride generator.
8. Check the waste water container and empty if necessary.

The procedure for running the computer is as follows:

1. Choose library, press select to choose methods and to see methods stored.

The method we use is:

2. If you wish to analyze samples or run blanks choose "analyze", "batch". Specify batch (sample) size. The computer will ask you whether the sample tray is in position and if you wish to change the sample tray. If you respond no twice, the instrument will then align the sample tray to the run you have specified. It will then be necessary to return to the analyze menu and check batch etc. You may then respond "YES" to the questions about tray position. This response will initiate the run. The instrument will analyze 50 samples. If you have more than 50 samples you must re-select analyze and you can choose a reference number which reflects the actual number of samples you are running.
3. If you wish to run standards, select "calibration". You may then choose to select a new curve, modify the old curve, etc.

Data Transfer

We do not find the program supplied with the AFS adequate for our needs. Specifically, there is not a direct method to correct for drift. In addition, the curve fitting function is not adequate for low level samples (< 1 ppt). We therefore after printing results from the machine, save the data as an asci file and transfer it into a Lotus spread sheet.

Data Handling

Calculation Standard Curve

Currently the NBS standard available for Hg is 1000µg/ml. With appropriate dilutions, standards can be made repeatable and reliably to 10ppt using standard dilution and pipetting procedures. The intercept location in the standard calculation becomes critical to proper calculation of concentrations. We have found the most reliable and reproducible results are generated by running a set of standards, and then assuming the standard regression is acceptable, ignoring all but the 50ppt standard and forcing the intercept through zero to generate the regression for linear estimate. This method is used for water samples and has also been found to be comparable to traditional estimating procedures used in sediment, soil and tissue analysis. All data is printed as hard copy and stored on computer disks. We maintain a back-up copy for each disk.

Appendix F

MSL/M-027-01

Standard Operating Procedures for

Total Mercury in Aqueous Samples by Cold Vapor Atomic Fluorescence

Battelle Pacific Northwest Laboratories
Marine Sciences Laboratory

May 28, 1993

Total Mercury in Aqueous Samples by Cold Vapor Atomic Fluorescence

1.0 Scope and Application

This is a peer-reviewed, published procedure and is applicable to the determination of total mercury in aqueous samples. All samples must be subjected to a BrCl UV oxidation step prior to analysis.

2.0 Summary of Method

The method is a cold vapor atomic fluorescence technique, based upon the emission of 254 nm radiation by excited Hg° atoms in an inert gas stream. Mercuric ions in the oxidized samples are reduced to Hg° with $SnCl_2$, and then purged onto gold-coated sand traps as a means of preconcentration and interference removal. Mercury vapor is thermally desorbed to a second "analytical" gold trap, and from that into the fluorescence cell. Fluorescence (peak area) is proportional to the quantity of mercury collected, which is quantified using a standard curve as a function of the quantity of sample purged.

Typical detection limit for the method is 0.200 ng/L as Hg.

3.0 Sample Collection, Preservation and Handling

3.1 Samples should be collected in teflon bottles. All teflon bottles should be washed and then boiled in concentrated HNO_3 for 24 hours. Vessels are thoroughly rinsed in tap water shown to contain negligible concentrations of mercury, then filled with 1% HCl in tap water and heated to 45±5° for 24 hours. This water is poured off and the vessels are refilled with 1% HCl and stored full until use. Just prior to use, vessels are emptied and dried in a clean drying oven at 75±5°C. Vessels to be shipped are packed in clean polyethylene bags. Samples can be collected in ashed or acid-cleaned borosilicate or quartz glass bottles with teflon lids. Under no circumstances should polyethylene, polypropylene, or vinyl containers be used.

3.2 Sample bottles should be rinsed once with sample water, and then filled. Samples should be preserved with 0.5% by volume of high purity HCl and refrigerated at 4±2°C, but not frozen.

4.0 Definitions

4.1 Atomic Fluorescence - detection based on fluorescent emission from excited atoms of a particular element at a characteristic wavelength. The amount of fluorescence, quantified by integration of peak area, is proportional to the concentration of the atom of interest.

4.2 Acid-cleaned - cleaned in nitric acid of the highest concentration and temperature which can be withstood by the item being cleaned. Glass and teflon containers are boiled in concentrated nitric acid for 48 hours.

5.0 Potential Interferences

5.1 Due to the strong oxidation step, followed by dual gold amalgamation, there are no observed interferences with the method. The potential exists for destruction of the gold traps (and consequently low values) if free halogens are purged onto them or if they are overheated (<500°C). When these instructions are followed, neither of these problems is likely to occur.

5.2 Water vapor may be collected on the gold traps, and be released on to the fluorescence cell where it condenses, giving a false peak due to scattering of the excitation radiation. This can be avoided by pre-drying the gold trap and by discarding traps which tend to absorb large quantities of water vapor.

5.3 As always with atomic fluorescence, the fluorescence intensity is strongly dependent upon the inertness of the carrier gas. The dual amalgamation technique eliminates quenching due to trace gases, but it still remains the analyst's responsibility to ensure high purity inert carrier gas and a leak-free analytical system.

6.0 Responsible Staff

6.1 Technician - sample prep, digestions
6.2 Analyst - analysis, calculations
6.3 QA/QC staff - data checking

7.0 Apparatus and Reagents

7.1 Apparatus

7.1.1 Cold Vapor Atomic Fluorescence Spectrophotometer (CVAF). The components of this detector include a four-watt low pressure mercury vapor lamp, a far UV quartz flow-through fluorescence cell (12 mm x 12 mm x 45 mm) with a 10 mm path length, and a UV-visible photomultiplier, sensitive to <230 nm isolated from outside light with a 254 nm interference filter. The carrier gas flow is controlled using a flowmeter with needle valve capable of keeping a constant flow of 25 ml/min.

7.1.2 Flowmeter/needle Valves. Flowmeter capable of controlling and measuring gas flow to the purge vessel at 200-500 ml/min.

7.1.3 Teflon Fittings. Connections between components and columns are made using 6.4 mm O.D. Teflon FEP tubing, and teflon friction-fit or threaded tubing connectors. Connections between components requiring mobility are made with 3.2 mm O.D. Teflon tubing due to its greater flexibility.

7.1.4 Acid Fume Pretrap. A 10 cm x 0.9 cm diameter teflon tube containing 2-3g of reagent grade, non-indicating 8-14 mesh soda lime, packed between silanized glass wool plugs. This trap is purged of Hg by placing it on the output of a clean cold vapor generator, filled with 1% HCl, and purging overnight with N_2 at 100 ml/min.

7.1.5 Cold Vapor Generator. A 250 ml or 125 ml florence flask with standard taper 24/40 neck, fitted with a purging stopper having a coarse glass fit which extends to within 0.2 cm of the flask bottom.

7.1.6 Gold-coated Sand Column. Made from 10 cm lengths of 6.5 mm O.D. X 4 mm I.D. Quartz tubing, with a coarse quartz frit or crimp 2.0 cm from one end. The tube is filled with 3.4 cm of gold-coated ashed (800°C for 6 hours) quartz sand (60/80 mesh). The end is then plugged with quartz wool. Gold is applied to the sand as a coating several atoms thick using as ion exchange gilding apparatus. The columns are heated to 450-500°C with a coil consisting of 24 gauge nichrome wire at a potential of 10 VAC.

7.1.7 Oxidation Bottles. Acid-cleaned, 135 ml teflon bottles and caps.

7.1.8 Pipetters. All plastic pneumatic fixed and variable volume pipetters in the range of 10 µl to 5 ml (calibrated).

7.1.9 Recorder. Multi-range chart-recorder/integrator with 0.1 - 5.0 mV input and variable speed.

7.2 Reagents

7.2.1 Water. Deep well tap water which has been determined to be very low (<0.02 ng/l) in mercury. The water is continuously monitored for mercury.

7.2.2 10% Stannous Chloride ($SnCl_2$). A solution containing 200 grams of $SnCl_2 \cdot 2H_20$ and 100 ml of concentrated HCl, brought to a final volume of 1 liter with mercury-free water. This solution is purged overnight with mercury-free nitrogen at 500 ml/min to remove all traces of mercury. Store tightly capped and in the dark.

7.2.3 5% Bromine Monochloride (BrCl). 27 g of KBr are added to a 2-liter bottle of concentrated HCl (<5 ng/Hg). A clean magnetic stir bar (teflon coated) is placed in the bottle and the solution is stirred for one hour in a fume hood. Next 38 g of pre-analyzed, low Hg $KBr0_3$ are slowly added to the acid as stirring continues. CAUTION: This process should always be carried out in a fume hood. The fumes from this reagent are very corrosive and a strong irritant. When all of the $KBr0_3$ has been added, the solution should have gone from yellow to red to orange. Loosely cap the bottle and allow to stir another hour before tightening the lid.

7.2.4 30% Hydroxylamine Hydrochloride. Dissolve 1 of $NH_2OH \cdot HCl$ in low Hg water to make 500 ml. This solution may be purified by adding 1.0 ml $SnCl_2$ reagent and purging overnight at 500 ml/min with Hg-free N_2.

7.2.5 Stock Mercury Standard. A commercially available 1000 mg Hg/l atomic absorption standard is used.

7.2.6 Secondary Standard Solution. 0.100 ml is diluted with Hg-free water containing 5 ml BrCl, to a final volume of 100 ml in a teflon bottle. This solution contains 1000 ng/liter and should be restandardized or replaced annually.

7.2.7 Working Standard Solution. Dilute 1.0 ml of the 2° mercury standard to 100 ml with Hg-free water containing 1% (by volume) bromine monochloride, using a 100 ml class A volumetric flask. This solution has a[Hg] 10.0 ng/ml and should be replaced semi-annually.

7.2.8 Nitrogen. Grad 4.5 nitrogen which has been further purified by theremoval of Hg using an in-line gold coated sand trap.

7.2.9 Helium or Argon. Grade 5.0 inert gas which has been further purified bythe removal of Hg using an in-line gold coated sand trap.

8.0 Procedure

8.1 Sample Preparation

8.1.1 Place a 50±5 ml aliquot of the acidified sample in a 125 ml teflon bottle, either by a pipette or by weight difference, recording the volume on a mercury datasheet. Add 0.5 ml of BrCl reagent to each sample, and place the bottle in the UV oxidation booth for 2 hours.

8.2 Analysis

8.2.1 Add 75 ml of low Hg water to each bubbler, followed by 1 ml of conc. HCl and 0.500 ml of $SnCl_2$ solution. The bubbler is purged with N_2 at 350 ml/min for 10 minutes, then a gold-coated sand trap is attached to the soda lime pretrap and purged for 20 minutes. This value is the bubbler blank. Just prior to analysis, add 0.250 ml $NH_2OH{\cdot}HCl$, or half volume of $NH_2OH{\cdot}HCl$ as BrCl, to the samples and let react for 5 minutes. The yellow color should disappear as the $NH_2OH{\cdot}HCl$ reacts with the BrCl. To analyze samples, attach a fresh gold-coated sand trap to the soda lime trap, then add 30±5ml of digestate to each bubbler (by weight difference). Record the sample number and the weight of the sample bottle before and after adding an aliquot to the bubbler on a mercury datasheet. Weigh the samples on a balance which is gently swirled, and the sample is purged for 20 minutes. New samples may be sequentially added to the bubblers, up to a maximum of 3 consecutive samples. After 3 samples, rinse the bubblers with clean low Hg water and the above sequence is repeated.

8.2.2 To analyze the mercury contained on the gold trap, the gold trap is placed inside a coil of nichrome wire and then inserted in-line with a mercury analyzer incoming Hg-free He and the second (analytical) gold-coated sand trap. The He flow rate should be about 30 ml/min. The system is purged for 2 minutes to dry off any condensed water vapor. 10 VAC is applied to the nicrome coil on the working gold-coated trap for 4 minutes, thermally desorbing the Hg as Hg^0 which is then resorbed by the analytical gold-coated sand column. The voltage to the working gold-coated sand trap is turned off, and a cooling stream of compressed air is directed towards the trap. 10 VAC is applied to the analytical gold-coated sand trap, and the integrator is activated. The analytical traps heated for 4.0 minutes, or 1 minute beyond the point where the mercury peak returns to baseline.

8.2.3 Following the integration of the mercury peak, the voltage to the analytical trap is turned off. A stream of cooling compressed air is directed towards the analytical trap. The sample gold-coated sand trap is removed from the gas stream, and the teflon end plugs are replaced until it is needed to collect another sample. The next sample gold-coated sand trap is placed inside the nichrome wire coil, placed in-line with the mercury analyzer incoming Hg-free, and the procedure is repeated. Under no circumstances should a sample gold-coated sand trap be heated while the analytical column is still warm, or analyte may be lost by passing through the analytical column.

8.2.4 Peaks generated using this technique should be very sharp and almost symmetrical. The peak comes off at approximately 1 minute and has a half-height width of about 4 seconds. Broad or asymmetrical peaks are indicative of an analytical problem possibly including: low gas flow, water vapor on the column, or an analytical column damaged by chemical fumes or overheating. If the analytical column has been damaged, replace the column and the tubing downstream due to the possibility of gold migration on the downstream surfaces.

8.2.5 Cold vapor atomic fluorescence for mercury is linear over at least five orders of magnitude (Bloom and Fitzgerald, 1988). This method is virtually interference free, so the method of standard additions is not routinely applied. To run standards, an aliquot of working standard solution in the range of 1 ng Hg is pipetted into a purged bubbler containing 0.5 ml of $SnCl_2$ solution, and analyzed as one would a sample.

8.2.6 Gold-coated sand traps should be kept track of by unique identifiers, so that any trap producing poor results can be quickly recognized and discarded. Occasionally due to inadvertent contact with halogen fumes, bubbler solution, organic fumes, or overheating, a sample gold-coated sand trap may become damaged irreproducible results. Suspect gold-coated sand traps should be checked with at least two consecutive standard runs before continued use.

8.2.7 The major cause of analytical problems with this method is from using the soda lime pretraps too long. These traps should be purged overnight as described and then used for only one day's analytical work. Longer use risks irreproducibility, as the traps may begin retarding the flow of Hg^0. Also, as the traps become very wet there is a risk of NaOH-saturated water drops coming off onto the gold-coated sand traps.

9.0 Quality Control

9.1 All quality control data should be maintained and available for each reference or inspection.

9.2 Quality assurance data must be composed of a minimum of 2 blanks and 2 certified reference materials. Such checks should be run at least twice (1 standard and 1 blank) per day, or every 20 samples, whichever is first.

9.3 Samples containing high analyte concentrations may be run either following dilution, or on a separate run at a lower instrumental sensitivity.

9.4 Duplicate or triplicate analyses (depending upon client preference) should be run once every 10 samples or once per batch, whichever comes first.

9.5 No certified materials exist for Hg in water near ambient levels. NRCC or NBS certified standard materials for mercury in tissues and sediments should be analyzed as a QA/QC measure in lieu of these.

9.6 Procedural spike recoveries should be run once per 10 samples or once per batch, whichever comes first, in the absence of a suitable certified standard tissue, or at the request of the client.

10.0 Calculations

10.1 Calculations may be made using either a best fit linear regression analysis of the standards and blanks or by using the average response factor method.

10.1.1 Average Response Factor Method:

$$\text{Ave Response Factor (RF)} = \frac{\sum((\text{std peak area} - \text{blk area})/[\text{Hg}]\text{ng}}{\#\ \text{std}}$$

$$[\text{Hg}]\text{ng/L} = \frac{\text{sam pk area}}{\text{RF} * \text{V}} - \text{blk}$$

(Where std peak area is the standard peak area, blk area is the average blank area, [Hg] is the Hg concentration in ng/L, sam pk area is the sample peak area, v is the sample aliquot analyzed in liters, and RF is the average response factor in area/ng and blank in ng/L)

10.1.2 Linear Regression Method:

$$[\text{Hg}]\text{ng/L} = \frac{\text{sam pk area}}{\text{slope} * \text{v}}$$

(Where slope is the slope of the standard regression line in area/ng. For an explanation of the other variables, refer to the average response method above.)

The final sample concentrations must be corrected for dilution by reagents as follows:

[Hg]ng/L = [Hg]ng/L(analytical)*
(total volume/initial volume)

where: total volume = volume of sample + reagents

initial volume = volume of sample only

10.2 Method Detection Limit (MDL):

The MDL is calculated by recording the results of a standard analyzed seven times, whose concentration is within 10 times of the actual method detection limit.

$$\text{MDL}[\text{Hg}]\text{ng} = \text{SD} * t_{(0.01(1)(n-1))}$$

(Where SD is the standard deviation of the [Hg]ng of the standards analyzed multiplied by the student t value for 99% one tailed confidence interval with n-1 degrees of freedom.)

Detection Limit [Hg]ng/L = MDL/sam vol (L)

(Where MDL is the method detection limit [Hg]ng/L and sam vol is the volume of the sample analyzed in liters.)

11.0 References

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Attachment 1 (page 1 of 1)

MERCURY DATA SHEET

DATE:

TYPE: ER TOTAL METHYL

ANALYST:

FILE NAME:

CALIBRATION:

CALIBRATION TYPE:

(SEE OVER)

PROJECT NAME(S):

Report #	Sample ID	Bubbler	Sample Volume	Units	[Hg]( )	Comments

Appendix G

Method 1631

Mercury in Water by Oxidation, Purge and Trap, and CVAFS

U.S. Environmental Protection Agency
Office of Water
Office of Science and Technology
Engineering and Analysis Division (4303)
401 M Street SW
Washington, DC 20460

DRAFT
January 1996

Mercury in Water by Oxidation, Purge and Trap, and CVAFS

Acknowledgments

This method was prepared under the direction of William A. Telliard of the Engineering and Analysis Division (EAD) within the U.S. Environmental Agency's (EPA's) Office of Science and Technology (OST). The method was prepared by Nicholas Bloom of Frontier GeoSciences under EPA Contract 68-C3-0337 with the DynCorp Environmental Programs Division. Additional assistance in preparing the method was provided by Interface, Inc.

Disclaimer

This sampling method has been reviewed and approved for publication by the Analytical Methods Staff within the Engineering and Analysis Division of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Questions concerning this method or its application should be addressed to:

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Introduction

This analytical method supports water quality monitoring programs authorized under the Clean Water Act. Section 304(a) of the Clean Water Act requires EPA to publish water quality criteria that reflect the latest scientific knowledge concerning the physical fate (e.g., concentration and dispersal) of pollutants, the effects of pollutants on ecological and human health, and the effect of pollutants on biological community diversity, productivity, and stability.

Section 303 of the Clean Water Act requires states to set a water quality standard for each body of water within its boundaries. A state water quality standard consists of a designated use or uses of a waterbody or a segment of a waterbody, the water quality criteria that are necessary to protect the designated use or uses, and an antidegradation policy. These water quality standards serve two purposes: (1) they establish the water quality goals for a specific waterbody, and (2) they are the basis for establishing water quality-based treatment controls and strategies beyond the technology-based controls required by Sections 301(b) and 306 of the Clean Water Act.

In defining water quality standards, the state may use narrative criteria, numeric criteria, or both. However, the 1987 amendments to the Clean Water Act required states to adopt numeric criteria for toxic pollutants (designated in Section 307(a) of the Act) based on EPA Section 304(a) criteria or other scientific data, when the discharge or presence of those toxic pollutants could reasonably be expected to interfere with designated uses.

In some cases, these water quality criteria are as much as 280 times lower than those achievable using existing EPA methods and required to support technology-based permits. Therefore, EPA developed new sampling and analysis methods to specifically address state needs for measuring toxic metals at water quality criteria levels, when such measurements are necessary to protect designated uses in state water quality standards. The latest criteria published by EPA are those listed in the National Toxics Rule (58 *FR* 60848) and the Stay of Federal Water Quality Criteria for Metals (60 *FR* 22228). These rules include water quality criteria for 13 metals, and it is these criteria on which the new sampling and analysis methods are based. Method 1631 was specifically developed to provide reliable measurements of mercury at EPA WQC levels.

In developing these methods, EPA found that one of the greatest difficulties in measuring pollutants at these levels was precluding sample contamination during collection, transport, and analysis. The degree of difficulty, however, is highly dependent on the metal and site-specific conditions. This analytical method, therefore, is designed to provide the level of protection necessary to preclude contamination in nearly all situations. It is also designed to provide the procedures necessary to produce reliable results at the lowest possible water quality criteria published by EPA. In recognition of the variety of situations to which this method may be applied, and in

recognition of continuing technological advances, the method is performance based. Alternative procedures may be used as long as those procedures are demonstrated to yield reliable results.

Requests for additional copies of this method should be directed to:

U.S. EPA NCEPI
11209 Kenwood Road
Cincinnati, OH 45242
1-800-490-9198

Note: This method is intended to be performance based, and the laboratory is permitted to omit any step or modify any procedure provided that all performance requirements set forth in this method are met. The laboratory is not allowed to omit any quality control analyses. The terms "must," "may," and "should" are included throughout this method and are intended to illustrate the importance of the procedures in producing verifiable data at water quality criteria levels. The term "must" is used to indicate the steps that are critical to production of reliable results; however, these procedures may be modified or omitted if the laboratory can demonstrate data quality is not affected.

1.0 Scope and Application

1.1 This method is for determination of total mercury (Hg) in filtered and unfiltered water by oxidation, purge and trap, desorption, and cold-vapor atomic fluorescence detection. This method is for use in EPA's data gathering and monitoring programs associated with the Clean Water Act, the Resource Conservation and Recovery Act, the Comprehensive Environmental Response, Compensation and Liability Act, and the Safe Drinking Water Act. The method is based on a contractor-developed method (Reference 1) and on peer-reviewed, published procedures for the determination of mercury and in aqueous samples, ranging from sea water to sewage effluent (References 2–5).

1.2 This method is accompanied by Method 1669: *Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels* (Sampling Method). The Sampling Method is necessary to ensure that contamination will not compromise trace metals determinations during the sampling process.

1.3 This method is designed for measurement of total Hg in the range of 0.2–100 ng/L and may be extended to higher levels by selection of a smaller sample size. This method is not intended for determination of metals at concentrations normally found in treated and untreated discharges from industrial facilities. Existing regulations (40 *CFR* Parts 400–500) typically limit concentrations in industrial discharges to the part-per-billion (ppb) range, whereas ambient mercury concentrations are normally in the low part-per-trillion (ppt) range.

1.4 The ease of contaminating ambient water samples with the metal(s) of interest and interfering substances cannot be overemphasized. This method includes suggestions for improvements in facilities and analytical techniques that should maximize the ability of the laboratory to make reliable trace metals determinations and minimize contamination. Section 4.0 gives these suggestions.

1.5 The detection limits and quantitation levels in this method are usually dependent on the level of background elements rather than instrumental limitations. The method detection limit (MDL; 40 *CFR* 136, Appendix B) for total mercury has been estimated to be 0.05 ng/L when no background elements or interferences are present. The minimum level (ML) has been established as 0.2 ng/L.

1.6 Clean and ultraclean—The terms "clean" and "ultraclean" have been applied to the techniques needed to reduce or eliminate contamination in trace metals determinations. These terms are not used in this method because they lack an exact definition. However, the information provided in this method is consistent with the summary guidance on clean and ultraclean techniques.

1.7 This method follows the EPA Environmental Methods Management Council's "Format for Method Documentation."

1.8 This method is "performance based." The analyst is permitted to modify the method to overcome interferences or lower the cost of measurements if all performance criteria are met. Section 9.1.2 gives the requirements for establishing method equivalency.

1.9 Any modification of this method, beyond those expressly permitted, shall be considered a major modification subject to application and approval of alternate test procedures under 40 *CFR* 136.4 and 136.5.

1.10 This method should be used only by analysts who are experienced in the use of CVAF analysis and who are thoroughly trained in the sample handling and instrumental techniques described in this method. Each analyst who uses this method must demonstrate the ability to generate acceptable results using the procedure in Section 9.2.

1.11 This method is accompanied by a data verification and validation guidance document, *Guidance on the Documentation and Evaluation of Trace Metals Data Collected for CWA Compliance Monitoring*. Data users should state data quality objectives (DQOs) required for a project before this method is used.

2.0 Summary of Method

2.1 A 100–2000 mL sample is collected directly into specially cleaned, pretested, fluoropolymer bottle(s) using sample handling techniques specially designed for collection of mercury at trace levels (Reference 6).

2.2 The sample is either field or laboratory-preserved by the addition of 5 mL of pretested 12 N HCl per liter of sample, depending on the time between sample collection and arrival at the laboratory.

2.3 Sample preparation and analysis are conducted at laboratory facilities specially designed for determination of mercury at 0.2–100 ng/L concentration. At this facility, a 100-mL sample aliquot is placed in a specially designed purge vessel.

2.4 Before analysis, 0.2 N BrCl solution is added to oxidize all Hg compounds to Hg(II).

2.5 After oxidation, the sample is sequentially prereduced with $NH_2OH{\cdot}HCl$ to destroy the free halogens, and then reduced with $SnCl_2$ to convert Hg(II) to volatile Hg(0).

2.6 The Hg(0) is separated from solution by purging with nitrogen onto a gold-coated sand trap.

2.7 The trapped Hg is thermally desorbed from the gold trap into an inert gas stream that carries the released Hg(0) into the cell of a cold-vapor atomic fluorescence spectrometer (CVAFS) for detection.

2.8 Quality is ensured through calibration and testing of the oxidation, purging, and detection systems.

3.0 Definitions

3.1 Total mercury as defined by this method means all BrCl-oxidizable mercury forms and species found in aqueous solution. This includes but is not limited to Hg(II), Hg(0), strongly organocomplexed Hg(II) compounds, adsorbed particulate Hg, and several tested covalently bound organomercurials (i.e., CH_3HgCl, $(CH_3)_2Hg$, and $C_6H_5HgOOCCH_3$). The recovery of Hg bound within microbial cells may require the additional step of UV photo-oxidation. In this context, "total" mercury refers to the forms and species of mercury, not to the total recoverable or dissolved fraction normally determined in an unfiltered or filtered sample, respectively. In this method, the total recoverable fraction will be referred to as "total recoverable" or "unfiltered."

3.2 Definitions of other terms used in this method are given in the glossary at the end of the method.

4.0 Contamination and Interferences

4.1 Preventing ambient water samples from becoming contaminated during the sampling and analytical process constitutes one of the greatest difficulties encountered in trace metals determinations. Over the last two decades, marine chemists have come to recognize that much of the historical data on the concentrations of dissolved trace metals in seawater are erroneously high because the concentrations reflect contamination from sampling and analysis rather than ambient levels. Therefore, it is imperative that extreme care be taken to avoid contamination when collecting and analyzing ambient water samples for trace metals.

4.2 Samples may become contaminated by numerous routes. Potential sources of trace metals contamination during sampling include: metallic or metal-containing labware (e.g., talc gloves that contain high levels of zinc), containers, sampling equipment, reagents, and reagent water; improperly cleaned and stored equipment, labware, and reagents; and atmospheric inputs such as dirt and dust. Even human contact can be a source of trace metals contamination. For example, it has been demonstrated that dental work (e.g., mercury amalgam fillings) in the mouths of laboratory personnel can contaminate samples that are directly exposed to exhalation (Reference 5).

4.3 Contamination Control

4.3.1 Philosophy—The philosophy behind contamination control is to ensure that any object or substance that contacts the sample is metal free and free from any material that may contain metals.

4.3.1.1 The integrity of the results produced cannot be compromised by contamination of samples. This method and the Sampling Method give requirements and suggestions for control of sample contamination.

4.3.1.2 Substances in a sample cannot be allowed to contaminate the laboratory work area or instrumentation used for trace metals measurements. This method gives requirements and suggestions for protecting the laboratory.

4.3.1.3 Although contamination control is essential, personnel health and safety remain the highest priority. The Sampling Method and Section 5 of this method give requirements and suggestions for personnel safety.

4.3.2 Avoiding contamination—The best way to control contamination is to completely avoid exposure of the sample to contamination in the first place. Avoiding exposure means performing operations in an area known

to be free from contamination. Two of the most important factors in avoiding/reducing sample contamination are (1) an awareness of potential sources of contamination and (2) strict attention to work being done. Therefore, it is imperative that the procedures described in this method be carried out by well-trained, experienced personnel.

4.3.3 Use a clean environment—The ideal environment for processing samples is a class 100 cleanroom (Section 6.1.1). If a cleanroom is not available, all sample preparation should be performed in a class 100 clean bench or a nonmetal glove box fed by mercury- and particle-free air or nitrogen. Digestions should be performed in a nonmetal fume hood situated, ideally, in the cleanroom.

4.3.4 Minimize exposure—The Apparatus that will contact samples, blanks, or standard solutions should be opened or exposed only in a cleanroom, clean bench, or glove box so that exposure to an uncontrolled atmosphere is minimized. When not being used, the Apparatus should be covered with clean plastic wrap, stored in the clean bench or in a plastic box or glove box, or bagged in clean zip-type bags. Minimizing the time between cleaning and use will also minimize contamination.

4.3.5 Clean work surfaces—Before a given batch of samples is processed, all work surfaces in the hood, clean bench, or glove box in which the samples will be processed should be cleaned by wiping with a lint-free cloth or wipe soaked with reagent water.

4.3.6 Wear gloves—Sampling personnel must wear clean, nontalc gloves (Section 6.9.7) during all operations involving handling of the Apparatus, samples, and blanks. Only clean gloves may touch the Apparatus. If another object or substance is touched, the glove(s) must be changed before again handling the Apparatus. If it is even suspected that gloves have become contaminated, work must be halted, the contaminated gloves removed, and a new pair of clean gloves put on. Wearing multiple layers of clean gloves will allow the old pair to be quickly stripped with minimal disruption to the work activity.

4.3.7 Use metal-free Apparatus—All Apparatus used for determination of metals at ambient water quality criteria levels must be nonmetallic, free of material that may contain metals, or both.

4.3.7.1 Construction materials—Only fluoropolymer containers should be used for samples that will be analyzed for mercury because mercury vapors can diffuse in or out of the other materials, resulting either in contamination or low-biased results. All materials, regardless of construction, that will directly or indirectly contact the sample must be cleaned using the procedures in this method and must be known to be clean and metal free before proceeding.

4.3.7.2 Serialization—It is recommended that serial numbers be indelibly marked or etched on each piece of Apparatus so that contamination can be traced, and logbooks should be maintained to track the sample from the container through the labware to injection into the instrument. It may be useful to dedicate separate sets of labware to different sample types; e.g., receiving waters vs. effluents. However, the Apparatus used for processing blanks and standards must be mixed with the Apparatus used to process samples so that contamination of all labware can be detected.

4.3.7.3 The laboratory or cleaning facility is responsible for cleaning the Apparatus used by the sampling team. If there are any indications that the Apparatus is not clean when received by the sampling team (e.g., ripped storage bags), an assessment of the likelihood of contamination must be made. Sampling must not proceed if it is possible that the Apparatus is contaminated. If the Apparatus is contaminated, it must be returned to the laboratory or cleaning facility for proper cleaning before any sampling activity resumes.

4.3.8 Avoid sources of contamination—Avoid contamination by being aware of potential sources and routes of contamination.

4.3.8.1 Contamination by carryover—Contamination may occur when a sample containing low concentrations of metals is processed immediately after a sample containing relatively high concentrations of these metals. To reduce carryover, the sample introduction system may be rinsed between samples with dilute acid and reagent water. When an unusually concentrated sample is encountered, it is followed by analysis of a laboratory blank to check for carryover. Samples known or suspected to contain the lowest concentration of metals should be analyzed first followed by samples containing higher levels.

4.3.8.2 Contamination by samples—Significant laboratory or instrument contamination may result when untreated effluents, in-process waters, landfill leachates, and other samples containing high concentrations of inorganic substances are processed and analyzed. This method is not intended for application to these samples, and samples containing high concentrations should not be permitted into the cleanroom and laboratory dedicated for processing trace metals samples.

4.3.8.3 Contamination by indirect contact—Apparatus that may not directly come in contact with the samples may still be a source of contamination. For example, clean tubing placed in a dirty plastic bag may pick up

contamination from the bag and subsequently transfer the contamination to the sample. Therefore, it is imperative that every piece of the Apparatus that is directly or indirectly used in the collection, processing, and analysis of ambient water samples be cleaned as specified in Section 11.

4.3.8.4 Contamination by airborne particulate matter—Less obvious substances capable of contaminating samples include airborne particles. Samples may be contaminated by airborne dust, dirt, particles, or vapors from unfiltered air supplies; nearby corroded or rusted pipes, wires, or other fixtures; or metal containing paint. Whenever possible, sample processing and analysis should occur as far as possible from sources of airborne contamination.

4.4 Interferences

4.4.1 Because all forms of Hg are oxidized in the BrCl oxidation step, there are no observed interferences with this method.

4.4.2 The potential exists for destruction of the gold trap if it is exposed to free halogens or if the trap is overheated (> 500°C).

4.4.3 Water vapor may collect in the gold trap and subsequently condense in the fluorescence cell upon desorption, giving a false peak due to scattering of the excitation radiation. Condensation can be avoided by predrying the gold trap, and by discarding those traps that tend to absorb large quantities of water vapor.

4.4.4 The fluorescence intensity is susceptible to the presence of foreign species in the carrier gas, which may cause "quenching" of the excited Hg atoms. The dual-trap technique in this method eliminates some quenching due to impurities in the carrier gas, but it remains the analyst's responsibility to ensure high-purity inert carrier gas and a leak-free analytical train.

5.0 Safety

5.1 The toxicity or carcinogenicity of each chemical used in this method has not been precisely determined; however, each compound should be treated as a potential health hazard. Exposure to these compounds should be reduced to the lowest possible level.

5.5.1.1 Chronic mercury exposure may cause kidney damage, muscle tremors, spasms, personality changes, depression, irritability and nervousness. Organomercurials may cause permanent brain damage. Because of the available toxicological and physical properties of the Hg, pure standards should be handled only by highly trained personnel thoroughly familiar with handling and cautionary procedures and the associated risks.

5.5.1.2 It is recommended that the laboratory purchase a dilute standard solution of the Hg in this method. If primary solutions are prepared, they shall be prepared in a hood, and a NIOSH/MESA-approved toxic gas respirator shall be worn when high concentrations are handled.

5.2 This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a current awareness file of OSHA regulations for the safe handling of the chemicals specified in this method. A reference file of material safety data sheets (MSDSs) should also be made available to all personnel involved in these analyses. It is also suggested that the laboratory perform personal hygiene monitoring of each analyst who uses this method and that the results of this monitoring be made available to the analyst. Additional information on laboratory safety can be found in References 7–10. The references and bibliography at the end of Reference 10 are particularly comprehensive in dealing with the general subject of laboratory safety.

5.3 Samples suspected to contain high concentrations of Hg are handled using essentially the same techniques employed in handling radioactive or infectious materials. Well-ventilated, controlled access laboratories are required. Assistance in evaluating the health hazards of particular laboratory conditions may be obtained from certain consulting laboratories and from State Departments of Health or Labor, many of which have an industrial health service. Each laboratory must develop a strict safety program for handling Hg.

5.3.1 **Facility**—When samples known or suspected of containing high concentrations of mercury are handled, all operations (including removal of samples from sample containers, weighing, transferring, and mixing) should be performed in a glove box demonstrated to be leaktight or in a fume hood demonstrated to have adequate airflow. Gross losses to the laboratory ventilation system must not be allowed. Handling of the dilute solutions normally used in analytical and animal work presents no inhalation hazards except in an accident.

5.3.2 **Protective equipment**—Disposable plastic gloves, apron or lab coat, safety glasses or mask, and a glove box or fume hood adequate for radioactive work should be used. During analytical operations that may give rise to aerosols or dusts, personnel should wear respirators equipped with activated carbon filters.

5.3.3 **Training**—Workers must be trained in the proper method of removing contaminated gloves and clothing without contacting the exterior surfaces.

5.3.4 **Personal hygiene**—Hands and forearms should be washed thoroughly after each manipulation and before breaks (coffee, lunch, and shift).

5.3.5 **Confinement**—Isolated work areas posted with signs, segregated glassware and tools, and plastic absorbent paper on bench tops will aid in confining contamination.

5.3.6 Effluent vapors—The effluent from the CVAFS should pass through either a column of activated charcoal or a trap containing gold or sulfur to amalgamate or react mercury vapors.

5.3.7 Waste handling—Good technique includes minimizing contaminated waste. Plastic bag liners should be used in waste cans. Janitors and other personnel must be trained in the safe handling of waste.

5.3.8 Decontamination

5.3.8.1 Decontamination of personnel—Use any mild soap with plenty of scrubbing action.

5.3.8.2 Glassware, tools, and surfaces—Sulfur powder will react with mercury to produce mercuric sulfide, thereby eliminating the possible volatilization of Hg. Satisfactory cleaning may be accomplished by dusting a surface lightly with sulfur powder, then washing with any detergent and water.

5.3.9 Laundry—Clothing known to be contaminated should be collected in plastic bags. Persons who convey the bags and launder the clothing should be advised of the hazard and trained in proper handling. If the launderer knows of the potential problem, the clothing may be put into a washer without contact. The washer should be run through a cycle before being used again for other clothing.

5.3.10 Wipe tests—A useful method of determining cleanliness of work surfaces and tools is to wipe the surface with a piece of filter paper. Extraction and analysis by this method can achieve a limit of detection of less than 1 ng per wipe. Less than 0.1 µg per wipe indicates acceptable cleanliness; anything higher warrants further cleaning. More than 10 µg on a wipe constitutes an acute hazard and requires prompt cleaning before further use of the equipment or work space, and indicates that unacceptable work practices have been employed.

6.0 Apparatus and Materials

Disclaimer: The mention of trade names or commercial products in this method is for illustrative purposes only and does not constitute endorsement or recommendation for use by the Environmental Protection Agency. Equivalent performance may be achievable using apparatus and materials other than those suggested here. The laboratory is responsible for demonstrating equivalent performance.

6.1 Sampling equipment

6.1.1 Sample collection bottles-Fluoropolymer, 125- to 1000-mL, with fluoropolymer or fluoropolymer-lined cap.

6.1.2 Cleaning—New bottles are cleaned by heating to 65–75°C in 4 N HCl for at least 48 h. The bottles are cooled, rinsed three times with reagent water, and filled with reagent water containing 1% HCl. These bottles are capped and placed in a clean oven at 60-70°C overnight. After cooling, they are rinsed three more times, filled with reagent water plus 0.4% (v/v) HCl, and placed in a mercury-free class 100 clean bench until dry. The bottles are then tightly capped (with a wrench) and double-bagged in new polyethylene zip-type bags until needed. After the initial cleaning, bottles are cleaned as above, except with only 6–12 h in the hot 4 N HCl step.

6.1.3 Filtration Apparatus

6.1.3.1 Filter—Gelman Supor 0.45-µm, 15-mm diameter capsule filter (Gelman 12175, or equivalent)

6.1.3.2 Peristaltic pump—115-V a.c., 12-V d.c., internal battery, variable-speed, single-head (Cole-Parmer, portable, "Masterflex L/S," Catalog No. H-07570-10 drive with Quick Load pump head, Catalog No. H-07021-24, or equivalent).

6.1.3.3 **Tubing for use with peristaltic pump**—styrene/ethylene/butylene/silicone (SEBS) resin, approx 3/8-in i.d. by approximately 3 ft (Cole-Parmer size 18, Catalog No. G-06464-18, or approximately 1/4-in i.d., Cole-Parmer size 17, Catalog No. G-06464-17, or equivalent). Tubing is cleaned by soaking in 5–10% HCl solution for 8–24 h, rinsing with reagent water in a clean bench in a cleanroom, and drying in the clean bench by purging with metal-free air or nitrogen. After drying, the tubing is double-bagged in clear polyethylene bags, serialized with a unique number, and stored until use.

6.2 Equipment for bottle and glassware cleaning

6.2.1 Vat, 100–200 L, high-density polyethylene (HDPE), half filled with 4 N HCl in reagent water.

6.2.2 Panel immersion heater, 500-W, all-fluoropolymer coated, 120 vac (Cole-Parmer H03053-04, or equivalent)

NOTE: Safety note: Read instructions carefully!! The heater will maintain steady state, without temperature feedback control, of 60–75°C in a vat of the size described. However, the equilibrium temperature will be higher (up to boiling) in a smaller vat. Also, the heater plate MUST be maintained in a vertical position, completely submerged and away from the vat walls to avoid melting the vat or burning out!

6.2.3 Laboratory sink in class 100 clean area, with high-flow reagent water (Section 7.1) for rinsing.

6.2.4 Clean bench, class 100, for drying rinsed bottles.

6.2.5 Oven, stainless steel, in class 100 clean area, capable of maintaining ± 5°C in the 60–70°C temperature range.

6.3 Cold vapor atomic fluorescence spectrometer (CVAFS): The CVAFS system used may either be purchased from a supplier, or built in the laboratory from commercially available components.

6.3.1 Commercially available: Tekran (Toronto, ON) Model 2357 CVAFS, or BrooksRand (Seattle, WA) Model 3 CVAFS, or equivalent.

6.3.2 Custom-built CVAFS (Reference 11). Figure 1 shows the schematic diagram. The system consists of the following:

6.3.2.1 Low-pressure 4-W mercury vapor lamp.

6.3.2.2 **Far UV quartz flow-through fluorescence cell**—12 mm x 12 mm x 45 mm, with a 10-mm path length (NSG cells or equivalent).

6.3.2.3 **UV-visible photomultiplier (PMT)**—sensitive to < 230 nm. This PMT is isolated from outside light with a 253.7-nm interference filter (Oriel Corp., Stanford, CT or equivalent).

6.3.2.4 Photometer and PMT power supply (Oriel Corp. or equivalent), to convert PMT output (nanoamp) to millivolts

6.3.2.5 **Black anodized aluminum optical block**—holds fluorescence cell, PMT, and light source at perpendicular angles, and provides collimation of incident and fluorescent beams (Frontier Geosciences Inc., Seattle, WA or equivalent).

6.3.2.6 Flowmeter, with needle valve capable of reproducibly keeping the carrier gas flow rate at 30 mL/min.

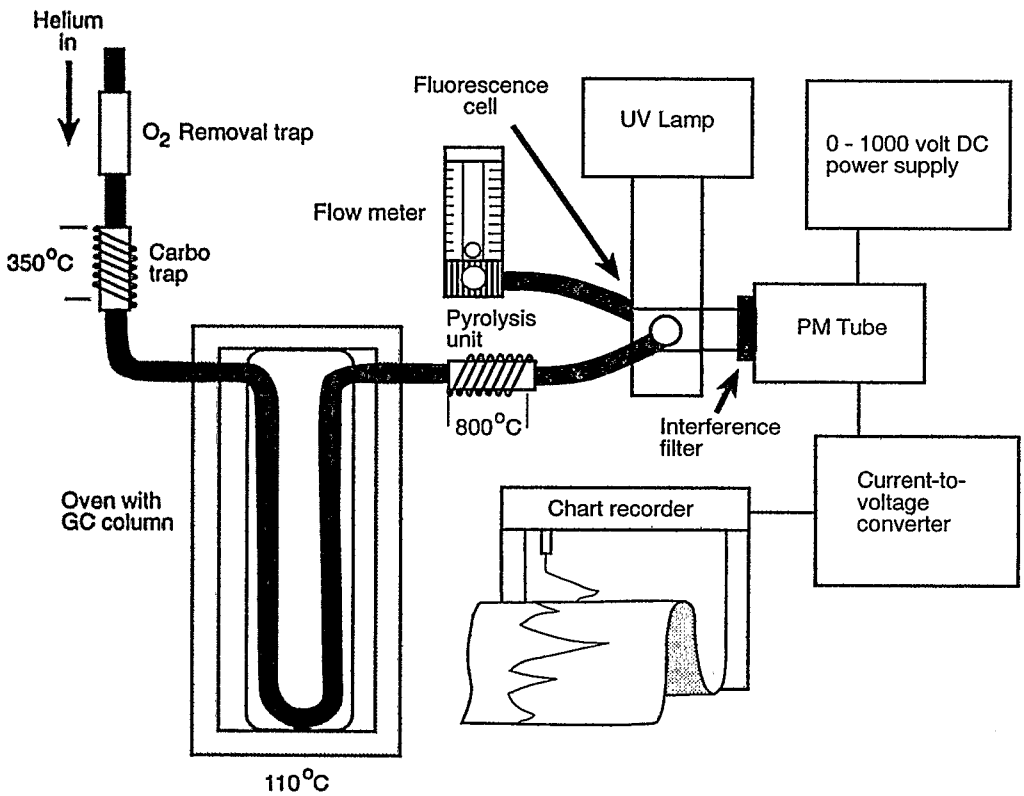

Figure 1. Schematic Diapor Atomic Fluorescence Spectrometer (CVAFS) Detector.

6.3.2.7 Ultra high-purity argon (grade 5.0)

6.4 Equipment for Hg purging system—Figure 2a shows the schematic diagram for the purging system. The system consists of the following:

6.4.1 Flow meter/needle valve—capable of controlling and measuring gas flow rate to the purge vessel at 350 (± 50) mL/min.

6.4.2 Fluoropolymer fittings—Connections between components and columns are made using 6.4-mm o.d. fluoropolymer tubing and fluoropolymer friction-fit or threaded tubing connectors. Connections between components requiring mobility are made with 3.2-mm o.d. fluoropolymer tubing because of its greater flexibility.

6.4.3 Acid fume pretrap—10-cm long x 0.9-cm i.d. fluoropolymer tube containing 2–3 g of reagent grade, nonindicating, 8–14 mesh soda lime chunks, packed between wads of silanized glass wool. This trap is cleaned of Hg by placing on the output of a bubbler and purging for 1 h with N_2 at 350 mL/min.

6.4.4 Bubbler—200-mL borosilicate glass (15 cm high x 5.0 cm diameter) with standard taper 24/40 neck, fitted with a sparging stopper having a coarse glass frit that extends to within 0.2 cm of the bubbler bottom.

6.5 Equipment for the Dual-trap Hg^0 Preconcentrating System

6.5.1 Figure 2b shows the schematic for the dual-trap amalgamation system (Reference 5).

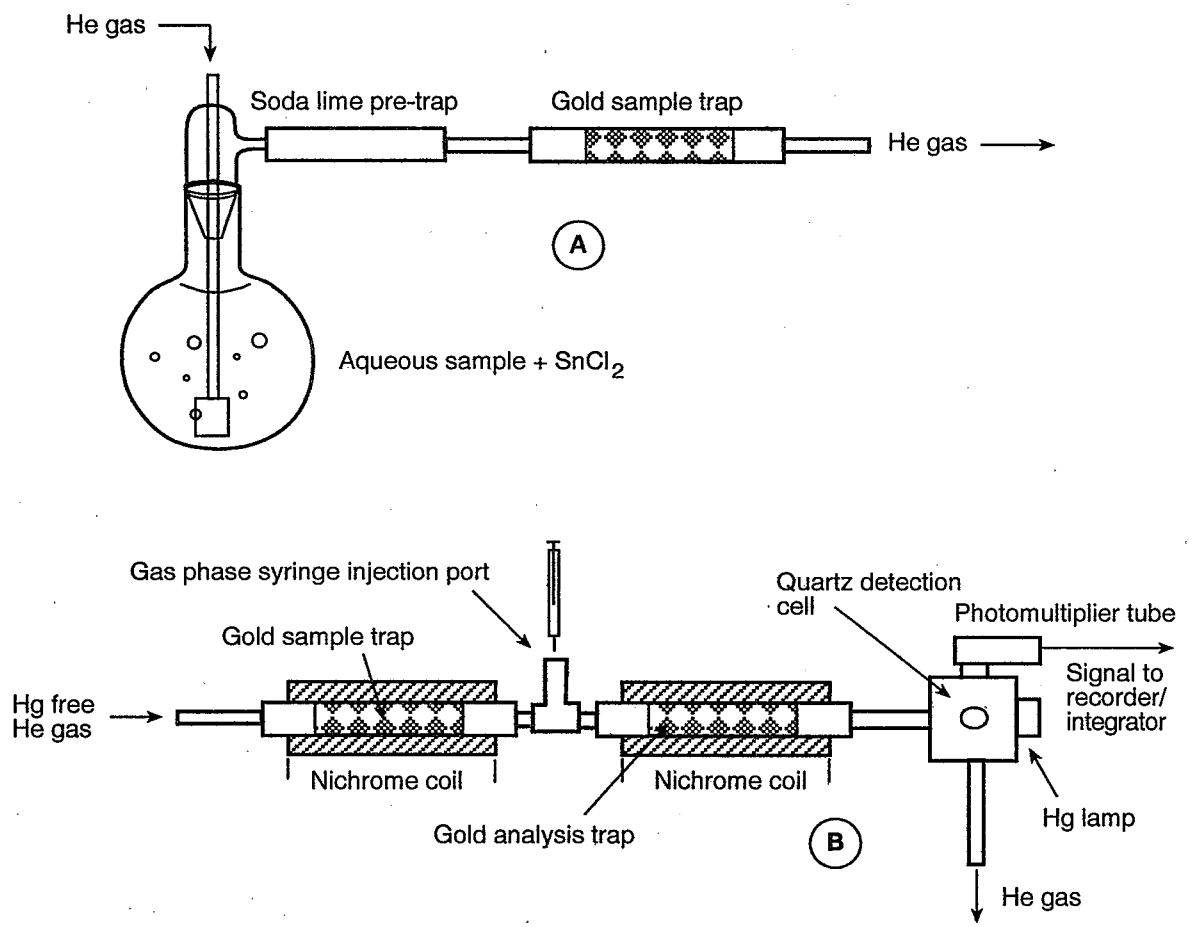

Figure 2. Schematic diagram of bubbler setup (A), and dual-amalystem (B), showing proper orientation of gold traps and soda lime pretraps.

6.5.2 **Gold-coated sand trap**—10-cm x 6.5-mm o.d. x 4-mm i.d. quartz tubing. The tube is filled with 3.4 cm of gold-coated 45/60 mesh quartz sand (Frontier Geosciences Inc., Seattle, WA or equivalent). The ends are plugged with quartz wool.

6.5.2.1 Traps are fitted with 6.5-mm i.d. fluoropolymer friction-fit sleeves for making connection to the system. When traps are not in use, fluoropolymer end plugs are inserted in trap ends to preclude contamination.

6.5.2.2 At least six traps are needed for efficient operation: one as the "analytical" trap, and the others to sequentially collect samples on.

6.5.3 **Heating of gold-coated sand traps**—To blank traps and desorb Hg collected on the traps, heat for 3.0 min to 450–500°C (a barely visible red glow when the room is darkened) with a coil consisting of 75 cm of 24-gauge Nichrome wire at a potential of 10 vac. Potential is applied and finely adjusted with an autotransformer.

6.5.4 **Timers**—The heating interval is controlled by a timer-activated 120-V outlet (Gralabor equivalent), into which the heating coil autotransformer is plugged. Two timers are required, one each for the "sample" trap and the "analytical" trap.

6.5.5 **Air blowers**—After heating, traps are cooled by blowing air from a small squirrel-cage blower positioned immediately above the trap. Two blowers are required, one each for the "sample" trap and the "analytical" trap.

6.6 **Recorder/integrator**—Any integrator with a range compatible with the CVAFS is acceptable.

6.7 **Pipettors**—All-plastic pneumatic fixed-volume and variable pipettors in the range of 10 uL to 5.0 mL.

6.8 Analytical balance capable of weighing to the nearest 0.01 g.

7.0 Reagents and Standards

7.1 **Reagent water**—Water in which mercury is not detected by this method; 18-MΩ ultrapure deionized water starting from a prepurified (distilled, R.O., etc.) source.

7.2 **Air**—It is very important that the laboratory air be low in both particulate and gaseous mercury. Ideally, mercury work should be conducted in a new laboratory with mercury-free paint on the walls. Outside air, which is very low in Hg, should be brought directly into the class 100 clean bench air intake. If this is impossible, air coming into the clean bench can be cleaned for mercury by placing a gold-coated cloth prefilter over the intake.

7.2.1 **Gold-coated cloth filter:** Soak 2 m^2 of cotton gauze in 500 mL of 2% gold chloride solution at pH 7. In a hood, add 100 mL of 30% $NH_2OH{\cdot}HCl$ solution, and homogenize into the cloth with gloved hands. As colloidal gold is precipitated, the material will turn black. Allow the mixture to set for several hours, then rinse with copious amounts of deionized water. Squeeze-dry the rinsed cloth, and spread flat on newspapers to air-dry. When dry, fold and place over the intake prefilter of your laminar flow hood.

CAUTION: Great care should be taken to avoid contaminating the laboratory with gold dust. This could cause interferences with the analysis if gold becomes incorporated into the samples or equipment. The gilding procedure should be done in a remote laboratory if at all possible.

7.3 **Hydrochloric acid**—trace-metal purified reagent HCl containing less than 5 pg/mL Hg.

7.4 **Hydroxylamine hydrochloride**—Dissolve 300 g of $NH_2OH{\cdot}HCl$ in reagent water and bring to 1.0 L. This solution may be purified by the addition of 1.0 mL of $SnCl_2$ solution and purging overnight at 500 mL/min with Hg-free N_2.

7.5 **Stannous chloride**—Bring 200 g of $SnCl_2{\cdot}2H_2O$ and 100 mL concentrated HCl to 1.0 L with reagent water. Purge overnight with mercury-free N_2 at 500 mL/min to remove all traces of Hg. Store tightly capped.

7.6 **Bromine monochloride (BrCl)**—Dissolve 27 g of reagent grade KBr in 2.5 L of low-Hg HCl. Place a clean magnetic stir bar in the bottle and stir for approximately 1 h in a fume hood. Slowly add 38 g reagent grade $KBrO_3$ to the acid with stirring. When all of the $KBrO_3$ has been added, the solution color should change from yellow to red to orange. Loosely cap the bottle, and allow to stir another hour before tightening the lid.

CAUTION: This process generates copious quantities of free halogens (Cl_2, Br_2, BrCl), which are released from the bottle. Add the $KBrO_3$ SLOWLY in a fume hood!

7.7 **Stock mercury standard**—NIST-certified 10,000-ppm aqueous Hg solution (NBS-3133). This solution is stable at least until the NIST expiration date.

7.8 **Secondary Hg standard**—Dilute 0.100 mL of the stock solution to 1.00 L of water containing 5 mL of BrCl. This solution contains 1.00 μg/mL (1.00 ppm) Hg. Keep in a tightly closed fluoropolymer bottle. This solution is stable indefinitely.

7.9 **Working Hg standard**—Dilute 5.00 mL of the secondary Hg standard to 1.00 L in a class A volumetric

flask with reagent water containing 0.5% by volume BrCl solution. This solution contains 5.0 ng/mL and should be replaced monthly.

7.10 Calibration solutions—Using the secondary Hg standard (Section 7.8), prepare five calibration solutions to contain Hg at a concentration of 0.2, 1.0, 5, 25, and 100 ng/L in reagent water (Section 7.1).

7.11 Nitrogen—Grade 4.5 (standard laboratory grade) nitrogen that has been further purified by the removal of Hg using a gold-coated sand trap.

7.12 Argon—Grade 5.0 (ultra high-purity, GC grade) inert gas that has been further purified by the removal of Hg using a gold-coated sand trap.

8.0 Sample Collection, Preservation, and Storage

8.1 Before samples are collected, consideration should be given to the type of data required, (i.e., dissolved or total recoverable), so that appropriate preservation and pretreatment steps can be taken. The pH of all aqueous samples must be tested immediately before aliquotting for processing or direct analysis to ensure the sample has been properly preserved. If properly acid-preserved, the sample can be held up to 6 months before analysis.

8.2 Samples are collected only into rigorously cleaned fluoropolymer bottles with fluoropolymer or fluoropolymer-lined caps. It is critical that the bottles have tightly sealing caps to avoid diffusion of atmospheric Hg through the threads (Reference 4). Clean bottles filled with high-purity 0.4% (v/v) HCl are dried, capped, and double bagged in new zip-type bags in the cleanroom, and stored in wooden or plastic boxes until use.

8.3 Collect samples using the Sampling Method (Reference 6). Procedures in the Sampling Method are based on rigorous protocols for collection of samples for mercury (References 4 and 11).

8.4 Sample filtration—For dissolved Hg, samples and field blanks are filtered through a 0.45-µm capsule filter at the field site. The Sampling Method describes filtering procedures. For the determination of total recoverable Hg, samples are filtered before preservation.

8.5 Preservation—Samples may be preserved by adding 5 mL/L of concentrated HCl (to allow both total and methyl Hg determination) or 5 mL/L BrCl solution, if total mercury only is to be determined. Acid- and BrCl-preserved samples are stabile for a minimum of 6 months.

8.5.1 Samples may be shipped to the laboratory unpreserved if they are (1) collected in fluoropolymer bottles, (2) filled to the top with no head space, (3) capped tightly, and (4) maintained at 0–4°C from the time of collection until preservation. The samples must be acid-preserved within 48 h after sampling.

8.5.2 Samples that are acid-preserved may lose Hg to coagulated organic materials in the water or the Hg may be condensed on the walls (Reference 12). Add BrCl directly to the sample bottle at least 24 h before analysis to prevent coagulation, condensation, or both. Aliquots for determination of other Hg species must be removed before BrCl is added. If BrCl cannot be added directly to the sample bottle, the bottle should be vigorously shaken before subsampling.

8.5.3 All handling of the samples in the laboratory should be undertaken in a mercury-free clean bench, after rinsing the outside of the bottles with reagent water and drying in the clean air hood.

8.5.4 If preserved in the laboratory, preserve a blank and OPR with each sample batch.

8.6 Storage—Sample bottles should be stored in polyethylene bags at 0–4°C until analysis.

9.0 Quality Control

9.1 Each laboratory that uses this method is required to operate a formal quality assurance program (Reference 13). The minimum requirements of this program consist of an initial demonstration of laboratory capability, ongoing analysis of standards and blanks as a test of continued performance, and the analysis of matrix spikes (MS) and matrix spike duplicates (MSD) to assess accuracy and precision. Laboratory performance is compared to established performance criteria to determine that the results of analyses meet the performance characteristics of the method.

9.1.1 The analyst shall make an initial demonstration of the ability to generate acceptable accuracy and precision with this method. This ability is established as described in Section 9.2.

9.1.2 In recognition of advances that are occurring in analytical technology, the analyst is permitted certain options to improve results or lower the cost of measurements. These options include automation of the dual-amalgamation system, direct electronic data acquisition, changes in the bubbler design (including substitution of a flow-injection system) to maximize throughput, and changes in the detector (i.e., CVAAS), where less sensitivity is acceptable or desired. Changes in the principle

of the determinative technique, such as the use of colorimetry, are not allowed. If an analytical technique other than the techniques specified in the method is used, that technique must have a specificity equal to or better than the specificity of the techniques in the method for the analytes of interest.

9.1.2.1 Each time this method is modified, the analyst is required to repeat the procedure in Section 9.2. If the change will affect the detection limit of the method, the laboratory is required to demonstrate that the MDL (40 *CFR* Part 136, Appendix B) is lower than one-third the regulatory compliance level or lower than the MDL of this method, whichever is higher. If the change will affect calibration, the analyst must recalibrate the instrument according to Section 10.

9.1.2.2 The laboratory is required to maintain records of modifications made to this method. These records include the following, at a minimum:

9.1.2.2.1 The names, titles, addresses, and telephone numbers of the analyst(s) who performed the analyses and modification, and the quality control officer who witnessed and will verify the analyses and modification.

9.1.2.2.2 A narrative stating the reason(s) for the modification(s).

9.1.2.2.3 Results from all quality control (QC) tests comparing the modified method to this method, including the following:

(a) Calibration (Section 10)

(b) Calibration verification (Section 9.5)

(c) Initial precision and recovery (Section 9.2)

(d) Analysis of blanks (Section 9.4)

(e) Accuracy assessment (Section 9.3)

(f) Ongoing precision and recovery (Section 9.6)

9.1.2.2.4 Data that will allow an independent reviewer to validate each determination by tracking the instrument output to the final result. These data are to include the following:

(a) Sample numbers and other identifiers

(b) Processing dates

(c) Analysis dates

(d) Analysis sequence/run chronology

(e) Sample weight or volume

(f) Copies of logbooks, chart recorder, or other raw data output

(g) Calculations linking raw data to the results reported

9.1.3 Analyses of MS and MSD samples are required to demonstrate the accuracy and precision and to monitor matrix interferences. Section 9.3 describes the procedure and QC criteria for spiking.

9.1.4 Analyses of laboratory blanks are required to demonstrate acceptable levels of contamination. Section 9.4 describes the procedures and criteria for analyzing a blank.

9.1.5 The laboratory shall, on an ongoing basis, demonstrate through analysis of the ongoing precision and recovery (OPR) sample and the quality control sample (QCS) that the system is in control. Sections 9.5 and 9.6 describe these procedures respectively.

9.1.6 The laboratory shall maintain records to define the quality of the data that are generated. Sections 9.3.7 and 9.6.3 describe the development of accuracy statements.

9.1.7 The determination of total Hg in water is controlled by an analytical batch. An analytical batch is a set of samples oxidized with the same batch of reagents, and analyzed during the same 12-hour shift. A batch may be from 1 to as many as 10 samples. Each batch must be accompanied by at least three bubbler blanks (Section 9.4), an OPR sample, and one MS and one MSD. If more than 10 samples are run during one 12-hour shift, an additional bubbler blank, OPR sample, and MS/MSD must be analyzed for each additional 10 or fewer additional samples. Reagent blanks for this determination are required when the batch of reagents (bromine monochloride plus hydroxylamine hydrochloride) are made, with verification in triplicate each month until a new batch of reagents is needed.

9.2 Initial demonstration of laboratory capability

9.2.1 Method detection limit—To establish the ability to detect Hg, the analyst shall determine the MDL determined according to the procedure in 40 *CFR* 136, Appendix B using the apparatus, reagents, and standards that will be used in the practice of this method. The laboratory must produce an MDL that is less than or equal to the MDL listed in Section 1.3 or one-third the regulatory compliance limit, whichever is greater. The MDL should be determined when a new operator begins work or whenever, in the judgment of the analyst, a change in instrument hardware or operating conditions would dictate that the MDL be redetermined.

9.2.2 Initial precision and recovery (IPR)—To establish the ability to generate acceptable precision and accuracy, the analyst shall perform the following operations:

9.2.2.1 Analyze four replicates of the working Hg standard (Section 7.9) according to the procedure beginning in Section 11.

9.2.2.2 Using the results of the set of four analyses, compute the average percent recovery (X), and the standard deviation of the percent recovery (s) for total Hg.

9.2.2.3 Compare s and X with the corresponding limits for initial precision and recovery in Table 1. If s and X meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however, s exceeds the precision limit or X falls outside the acceptance range, system performance is unacceptable. Correct the problem and repeat the test (Section 9.2.2.1).

Table 1. Acceptance Criteria For Performance Test

Acceptance Criterion	Section	Limits
Method Detection Limit	9.2.1	<0.2 ng/L
Initial Precision and Recovery	9.9.2	
Precision (s)	9.2.2.3	± 21%
Recovery (X)	9.2.2.3	79–121%
Interlaboratory Intercomparison	9.2.2.2	75–125%
Matrix Spike/Matrix Spike Duplicate	9.3	
Recovery	9.3.4	75–125%
Relative Percent Difference	9.3.6	± 24%
Bubbler Blanks	9.4	
Maximum	9.4.1.2	< 50 pg
Mean	9.4.1.3	< 25 pg
Ongoing Precision and Recovery	9.5	77–123%

9.3 Matrix spike (MS) and matrix spike duplicate (MSD)—To assess the performance of the method on a given sample matrix, the laboratory must spike, in duplicate, a minimum of 10% (1 sample in 10) from a given sampling site or, if for compliance monitoring, from a given discharge. Blanks (e.g., field blanks) may not be used for MS/MSD analysis.

9.3.1 The concentration of the spike in the sample shall be determined as follows:

9.3.1.1 If, as in compliance monitoring, the concentration of total Hg in the sample is being checked against a regulatory concentration limit, the spiking level shall be at that limit or at 1–5 times higher than the background concentration of the sample (determined in Section 9.3.2), whichever concentration is higher.

9.3.1.2 If the concentration of total Hg in a sample is not being checked against a limit, the spike shall be at the 7.9) or at 1–5 times the background concentration, whichever concentration is higher.

9.3.2 To determine the background concentration (B), analyze 1 sample aliquot from each set of 10 samples from each site or discharge according to the procedure in Section 11. If the expected background concentration is known from previous experience or other knowledge, the spiking level may be established a priori.

9.3.2.1 If necessary, prepare a standard solution appropriate to produce a level in the sample at the regulatory compliance limit or at 1–5 times the background concentration (Section 9.3.1).

9.3.2.2 Spike two additional sample aliquots with the spiking solution and analyze these aliquots to determine the concentration after spiking (A).

9.3.3 Calculate the percent recovery (P) in each aliquot using the following equation:

$$P = 100\frac{(A - B)}{T}$$

where:

A = Measured concentration of analyte after spiking
B = Measured concentration of analyte before spiking
C = True concentration of the spike

9.3.4 Compare the percent recovery (P) with the QC acceptance criteria in Table 1.

9.3.4.1 If the results of spike fail the acceptance criteria, and recovery for the OPR standard (Section 9.6) for the analytical batch is within the acceptance criteria in Table 1, an interference may be present. The result may not be reported for regulatory compliance purposes. If the interference can be attributed to sampling, the site or discharge should be resampled. If the interference can be attributed to a method deficiency, the analyst must modify the method, repeat the test required in Section 9.1.2, and repeat analysis of the sample and MS/MSD. However, when this method was written, there were no known interferences in the determination of total Hg using this method. If such a result is observed, the analyst should investigate it thoroughly.

9.3.4.2 If the results of both the spike and the OPR test fail the acceptance criteria, the analytical system is judged to be out of control. The analyst must identify and correct the problem and reanalyze the sample batch.

9.3.5 Relative percent difference between duplicates—Compute the relative percent difference (RPD)

between the MS and MSD according to the following equation using the concentrations found in the MS and MSD. Do not use the recoveries calculated in Section 9.3.3 for this calculation because the RPD is inflated when the background concentration is near the spike concentration.

$$RPD = 200x\frac{(|D1 - D2|)}{(D1 + D2)}$$

where:
D1 = concentration of Hg in the MS sample
D2 = concentration of Hg in the MSD sample

9.3.6 The RPD for the MS/MSD pair shall meet the acceptance criterion in Table 1. If the criterion is not met, the system is judged to be out of control. The problem must immediately be identified and corrected, and the analytical batch reanalyzed.

9.3.7 As part of the QC program for the laboratory, method precision and accuracy for samples should be assessed and records maintained. After analyzing five samples in which the recovery passes the test in Section 9.3.4, compute the average percent recovery (P_a) and the standard deviation of the percent recovery (s_p). Express the accuracy assessment as a percent recovery interval from $P_a - 2s_p$ to $P_a + 2s_p$. For example, if P_a = 90% and s_p = 10% for five analyses, the accuracy interval is expressed as 70–110%. Update the accuracy assessment regularly (e.g., after every five to ten new accuracy measurements).

9.4 **Blanks**—Blanks are critical to the reliable determination of Hg at low levels. The sections below give the minimum requirements for analysis of blanks. However, it is suggested that additional blanks be analyzed as necessary to pinpoint sources of contamination in, and external to, the laboratory.

9.4.1 **Bubbler blanks**—Bubbler blanks are analyzed to demonstrate freedom from system contamination.

9.4.1.1 Immediately after analyzing a sample for total Hg, place a clean gold trap on the bubbler, analyze the sample a second time using the procedure in Section 11, and determine the amount of Hg remaining in the system.

9.4.1.2 If the bubbler blank is found to contain more than 50 pg Hg, the system is out of control. The problem must be investigated and remedied, and the samples run on that bubbler must be reanalyzed. The remedy for a contaminated bubbler usually involves cleaning the bubbler, changing the soda lime trap on the affected bubbler, or both. If the blank from another bubbler contains less than 50 pg Hg, the data associated with that bubbler remain valid.

9.4.1.3 The mean result for all bubbler blanks (from bubblers passing the specification in Section 9.4.2) in an analytical batch (at least three bubbler blanks) is calculated at the end of the batch. The mean result must be < 25 pg with a standard deviation of < 10 pg for the batch to be considered valid. If the mean is < 25 pg, the value is subtracted from all raw data before results are calculated.

9.4.2 **Reagent blanks**—Since even reagent water often contains measurable Hg, blanks must be determined on solutions of reagents by adding these reagents to previously purged reagent water in the bubbler.

9.4.2.1 Add aliquots of BrCl (0.5 mL), NH_2OH (0.2 mL) and $SnCl_2$ (0.5 mL) individually to previously purged reagent water in the bubbler.

9.4.2.2 The presence of more than 25 pg of Hg indicates a problem with the reagent solution. The purging of reagent solutions with mercury-free nitrogen or argon can reduce Hg to acceptable levels.

9.4.3 **Field blanks**

9.4.3.1 Analyze the field blank(s) shipped with each set of samples (samples collected from the same site at the same time, to a maximum of 10 samples). Analyze the blank immediately before analyzing the samples in the batch.

9.4.3.2 If Hg or any potentially interfering substance is found in the field blank at a concentration equal to or greater than the ML (Table 1), or greater than one-fifth the level in the associated sample, whichever is greater, results for associated samples may be the result of contamination and may not be reported for regulatory compliance purposes.

9.4.3.3 Alternatively, if a sufficient number of field blanks (three minimum) are analyzed to characterize the nature of the field blank, the average concentration plus two standard deviations must be less than the regulatory compliance level or less than one-half the level in the associated sample, whichever is greater.

9.4.3.4 If contamination of the field blanks and associated samples is known or suspected, the laboratory should communicate this to the sampling team so that the source of contamination can be identified and corrective measures taken before the next sampling event.

9.4.4 **Equipment blanks**—Before any sampling equipment is used at a given site, the laboratory or cleaning facility is required to generate equipment blanks to demonstrate that the sampling equipment is free from

contamination. Two types of equipment blanks are required: bottle blanks and sampler check blanks.

9.4.4.1 Bottle blanks—After undergoing the cleaning procedures in this method, bottles should be subjected to conditions of use to verify the effectiveness of the cleaning procedures. A representative set of sample bottles should be filled with reagent water acidified to pH < 2 and allowed to stand for a minimum of 24 h. Ideally, the time that the bottles are allowed to stand should be as close as possible to the actual time that the sample will be in contact with the bottle. After standing, the water should be analyzed for any signs of contamination. If any bottle shows signs of contamination, the problem must be identified, the cleaning procedures corrected or cleaning solutions changed, and all affected bottles recleaned.

9.4.4.2 Sampler check blanks—Sampler check blanks are generated in the laboratory or at the equipment cleaning contractor's facility by processing reagent water through the sampling devices using the same procedures that are used in the field (see Sampling Method). Therefore, the "clean hands/dirty hands" technique used during field sampling should be followed when preparing sampler check blanks at the laboratory or cleaning facility.

9.4.4.2.1 Sampler check blanks are generated by filling a large carboy or other container with reagent water (Section 7.1) and processing the reagent water through the equipment using the same procedures that are used in the field (see Sampling Method). For example, manual grab sampler check blanks are collected by directly submerging a sample bottle into the water, filling the bottle, and capping. Subsurface sampler check blanks are collected by immersing the sampler into the water and pumping water into a sample container.

9.4.4.2.2 The sampler check blank must be analyzed using the procedures in this method. If any metal of interest or any potentially interfering substance is detected in the blank, the source of contamination or interference must be identified, and the problem corrected. The equipment must be demonstrated to be free from the metal(s) of interest before the equipment may be used in the field.

9.4.4.2.3 Sampler check blanks must be run on all equipment that will be used in the field. If, for example, samples are to be collected using both a grab sampling device and a subsurface sampling device, a sampler check blank must be run on both pieces of equipment.

9.5 Ongoing precision and recovery (OPR)—To demonstrate that the analysis system is in control and that acceptable precision and accuracy is being maintained within each analytical batch, the analyst shall perform the following operations:

9.5.1 Analyze the low-level Hg working standard (Section 7.9) and a bubbler blank before analysis of each analytical batch according to the procedure beginning in Section 11. Subtract the peak area of the bubbler blank from the area for the standard and compute the concentration for the blank-subtracted standard.

9.5.2 Compare the concentration with the limits for ongoing precision and recovery in Table 1. If the concentration is in the range specified, the analysis system is in control and analysis of samples and blanks may proceed. If, however, the concentration is not in the specified range, the analytical process is not in control. Correct the problem and repeat the ongoing precision and recovery test.

9.5.3 The laboratory should add results that pass the specification in Section 9.5.2 to IPR and previous OPR data and update QC charts to form a graphic representation of continued laboratory performance. The laboratory should also develop a statement of laboratory data quality for each analyte by calculating the average percent recovery (R) and the standard deviation of the percent recovery (s_r). Express the accuracy as a recovery interval from $R - 2s_r$ to $R + 2s_r$. For example, if R = 95% and s_r = 5%, the accuracy is 85–105%.

9.6 Quality control sample (QCS)—It is suggested that the laboratory obtain a QCS from a source different from the Hg used to produce the standards used routinely in this method (Sections 7.7–7.10), and that the QCS be analyzed periodically to verify the concentration of these standards.

9.7 Depending on specific program requirements, the laboratory may be required to analyze field duplicates and field spikes collected to assess the precision and accuracy of the sampling, sample transportation, and storage techniques. The relative percent difference (RPD) between field duplicates should be less than 20%. If the RPD of the field duplicates exceeds 20%, the laboratory should communicate this to the sampling team so that the source of error can be identified and corrective measures taken before the next sampling event.

10.0 Calibration and Standardization

10.1 Establish the operating conditions necessary to purge Hg from the bubbler and to desorb Hg from the trap in a sharp peak. The system is calibrated using the external standard technique as follows:

10.1.1 Initial calibration—Analyze each calibration standard (Section 7.10) according to the procedure in Section 11. After the analysis of each standard, analyze a bubbler blank (Section 9.4.1) on the same bubbler used for the standard. Subtract the peak area of the bubbler blank from the area of each respective stan-

dard. Tabulate the resulting peak area against the respective concentration of each solution to form five calibration factors. Calculate the relative standard deviation (RSD) of the calibration factor over the five-point range.

10.1.2 Linearity—If the calibration factor is constant (< 20% RSD) over the five-point calibration range, linearity through the origin can be assumed and the average calibration factor can be used; otherwise, a complete calibration curve must be used over the five-point range.

10.2 Calibration verification and ongoing precision and recovery—The ongoing precision and recovery standard (Section 9.5) is used to verify the working calibration curve or calibration factor at the beginning of each 12-hour working shift on which samples are analyzed.

11.0 Procedure

11.1 Sample Preparation

11.1.1 Pour a 100-mL aliquot from a thoroughly shaken, acidified sample, into a 125-mL fluoropolymer bottle. Add bromine monochloride (BrCl), cap the bottle, and digest at room temperature for 12 hs minimum.

11.1.1.1 For clear water and filtered samples, add 0.5 mL of BrCl; for brown water and turbid samples, add 1.0 mL of BrCl. If the yellow color disappears because of consumption by organic matter or sulfides, more BrCl should be added until a permanent (12-h) yellow color is obtained.

11.1.1.2 Some highly organic matrices, such as sewage effluent, will require high levels of BrCl (i.e., 5 mL/100 mL of sample), and longer oxidation times, or elevated temperatures (i.e.; place sealed bottles in oven at 50°C for 6 h). The oxidation always must be continued until a permanent yellow color remains.

11.1.2 Matrix spikes and matrix spike duplicates—For each 10 or fewer samples, pour two additional 100-mL aliquots from a randomly selected sample, spike at the level specified in Section 9.3, and process in the same manner as the samples.

11.2 Hg reduction and purging—Place 100 mL of reagent water in each bubbler, add 1.0 mL of $SnCl_2$, and purge with Hg-free N_2 for 20 min at 300–400 mL/min.

11.2.1 Connect a gold/sand trap to the output of the soda lime pretrap, and purge the water another 20 min to obtain a bubbler blank. Discard the water in the bubbler.

11.2.2 Add 0.2 mL of 30% NH_2OH to the BrCl-oxidized sample in the 125-mL fluoropolymer bottle. Cap the bottle and swirl the sample. The yellow color will disappear, indicating the destruction of the BrCl. Allow the sample to react for 5 min with periodic swirling to be sure that no traces of halogens remain.

NOTE: *Purging of halogens onto the gold trap will result in damage and low or irreproducible results.*

11.2.3 Connect a fresh trap to the bubbler, pour the reduced sample into the bubbler, add 0.5 mL of 20% $SnCl_2$ solution, and purge the sample with N_2 for 20 min.

11.3 Desorption of Hg from the gold trap

11.3.1 Remove the gold (sample) trap from the bubbler, place the Nichrome wire coil around the sample trap and connect the sample trap into the analyzer train between the incoming Hg-free argon and the second gold-coated (analytical) sand trap (Figure 1a).

11.3.2 Pass argon through the sample and analytical traps at a flow rate of approximately 30 mL/min for approximately 2 min to drive off condensed water vapor.

11.3.3 Apply electrical current to the coil around the sample trap for 3 minutes to thermally desorb the mercury (as Hg^0) from the sample trap onto the analytical gold trap.

11.3.4 After the 3-min desorption time, turn off the current to the Nichrome coil, and cool the sample trap using the cooling fan.

11.3.5 Apply electrical current to the Nichrome wire coil around the analytical trap and begin data collection. Heat the analytical trap for 3 min or for 1 min beyond the point at which the peak returns to baseline, whichever is greater.

11.3.6 Stop data collection, turn off the current to the Nichrome coil, and cool the analytical trap to room temperature using the cooling fan.

11.3.7 Place the next sample trap in line and proceed with analysis of the next sample.

NOTE: *The analytical trap must be at or near room temperature when the sample trap is heated; otherwise, Hg^0 may be lost by passing through the analytical trap.*

11.4 Peaks generated using this technique should be very sharp and almost symmetrical. Mercury elutes at approximately 1 min and has a width at half-height of about 5 seconds.

11.4.1 Broad or asymmetrical peaks indicate a problem with the desorption train, such as low gas flow rate, water vapor on the trap(s), or an analytical column damaged by chemical fumes or overheating.

11.4.2 Damage to an analytical trap is also indicated by a sharp peak, followed by a small, broad peak.

11.4.3 If the analytical trap has been damaged, the trap and the fluoropolymer tubing should be discarded because of the possibility of gold migration onto downstream surfaces of the instrument tubing and analytical system.

11.4.4 Gold-coated sand traps should be tracked by unique identifiers so that any trap producing poor results can be quickly recognized and discarded.

12.0 Data Analysis and Calculations

12.1 Subtract the peak area of the mean of a minimum of three bubbler blanks (Section 9.4.1.3) from the peak area of each sample.

12.2 Using the blank-subtracted area, calculate the concentration of Hg in each sample directly from the mean calibration factor if a linear calibration is used, or from the calibration curve if the calibration factor does not meet the criterion in Section 10.1.2.

12.3 Reporting—Report results for samples in ng/L to three significant figures for total Hg found above the ML (Section 1.3). Report results below the ML as < 0.2 ng/L, or as required by the permitting authority or in the permit.

13.0 Method Performance

The data in Table 2 gives an example of the performance of the method under actual operating conditions by several different analysts over a period of 1 year. In addition to such data, this methodology has been intercompared with other techniques for low-level mercury determination in water under a variety of studies, including ICES-5 (Reference 14) and the International Mercury Speciation Intercomparison Exercise (Reference 15).

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollution prevention exist in laboratory operation. EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option. The acids used in this method should be reused as practicable by purifying by electrochemical techniques. The only other chemicals used in this method are the neat materials used in preparing standards. These standards are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the disposal of excess volumes of expired standards.

14.2 For information about pollution prevention that may be applied to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Governmental Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872–4477.

15.0 Waste Management

15.1 The laboratory is responsible for complying with all federal, state, and local regulations governing waste management, particularly hazardous waste identification rules and land disposal restrictions, and for protecting the air, water, and land by minimizing and controlling all releases from fume hoods and bench operations. Compliance with all sewage discharge permits and regulations is also required.

15.2 Acids, samples at pH < 2, and BrCl solutions must be neutralized before being disposed of, or must be handled as hazardous waste.

15.3 For further information on waste management, consult *The Waste Management Manual for Laboratory Personnel* and *Less is Better: Laboratory Chemical Management for Waste Reduction*, both available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington, DC 20036.

16.0 References

1 Frontier Geosciences, Inc., Purchase Order 8762 from DynCorp Viar, Inc., August 22, 1994.

2 Fitzgerald, W.F.; Gill, G.A. "Sub-Nanogram Determination of Mercury by Two-Stage Gold Amalgamation and Gas Phase Detection Applied to Atmospheric Analysis," *Anal. Chem.* 1979, *15,* 1714.

3 Bloom, N.S; Crecelius, E.A. "Determination of Mercury in Sea water at Subnanogram per Liter Levels," *Mar. Chem.* 1983, *14,* 49.

4 Gill, G.A.; Fitzgerald, W.F. "Mercury Sampling of Open Ocean Waters at the Picogram Level," *Deep Sea Res* 1985, *32,* 287.

5 Bloom, N.S; Fitzgerald, W.F. "Determination of Volatile Mercury Species at the Picogram Level by Low-Temperature Gas Chromatography with Cold-Vapor Atomic Fluorescence Detection," *Anal. Chim. Acta.* 1988, *208,* 151.

Table 2. Typical QC Results for Routine Water Analysis (Frontier Geosciences Inc., February–August 1993)

Parameter	Units	Mean	SD	N
Reagent Blanks	ng/L	0.14	0.04	36
Matrix Spike Recoveries	%	99.6	6.3	60
Laboratory Duplicates	RPD	4.9	6.6	49
Field Duplicates	RPD	13.3	14.6	33
Intercomparison Exercise	% Diff	0.0	13.3	18 labs

6 Method 1669, "Method for Sampling Ambient Water for Determination of Metals at EPA Ambient Criteria Levels," U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Engineering and Analysis Division (4303), 401 M Street SW, Washington, DC 20460, April 1995 with January 1996 revisions.

7 "Working with Carcinogens," Department of Health, Education, and Welfare, Public Health Service. Centers for Disease Control. NIOSH Publication 77-206, Aug. 1977, NTIS PB-277256.

8 "OSHA Safety and Health Standards, General Industry," OSHA 2206, 29 *CFR* 1910.

9 "Safety in Academic Chemistry Laboratories," ACS Committee on Chemical Safety, 1979.

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17.0 Glossary

The definitions and purposes below are specific to this method, but have been conformed to common usage as much as possible.

17.1 Ambient Water—Waters in the natural environment (e.g., rivers, lakes, streams, and other receiving waters), as opposed to effluent discharges.

17.2 Analytical Shift—All of the 12-hour period during which analyses are performed. The period begins with the purging of the OPR standard and ends exactly 12 hours later. All analyses both started and completed within this 12-hour period are valid.

17.3 Bubbler Blank—The process of analyzing water in the bubbler, including purging Hg from the water, trapping the Hg purged on a sample trap, desorbing the Hg onto an analytical trap, desorbing the Hg from the analytical trap, and determining the amount of Hg present. The blank is somewhat different between days, and the average of a minimum of the results from three bubbler blanks must be subtracted from all standards and samples before reporting the results for these standards and samples.

17.4 Intercomparison Study—An exercise in which samples are prepared and split by a reference laboratory, then analyzed by one or more testing laboratories and the reference laboratory. The intercomparison, with a reputable laboratory as the reference laboratory, serves as the best test of the precision and accuracy of the analyses at natural environmental levels.

17.5 Matrix Spike (MS) and Matrix Spike Duplicate (MSD)—Aliquots of an environmental sample to which known quantities of the analyte(s) of interest is added in the laboratory. The MS and MSD are analyzed exactly like a sample. Their purpose is to quantify the bias and precision caused by the sample matrix. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the MS and MSD corrected for these background concentrations.

17.6 Must—This action, activity, or procedural step is required.

17.7 Quality Control Sample (QCS)—A sample containing Hg at known concentrations. The QCS is obtained from a source external to the laboratory, or is prepared from a source of standards different from the source of calibration standards. It is used to check laboratory performance with test materials prepared external to the usual preparation process.

17.8 Reagent Water—Water known not to contain the analyte(s) of interest at the detection limit of this method. For this method, the Hg level is made as low as possible in mercury usually by double deionization. The reagent water is used to wash bottles and as trip and field blanks.

17.9 Should—This action, activity, or procedure is suggested, but not required.

17.10 Stock Solution—A solution containing an analyte that is prepared from a reference material traceable to EPA, NIST, or a source that will attest to the purity and authenticity of the reference material.

17.11 Ultraclean Handling—A series of established procedures designed to ensure that samples are not contaminated for Hg during sample collection, storage, or analysis.

Appendix H

RTI/6302/04

Standard Operating Procedures for the Operation and

Maintenance of a Trace Metal Cleanroom

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Operation and Maintenance of a Trace Metal Cleanroom

1.0 Scope and Application

1.1 This method is for the operation and maintenance of a Trace Metal Cleanroom facility. The ultimate goal of cleanroom utilization is to prevent contamination of samples or products by airborne particulates. The method may be used to establish and maintain the cleanliness of a cleanroom facility in which samples are prepared and analyzed for metals at EPA water quality criteria (WQC) levels. It is also suited for analysis of other types of environmental samples that require a high degree of protection from ambient air contamination.

1.2 Typically, airborne particulates have high metal concentrations and are a major source of sample contamination. In order to eliminate this problem, specially designed trace metal cleanrooms are used. Cleanrooms are rooms that have a high flow rate of purified air which continuously blankets samples and materials in a clean atmosphere. They are equipped with High Efficiency Particulate Air (HEPA) filters and/or Ultra Low Penetration Air (ULPA) filters that remove almost all particles from the air. These cleanrooms are continually flushed with purified air so that samples can be processed without atmospheric contamination. Use of cleanrooms has enabled metal quantitation in the parts per trillion range for some elements.

1.3 This method addresses minimization of contamination of samples, reagents, and labware from metals found in air, construction materials, laboratory apparatus, and sample and reagent containers, and from airborne metals generated by laboratory personnel or operations.

1.4 This method is applicable to all metallic elements since all are susceptible to atmospheric transport to some extent. While most metallic elements are transported through the atmosphere attached to particles, some elements (such as mercury) are transported in the vapor phase. Different cleanroom operations that are required as a result of differences in the chemical behavior of particle-borne and vapor phase metals are included.

1.5 The method is intended for analysis of metals at WQC concentrations, which are typically in the parts-per-trillion (ppt) to low parts-per-billion (ppb) range. It does not apply to metals at high concentrations such as those associated with discharges from industrial facilities, but does apply to industries that have permits based on water quality guidelines.

1.6 The method is based on the research and experience of analysts who determine metal concentrations at "ultra-trace" concentrations using cleanroom laboratories. Selected references are provided (1-4). Additional information about ultraclean techniques may be found in EPA technical guidance (Reference 5) and about cleanroom design, use, and maintenance in References 6 and 7.

1.7 This method includes procedures to be used to maintain a trace metal cleanroom, and to prepare labware, sampling supplies, and apparatus for analysis of metals at WQC levels.

Other EPA methods and guidance documents that address clean sampling and analysis techniques for trace metals in ambient waters are listed in Table 1.

2.0 Summary of Method

2.1 This method presents restrictions for personnel entrance to a cleanroom facility, requirements for cleanroom garb, procedures for handling and transporting materials into the cleanroom, labware cleaning references, and procedures for maintaining and monitoring cleanliness in the cleanroom facility.

2.2 Definitions of cleanroom terminology, descriptions of cleanroom facilities, and minimum requirements for cleanrooms are presented.

3.0 Definitions

3.1 A general definition of a cleanroom is a room which has a gentle shower of highly filtered air for the purposes of transporting airborne particulate contaminants away from sensitive samples or apparatus, and maintaining a clean environment with low atmospheric metal concentrations.

3.2 A cleanroom is defined in U.S. Federal Standard 209E as "a room in which the concentration of airborne particles is controlled and which contains one or more clean zones" (Reference 8).

Table 1.

EPA Method	Title	Document Number
1631	*Total Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*	EPA 821-R-95-027
1636	*Determination of Hexavalent Chromium by Ion Chromatography*	EPA-821-R-95-029
1637	*Determination of Trace Elements in Ambient Waters by Chelation Preconcentration and Graphite Furnace Atomic Absorption*	EPA 821-R-95-030
1638	*Determination of Trace Elements in Ambient Waters by Inductively Coupled Plasma-Mass Spectrometry*	EPA 821-R-95-031
1639	*Determination of Trace Elements in Ambient Waters by Stabilized Temperature Graphite Furnace Atomic Absorption*	EPA 821-R-95-032
1640	*Determination of Trace Elements in Ambient Waters by On-Line Chelation Preconcentration and Inductively Coupled Plasma-Mass Spectrometry*	EPA 821-R-95-033
1669	*Sampling Ambient Water for Determination of Trace Metals at EPA Water Quality Criteria Levels*	EPA 821-R-95-034
	Guidance on the Documentation and Evaluation of Trace Metals Data Collected for Clean Water Act Compliance Monitoring	EPA 821-B-95-002
	Guidance on Establishing Trace Metal Cleanrooms in Existing Facilities	EPA 821-B-95-001

3.3 A clean zone is defined in U.S. Federal Standard 209E as "a defined space in which the concentration of airborne particles is controlled to meet a specified airborne particulate cleanliness class" (Reference 8).

3.4 Particulate cleanliness classes are defined at a maximum number of particles of a given size per unit volume of air. For example, a class 100 cleanroom has a maximum of 100 particles, 0.5 µm or larger, per cubic foot of air. Table 1 lists maximum particle concentrations permissible for different cleanliness class designations (Reference 8).

3.5 High efficiency filters used to remove submicron particles are typically High Efficiency Particulate Air (HEPA) filters or Ultra Low Penetration Air (ULPA) filters.

3.6 HEPA filters have a minimum particle-collection efficiency of 99.97% for 0.3 µm particles (Reference 9).

3.7 ULPA filters have a minimum particle-collection efficiency of 99.999% for particles in the size range of 0.1 to 0.2 µm (Reference 9).

3.8 Efficiency is defined as the ratio of the difference in particle concentrations (upstream - downstream) to the upstream particle concentration:

$$\text{Efficiency (\%)} = \frac{U - D}{U} \times 100$$

where
U = upstream particle concentration (particles/unit volume)
D = downstream particle concentration (particles/unit volume).

This means that if the particle count upstream of a HEPA filter is 100,000 particles per cubic foot (0.3 µm), then the count downstream of the filter will be a maximum of 30 particles per cubic foot. Similarly for the ULPA filters, if there are 100,000 particles per cubic foot (0.12 µm) upstream of the filters, then the count downstream will be a maximum of 1 particle per cubic foot (Reference 10).

3.9 Laminar air flow is defined as the movement of air in parallel sheets or columns which are separate from adjacent sheets or columns of air. Air in one laminar flow sheet does not mix or interact with air in any other laminar flow sheet.

4.0 Contamination and Interferences

4.1 Sources of contamination for metals at WQC levels include three primary routes:

1. contamination from airborne particles and metal-containing vapors;
2. contamination from particles generated by personnel, clothing, equipment, or apparatus, and
3. improperly cleaned labware, sample containers, apparatus, and reagents. A properly designed and functioning cleanroom minimizes contamination from airborne particles and vapors, but use of a cleanroom does guarantee that samples will be uncontaminated. The behaviors of personnel, procedures used to clean and store labware and apparatus, and the purity of reagents are also very important in providing contaminant-free analyses.

4.2 Contamination of samples from elements associated with airborne particles or vapors can be substantially reduced in a cleanroom facility by high efficiency air filtration combined with vapor phase sorption. Most particles and metal-containing vapors are removed from the air, and the resulting clean, filtered air continuously bathes samples and apparatus.

4.3 In a properly functioning cleanroom with sufficient flow of clean, filtered air to maintain laminar flow conditions, the major sources of contamination remaining are personnel, labware, and reagents. Personnel must be trained to understand the air flow patterns, cleanliness regions and requirements, and material handling procedures and requirements in addition to the analytical procedures they must use. Great care must be paid by personnel to avoid sources of contamination, to decontaminate materials before allowing them to enter the cleanroom, and to continually maintain the clean status of their environment. The importance of training and vigilance should not be underestimated.

4.4 A compendium of standards, practices, and methods approved by the Institute of Environmental Sciences for contamination control is provided in Reference 11. This compendium provides much useful information for cleanrooms in general and for those used in the microelectronics industry in particular. It does not contain information specific to trace metal cleanrooms, but does provide approaches that have been demonstrated to reduce particle contamination in cleanrooms.

5.0 Safety

5.1 Acid Baths

5.1.1 Acid baths are used in trace metal laboratories for leaching labware. These baths may contain HNO_3, HCl, HBr, H_2SO_4, HF or mixtures of these acids at high concentrations and may be used at room temperature or elevated temperature. The baths should be constructed of a material that is suited to the type of acid contained so that it will not become brittle or leak after continued exposure to the acid. Check chemical compatibility lists for materials provided by the vendor. Additionally, acid baths should have lids to contain fumes and be located in a polypropylene exhaust cabinet or fume hood.

5.1.2 Care must be taken to avoid any acid spill when removing the acid-leached labware from acid baths. In the event of accidental spill, a proper cleanup procedure should be followed.

5.1.3 Acid baths must be properly labeled to indicate the type of acid(s) and the concentration of the acid(s).

5.2 Hot Plates

5.2.1 Most trace metal cleanrooms use hot plates to heat acid baths and to regulate the digestion of samples. The large amounts of acid used and acid vapors generated can create a safety hazard for electrical components, such as hot plates.

5.2.2 Hot plates should be inspected regularly by laboratory personnel to ensure the absence of corrosion or other damage to electrical leads and connections. In some commercially available hot plates, an acid-corroded electrical lead can result in the hot plate cycling to its highest setting, potentially leading to a fire. In others, corroded electrical leads can result in electrical shock. Any defects in a hot plate or controller should be corrected immediately and the unit replaced if necessary.

5.2.3 Hot plates in polypropylene hoods should never be operated above a temperature of 400°C, as the heat may damage the hood material. Excessive heat can lead to a fire.

5.3 Handling Chemicals

5.3.1 When handling chemicals, guidelines given in the material handling data sheets (MSDS) should be followed at all times. MSDS sheets are available through the manufacturer.

5.3.2 Because the room air inside a cleanroom is recirculated (to increase particle removal efficiency), a safety hazard could rapidly be created if toxic fumes were present in the laboratory. It is therefore very important that an exhausting fume hood be used for all volatile chemicals and vapor-generating procedures.

5.3.3 All prepared solutions should be properly labeled to reflect their contents (chemical name, matrix, solvent, preparation date, storage conditions, preparer).

5.3.4 Wastes that are generated must be stored in appropriate containers. Waste containers must be labeled to indicate their contents and approximate concentration whenever possible.

5.4 Safety Garments

5.4.1 Safety garments are required in the cleanroom to protect personnel from exposure to concentrated acids and oxidizers used in sample digestion and from exposure to sample materials which may be partly or totally uncharacterized. Safety glasses, lab coat, appropriate shoes, and gloves are required.

5.4.2 Additionally, lab personnel may use a face shield, lab apron, respirator or other safety equipment for certain tasks.

5.4.3 Routine safety equipment must meet Class 100 standards of cleanliness before being brought into the cleanroom. However, personnel safety is always the primary consideration and in some cases it may be necessary to use non-cleanroom equipment or supplies.

6.0 Facility, Equipment, and Supplies

6.1 Ideally, the Cleanroom Facility will consist of a suite of rooms, each provided with high purity laminar-flow air of designated cleanliness classes, including a laboratory and anteroom at class 100 or better. Minimally,

it will consist of two rooms, an anteroom and a cleanroom laboratory, both provided with high purity laminar-flow air at class 100 or better.

6.2 Ideally, the cleanroom laboratory used for preparation of samples and apparatus will contain one or more banks of HEPA or ULPA filters in the ceiling providing vertical down-flow laminar air. Minimally, the cleanroom will contain a series of clean zones in which HEPA or ULPA-filtered air is supplied in laminar flow at a designated cleanliness class.

6.3 Particulate Cleanliness Class: Class 100 air is the minimum particulate cleanliness class appropriate to preparation of apparatus and samples for metal analysis at WQC levels.

6.4 Fume hoods:

6.4.1 Non-metallic exhausting fume hoods are required for all operations with acids. Suitable construction materials are polypropylene (preferred) or fiberglass (alternate).

6.4.2 Class 100 (or better) HEPA-filtered non-metallic exhausting fume hoods are required for acid digestion of samples or other operations in which samples are present in open containers and acid vapors are generated.

6.4.3 Operations using volatile organic compounds should be performed in an exhaust hood with appropriate solvent resistance. When a stainless steel hood is required, the hood should be located in a cleanroom laboratory where acids are not used.

6.4.4 Operations involving hot plates must be performed within the thermal limitations of the fume hood construction materials. Heat-tolerant polypropylene and teflon inserts are commercially available for hot plate regions in polypropylene fume hoods. Heat-tolerant ceramic inserts and plates are also useful for cooling samples while they continue to evolve corrosive fumes.

6.4.5 The pattern of air flow in HEPA-filtered fume hoods is critically important. Clean, HEPA-filtered air should flow downward in a laminar flow to protect samples from contamination. All air inside the hood should be one-pass air, vented to the outside of the building. If significant acid vapors are generated in the hood, it should be equipped with a water wash-down for the exhaust plenum or an acid scrubber at the outlet of the exhaust. Air exhaust should be initiated at hand level at both the front of the work surface and the back of the work surface inside the hood. In this way, samples are exposed to clean, HEPA-filtered air only, and acid vapors are efficiently removed.

6.5 Clean Benches

6.5.1 Clean benches are HEPA-filtered non-exhausting enclosures located within a laboratory. Alternately they may be non-exhausting work surface regions within HEPA-filtered rooms.

6.5.2 The minimum cleanliness designation for a clean bench suitable for metal analysis at WQC levels is class 100.

6.5.3 Vertical laminar flow of air is the preferred flow pattern for all cleanroom operations, but in some cases, horizontal flow may be adequate. However, the likelihood of cross-contamination of samples or standards is much higher with a horizontal laminar flow design than with a vertical laminar flow design, and the analyst must be specifically trained to understand the limitations to the use of a horizontal flow clean bench.

6.5.4 Construction materials must be able to resist acids used in sample and standard preparation. In practice, polypropylene is typically the preferred material.

6.6 Anteroom:

6.6.1 The anteroom is a changing room for personnel and equilibration room for materials entering the class 100 laboratory. The anteroom should be maintained at class 100.

6.6.2 Cleanroom garb should be stored in the anteroom so that personnel can dress in clean, non-shedding jackets, head covers, foot covers, gloves, and if necessary face masks, before entering the class 100 laboratory.

6.6.3 Cleanroom garb should never be allowed to enter a region of dirtier air.

6.6.4 Vertical, down-flow, HEPA-filtered air should be provided in the anteroom to wash garb, personnel, and materials before admittance into the class 100 laboratory.

6.6.5 Storage and equilibration areas within the anteroom should be designed to provide minimal disruption to the laminar flow of air. Perforated shelves, hangers for lab coats, clips for head covers, etc, should be made of non-shedding, non-corroding material, such as plastic, and arranged within the anteroom to permit continued down-flow of air with minimal turbulence.

6.7 Construction Materials

6.7.1 All construction materials must be non-shedding, non-corroding materials. Due to the quantity and concentration of acids used in trace metal sample preparation and analysis, the preferred material for most components is plastic. Polypropylene wall laminates, flooring, benches, hoods, and ceiling grids are commercially available. Polypropylene, poly(vinylchloride), and teflon are suitable for specific plumbing purposes. Seams and joints should be heat-welded, to the extent possible, to ensure continuous seal without outgassing of solvents. Other plastics may be suitable for specific components, but should be tested for acid and solvent resistivity, thermal tolerance, outgassing, and metal leachability prior to use.

6.7.2 Epoxy paints may be used to provide a non-corroding coating for traditional construction materials (such

as metal, wood, cinder block, etc.) in the cleanroom. However, use of epoxy paints is considered a temporary solution to the problem of particle generation. Two warnings are presented regarding the use of epoxy paints:

6.7.2.1 Epoxy paints do not provide as permanent a barrier to particle generation as do plastics. Acids will corrode epoxy paints and the paints will degrade with age. Surfaces coated with epoxy paints will need to be checked frequently for signs of corrosion. At the first sign of corrosion, painted metal components (such as door hinges, drawer pulls, etc.) must be removed from the lab and replaced or re-finished with fresh epoxy paint. Painted wall surfaces and wooden components present less obvious signs of the onset of degradation, but must be repainted at a frequency that will prevent particle generation within the laboratory.

6.7.2.2 Laboratories that analyze mercury or other vapor-phase metal species must test the epoxy paints for their metal concentration prior to use. The volatile metal species will outgas and contaminate the laboratory if present in the paints.

6.8 Additional information about cleanroom philosophy, design, construction materials, components, etc. may be found in References 1-8 and in guidance documents and standards published through the Institute of Environmental Sciences (References 9-12).

7.0 Reagents and Standards

7.1 Reagents and standards appropriate to the EPA sampling and analysis method(s) followed and analyte(s) determined should be used. This method does not specify the analysis procedure, reagents, or standards.

8.0 Sample Collection, Preservation, and Storage

8.1 Sample collection, preservation, and storage procedures specified in the appropriate EPA method should be followed. This method does not specify the types of samples to be collected nor the preservation and storage procedures to be followed.

9.0 Quality Control

9.1 Quality control procedures appropriate to the EPA method(s) followed and analyte(s) determined should be used. This method does not specify the analysis or quality control procedure(s).

10.0 Calibration and Standardization

10.1 Calibration and standardization procedures specified in the appropriate EPA method should be followed. This method does not specify the analytical instrumentation nor procedures to be used.

11.0 Procedures

11.1 Personnel Entry and Garb

11.1.1 One of the most significant sources of particles and contamination in a cleanroom laboratory is personnel. Particles are transported on and generated by shoes, clothing, hair, skin, and supplies. Thus one of the simplest methods used to reduce airborne particle concentrations in the laboratory is to restrict entrance to the laboratory to only those personnel who need to work there. At the discretion of the laboratory manager, escorted visitors who are trained in cleanroom protocols and appropriately garbed may be allowed entrance.

11.1.2 Cleanrooms are restricted access areas. Appropriate signs should be posted outside the rooms so that unauthorized personnel do not enter.

11.1.3 Appropriate cleanroom garb must be worn by all personnel in the facility. Cleanroom shoe covers, jackets, hair covers, face masks, gloves, etc. are commercially available through cleanroom vendors.

11.1.3.1 All personnel must either cover street shoes with disposable cleanroom shoe covers or wear shoes designated and reserved for cleanroom use only. If there are multiple cleanrooms within the facility at different cleanliness designations, such as class 10,000 and class 100, then shoe covers should be changed between rooms such that the cleanest covers are worn in the room with the lowest class designation.

11.1.3.2 All personnel must wear non-shedding, cleanroom lab jackets or coveralls in a class 100 (or lower) cleanroom or clean zone.

11.1.3.3 All personnel must wear disposable, non-shedding, cleanroom head covers in a class 100 (or lower) cleanroom or clean zone.

11.1.3.4 Clean, powder-free gloves must be worn in a class 100 (or lower) cleanroom or clean zone.

11.1.3.5 Laboratories that analyze only mercury do not have the same restrictions as those that analyze other metals. Because mercury is primarily transported in the vapor phase and not attached to airborne particles, it is not necessary to wear all of the particle-barrier layers described above. Nevertheless, cleanroom foot covers and powder-free gloves should be worn in mercury cleanrooms. In addition, breath and saliva may contain high concentrations of mercury (Reference 13), and it may be prudent to wear a cleanroom face mask.

11.2 Materials Handling and Exchange Procedures

11.2.1 In order to prepare and analyze samples in a cleanroom environment, it is necessary to transport

samples, reagents, supplies, etc. from a "non-clean" environment to the cleanroom environment. The transfer should be accomplished in a series of decontamination steps with each step moving the materials to a progressively cleaner area.

11.2.2 Each area of the facility should contain supplies appropriate to the decontamination to be performed in that region.

11.2.3 A service room or receiving area should be used to receive materials, remove outer shipping containers, and remove any outside dust or contamination.

11.2.4 Supplies and apparatus that cannot be adequately cleaned to allow them in a cleanroom, such as cylinders of compressed gas, air driers, refrigerators, etc., should be stored in an outer room such as a service room. Gas and water lines can be plumbed from the outer room to the cleanroom, as appropriate.

11.2.5 A HEPA-filtered room or zone should be used to remove intermediate layers of wrap such as the outer bag of double-bagged samples or supplies. Water and lint-free wipes should be available in this region to rinse and dry materials, such as reagent bottles or other non-cleanroom packaged supplies.

11.2.6 In addition to laboratory reagents and supplies, ordinary materials brought into the cleanroom, such as notebooks, pens, calculators, written protocols, etc., should be appropriately decontaminated in a HEPA-filtered room or zone before they are brought into the cleanroom. All materials in ambient air are contaminated with atmospheric dust that adheres loosely to their surface. The purpose of decontaminating ordinary materials is to remove this dust so that it is not transported into the cleanroom. In practice, it is usually best to leave a supply of cleaned, ordinary materials in the cleanroom.

11.2.7 Materials that are purchased from cleanroom suppliers with "Class 100 packaging" should be brought into a class 100 zone, such as an anteroom to a class 100 laboratory, to be opened.

11.2.8 Cleanroom garb and supplies should be stored in a HEPA-filtered anteroom or region where their cleanliness can be maintained.

11.2.9 In order to prevent contamination of an ultra-clean region or the supplies kept in that region, separate areas should be used for "high level" reagents and standards, i.e. those containing more than 1 part-per-million (ppm) of metals, and "low level" reagents and standards, containing less than 1 ppm of metals. In practice, this may be accomplished by keeping the chemicals and associated labware in separate rooms or by designating separate areas within a single cleanroom for "high level" and "low level" operations. In either case, separate labware should be maintained. For example, flasks and pipets that are used for high concentrations of metals should never be used for solutions that contain low levels of metals. Separate wash basins, acid leaching baths, and storage cabinets should be used for high level and low level labware.

11.3 Procedures for Cleaning Labware and Apparatus

11.3.1 For preparing labware and apparatus for analysis of metals other than mercury, follow the cleaning procedures specified in EPA Method 1640: *Determination of Trace Elements in Ambient Waters by On-Line Chelation Preconcentration and Inductively Coupled Plasma-Mass Spectrometry*, Section 11.0.

11.3.2 For preparing labware and apparatus for analysis of mercury, follow the cleaning procedures specified in EPA Method 1631: *Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry*, Section 6.0.

11.4 Procedures for Maintaining Cleanliness

11.4.1 All exposed bench surfaces and hood surfaces in the cleanroom must be wiped daily with a lint-free cleanroom wiper wetted with deionized water. This should be performed prior to initiating work each day and again as needed during the day to maintain clean work surfaces.

11.4.2 The cleanroom floor should be mopped with tap water on the first working day of every week, or more often if needed. A suitable metal free detergent may be used if necessary, but generally, clean detergent-free water is preferred so that no residual, particle-generating film is left. The purpose of mopping this floor is to remove the small accumulation of dust particles or laboratory debris, rather than to remove any significant amount of dirt or mud. The mop used for this purpose should be reserved for cleanroom use only, and should be constructed of plastic and sponge with a minimum of metal parts. It should be stored in a class 100 anteroom or equivalent space between uses so that it remains sufficiently clean for use.

11.4.3 Large adhesive mats kept at the entrance to cleanroom areas must be replaced periodically. The frequency of replacement depends on the traffic through the area, but should be sufficient to trap dirt and lint from the shoes of personnel. Frequent replacement of these mats will minimize the transport of dirt into clean areas.

11.4.4 If HEPA or ULPA filters are functioning properly, the pressure differential across the filter face should be within a range specified by the manufacturer. If the pressure drop is outside of the target range, the filter should be replaced. Pressure differential is routinely monitored using a manometer.

11.4.5 Prefilters used to trap large particles should be examined and changed periodically to ensure that the relatively expensive HEPA and ULPA filters do not become clogged prematurely.

11.4.6 Acid baths used to clean labware must be changed periodically to ensure that the concentration of metals is sufficiently low. It is recommended that the baths

be changed when any of the analytes of interest reach a concentration greater than 1 ppm or in the event of known contamination. For routine use, only clean plastic tongs should be inserted into an acid bath to retrieve labware.

11.4.7 All laboratory operations performed can potentially impact the cleanliness of the facility by generating or transporting particles and/or vapors, or by contaminating labware or acid baths. The location for each laboratory activity should be selected carefully so that the cleanliness of samples, labware, and apparatus is maintained and so that the area does not become contaminated or hazardous as a result of the operation.

1.5 Procedures for Monitoring Cleanliness

11.5.1 Monitoring the cleanliness of the cleanroom facility requires measurement of two sets of parameters. The first set are physical parameters that indicate if the air handling system is functioning properly. It includes measurement of temperature, pressure, relative humidity, and the alarm status of various fans, motors, and smoke detectors. The second set are chemical parameters that indicate if the cleanroom is sufficiently clean in selected analytes to enable contaminant-free analyses. It includes measurement of chemical concentrations in acid baths and traps for settled particles.

11.5.2 Initial certification of the cleanliness class of a cleanroom facility should be performed using standard cleanroom tests specified by the Institute of Environmental Sciences (Reference 12). Independent testing agencies have appropriate equipment and experience to conduct these tests and certify the cleanliness class. Certification should be repeated periodically; annual re-certification is recommended.

11.5.3 After the initial certification, proper functioning of the air handling system should be ascertained at least once daily.

11.5.3.1 In cleanrooms or clean zones equipped with a manometer on each HEPA or ULPA filter, the pressure differential should be noted for each filter. If the pressure drop is not within the target range, the filter should be replaced.

11.5.3.2 Because air moves from regions of higher pressure to regions of lower pressure, airborne contaminants will be transported along pressure differentials such that the cleanest regions will be associated with the highest pressure, and the dirtiest regions will be associated with the lowest (ambient) pressure. Proper air flow in a cleanroom facility can thus easily be monitored by measuring static pressure in each room of the facility. The cleanest region (lowest class designation) should have the highest static pressure, and each progressively "dirtier" region should have a correspondingly lower static pressure. Using this method, the values of static pressure measured in each room will depend on atmospheric conditions, but the pressure trends should be maintained.

11.5.3.3 The temperature and relative humidity in the cleanroom facility may be monitored to ensure that the air handling system is functioning properly, but unless extreme conditions are observed indicating HVAC breakdown, they should not have a significant impact on the cleanliness of the facility.

11.5.3.4 Particle concentration measurements should be made annually and compared to those obtained during the initial certification of the facility. High particle counts in any region of the facility are indicative of either localized particle generation or failure of the HEPA or ULPA filter(s) in that region. In either case, corrective actions should be taken to locate and remove the source of the particles.

11.5.4 In addition to the physical parameters used to monitor the cleanliness of the facility, periodic measurements should be made to determine the background concentration of specific metals within the laboratory. The list of metals monitored should be selected according to the analysis needs and schedule of the laboratory.

11.5.4.1 Sampling of facility background should be performed by placing four clean teflon beakers, two open (samples) and two covered (blanks), at each sampling location within the cleanroom facility.

11.5.4.2 The sampling locations should be selected to correspond to regions where sample preparation activities typically occur.

11.5.4.3 Sampling should be performed every six months or more frequently.

11.5.4.4 A small amount of highest purity acid is added to each beaker and warmed to dissolve any settled particles. The solution should then be diluted and analyzed using the method most appropriate for the metals being monitored.

11.5.4.5 Maximum acceptable background limits are typically 0.1 ng cm^{-2} day^{-1}. After a series of measurements are made in a cleanroom facility, a more appropriate limit value should be substituted for each element of interest.

12.0 Data Analysis and Calculations

12.1 Data analysis and calculation methods appropriate to each metal determined and analysis method employed should be followed. Consult the appropriate EPA method for specific guidance.

13.0 Method Performance

13.1 Consult the appropriate EPA method for an evaluation of the performance of the analytical method.

14.0 Pollution Prevention

14.1 Pollution prevention encompasses any technique that reduces or eliminates the quantity or toxicity of waste at the point of generation. Many opportunities for pollu-

tion prevention exist in laboratory operation. The EPA has established a preferred hierarchy of environmental management techniques that places pollution prevention as the management option of first choice. Whenever feasible, laboratory personnel should use pollution prevention techniques to address their waste generation. When wastes cannot be feasibly reduced at the source, the Agency recommends recycling as the next best option. The acids used in this method should be reused as practicable by purifying by electrochemical techniques. The only other chemicals used in this method are the metal standards. These standards are used in extremely small amounts and pose little threat to the environment when managed properly. Standards should be prepared in volumes consistent with laboratory use to minimize the volume of expired standards to be disposed.

14.2 For information about pollution prevention that may be applicable to laboratories and research institutions, consult *Less is Better: Laboratory Chemical Management for Waste Reduction*, available from the American Chemical Society's Department of Government Relations and Science Policy, 1155 16th Street NW, Washington DC 20036, 202/872-4477.

15.0 Waste Management

15.1 The Environmental Protection Agency requires that laboratory waste management practices be conducted consistent with all applicable rules and regulations. The Agency urges laboratories to protect the air, water, and land by minimizing and controlling all releases from hoods and bench operations, complying with the letter and spirit of any sewer discharge permits and regulations, and by complying with all solid and hazardous waste regulations, particularly the hazardous waste identification rules and land disposal restrictions. For further information on waste management consult *The Waste Management Manual for Laboratory Personnel*, available from the American Chemical Society at the address listed in Section 14.2.

16.0 References

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Table 1. Airborne Particulate Cleanliness Classes

Class limits are given for each class name. The limits designate specific concentrations (particles per unit volume) of airborne particles with sizes equal to and larger than the particle sizes shown.[a]

Class Name[b]		Class Limits									
		0.1 µm Volume Units		0.2 µm Volume Units		0.3 µm Volume Units		0.5 µm Volume Units		5 µm Volume Units	
SI	English[c]	(m^3)	(ft^3)	(m^3)	(ft^3)	(m^3)	(ft^3)	(m^3)	(ft^3)	(m^3)	(ft^3)
M1		350	9.91	75.7	2.14	30.9	0.875	10.0	0.283	-	-
M1.5	1	1,240	35.0	265	7.50	106	3.00	35.3	1.00	-	-
M2		3,500	99.1	757	21.4	309	8.75	100	2.83	-	-
M2.5	10	12,400	350	2,650	75.0	1,060	30.0	353	10.0	-	-
M3		35,000	991	7,570	214	3,090	87.5	1,000	28.3	-	-
M3.5	100	-	-	26,500	750	10,600	300	3,530	100	-	-
M4		-	-	75,700	2,140	30,900	875	10,000	283	-	-
M4.5	1,000	-	-	-	-	-	-	35,300	1,000	247	7.00
M5		-	-	-	-	-	-	100,000	2,830	618	17.5
M5.5	10,000	-	-	-	-	-	-	353,000	10,000	2,470	70.0
M6		-	-	-	-	-	-	1,000,000	28,300	6,180	175
M6.5	100,000	-	-	-	-	-	-	3,530,000	100,000	24,700	700
M7		-	-	-	-	-	-	10,000,000	283,000	61,800	1,750

[a] The class limits shown are defined for classification purposes only and do not necessarily represent the size distribution to be found in any particular situation.

[b] Concentration limits for intermediate classes can be calculated, approximately, from the following equations:

$$particles/m^3 = 10^M(0.5/d)^{2.2}$$

where **M** is the numerical designation of the class based on SI units, and **d** is the particle size in micrometers, or

$$particles/ft^3 = N_c(0.5/d)^{2.2}$$

where N_c is the numerical designation of the class based on English (U.S. customary) units, and **d** is the particle size in micrometers.

[c] For naming and describing the classes, SI names and units are preferred; however, English (U.S. customary) units may be used.

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