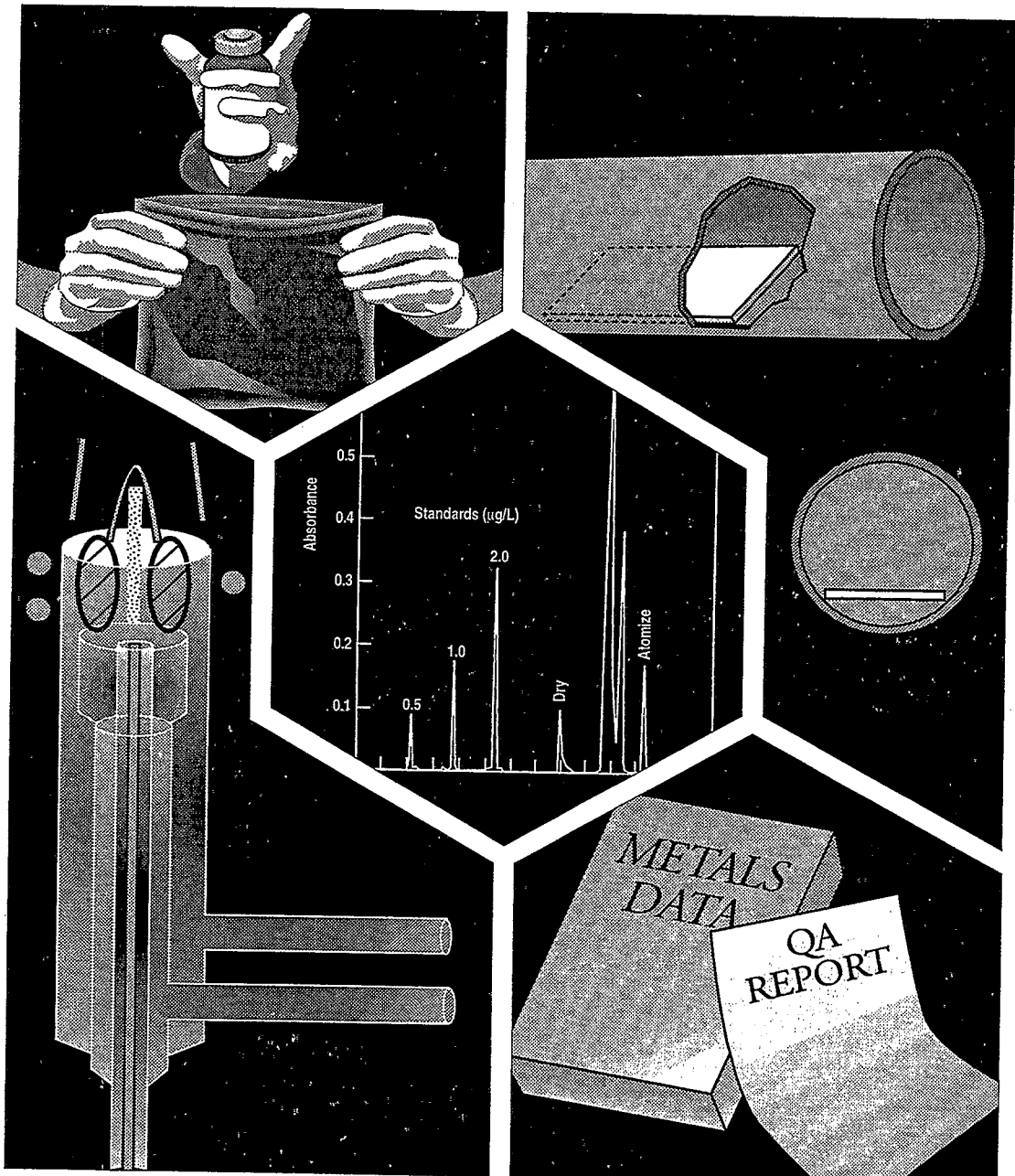
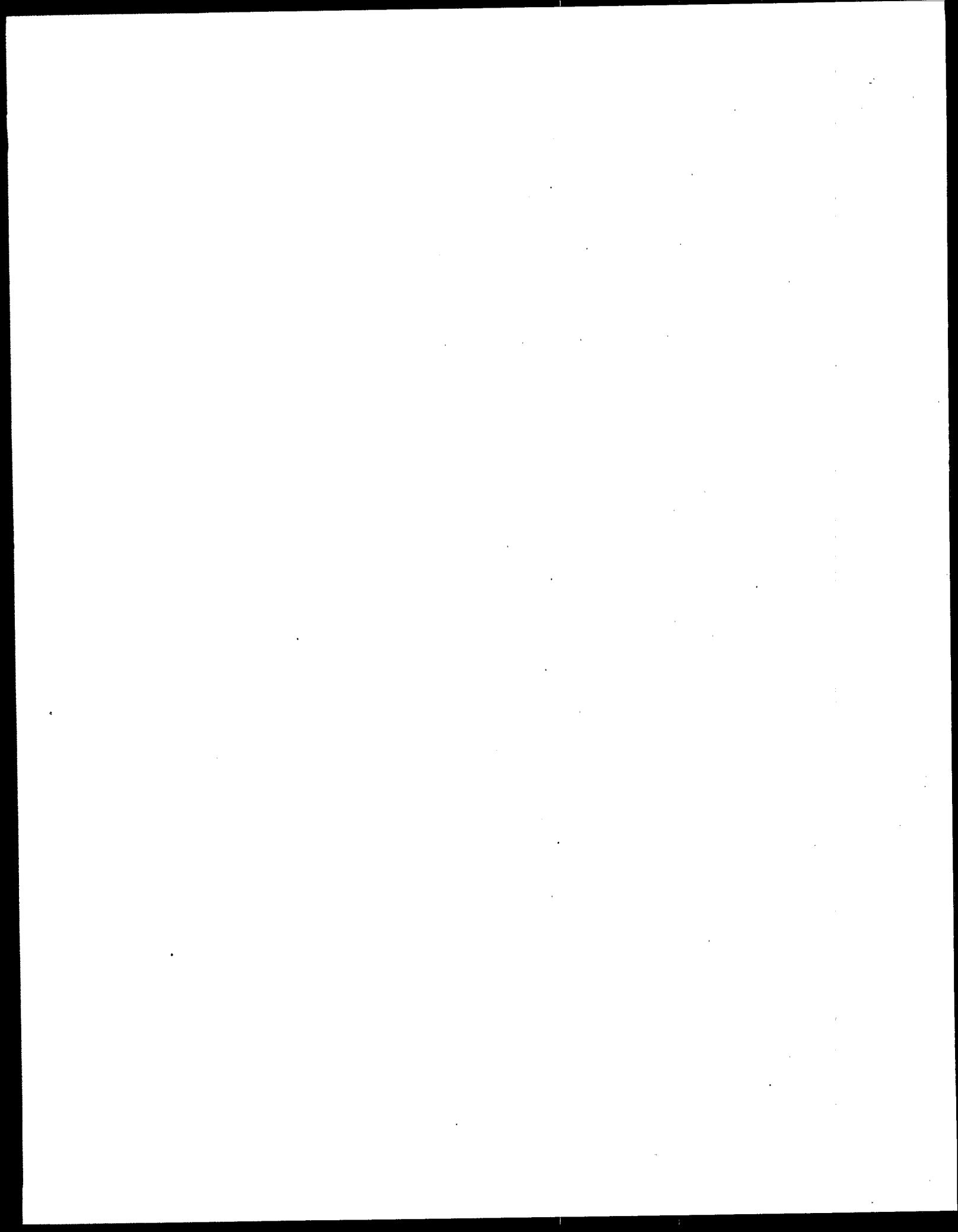




# Guidance on Establishing Trace Metal Clean Rooms in Existing Facilities



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## Section 1 Introduction

The concept of "trace metal clean" laboratories for environmental analyses was pioneered by Clair C. Patterson at the California Institute of Technology. He recognized that measurements of environmental lead concentrations were often erroneously high because of the inadvertent introduction of contaminant lead to the samples (Patterson, 1965). He then determined that contamination occurred during sampling in the field, during storage of the samples prior to analysis, and during analysis of the samples in the laboratory. This resulted in his development of rigorous trace metal "clean techniques" for elemental analyses of environmental samples (Patterson and Settle, 1976). Prominent among those techniques was the establishment of trace metal clean facilities for cleaning reagents and materials used for sampling and analyses and for storing, processing, and analyzing samples.

While the importance of trace metal clean laboratories is now widely recognized, Patterson received a great deal of criticism while he was developing those facilities. Much of that criticism was focused on the inordinate effort and cost involved in trace metal clean analyses compared to standard analyses. Indeed, the level of expertise, time, and cost invested in analyses in Patterson's laboratories was prohibitively expensive for most other research groups, much less environmental monitoring laboratories.

However, Patterson and others who adopted his trace metal clean techniques demonstrated that much of the environmental trace element data collected without those techniques were erroneously high. This phenomenon has been repeatedly demonstrated by interlaboratory calibrations and through comparisons with data in the literature. Consequently, trace metal clean techniques are now considered to be essential for many environmental monitoring and research programs.

As a result, the construction of trace metal clean facilities has developed into a multimillion-dollar-a-year business. Manufacturers now compete to design and build facilities that are "ultra trace metal clean," in response to requests for a laboratory that is "better than Patterson's." Actually, many people now using trace metal clean laboratories do not know who Patterson is, and his laboratory would not be acceptable by today's standards.

The problem with Patterson's laboratory was that it was constructed in the 1950s within a building that had been constructed in the 1930s. The building's construction made it impossible to have the most effective flow of HEPA (high efficiency particle attenuation) air through his laboratory. Instead, filtered air entered through metal ducts in the center of the rooms, and drying ovens were flushed with filtered nitrogen pumped up from tanks in the basement three floors below. The building's construction also precluded the use of modern plastics in the construction of acid hoods and laboratory furniture. The hoods were constructed of stainless steel and covered with epoxy paint, and the counters, which were also stainless steel, were covered with a fresh sheet of plastic each day. Moreover, Patterson's clean water system was a handmade quartz still that took years to perfect, cost tens of thousands of dollars to construct, and occupied an entire room.

Although Patterson's now antiquated laboratory could be subjected to ridicule today, the trace

metal data generated in that laboratory are still the benchmark for accuracy. This apparent inconsistency illustrates both the importance and limitations of trace metal clean facilities. Trace metal clean facilities are required for accurate analyses of many trace element concentrations in environmental matrices, but the use of those facilities does not ensure the analyses will be accurate.

Similarly, the accuracy of trace element analyses does not necessarily improve as the size and cost of the laboratory increase. This discrepancy has been illustrated by the success of some of Patterson's early apostles. They include Ed Boyle, Ken Bruland, and John Martin, who made several of the first accurate measurements of part-per-billion trace element concentrations in the oceans. Boyle's first trace metal clean facilities at the Massachusetts Institute of Technology consisted of a single HEPA laminar flow hood in the back of his laboratory; Bruland's first trace metal clean facilities at the University of California at Santa Cruz consisted of wooden carnival-type booths with used HEPA filters that were scavenged from a computer company; and Martin's first trace metal clean laboratory at Moss Landing Marine Laboratories was in an old aquarium. The experiences of these and other researchers demonstrate that adequate trace metal clean facilities can be constructed within existing structures and at relatively little cost.

## Section 2

# General Considerations

The most important consideration in the construction of a trace metal clean facility is the hierarchy of cleanliness within the laboratory. There should be a well-defined gradient of increasing cleanliness from the regular laboratory facilities to the cleanest facilities at the back of the clean room(s). Inputs of HEPA-filtered air should be located in that area and should establish a net positive-pressure flow of air through the rest of the clean room(s) and into the general laboratory. Conversely, to limit the amount of contamination introduced in the cleaner areas, most personnel activities should be located in the regular laboratory facilities. Moreover, activities in the clean areas should be strictly controlled, with limited access and mandatory precautions.

This hierarchy is most easily addressed with the construction of a set of rooms in sequence (see Figure 1). This set could include a regular laboratory, change room, "clean" room, "cleaner" room, and "cleanest" room; a parallel hierarchy of cleanliness should exist within each room. Such a construction would provide the optimal physical and psychological barriers to the transport of contaminants into the clean rooms.

Often such construction is not necessary, as evidenced by the utility of the much more modest trace metal clean facilities of Boyle, Bruland, and Martin noted previously. In addition, only modest facilities are required for many trace metal analyses. These include analyses of part-per-million concentrations of trace elements in sewage, sediments, and biological tissues. A single trace metal clean area with its own hierarchy of cleanliness is sufficient in those cases. More extensive trace metal clean facilities are required for analyses of part-per-billion concentrations and below in most laboratories, and are advised for most trace metal analyses whenever possible. This is because they provide both physical and psychological barriers to the introduction of contaminants to the samples within the laboratory.

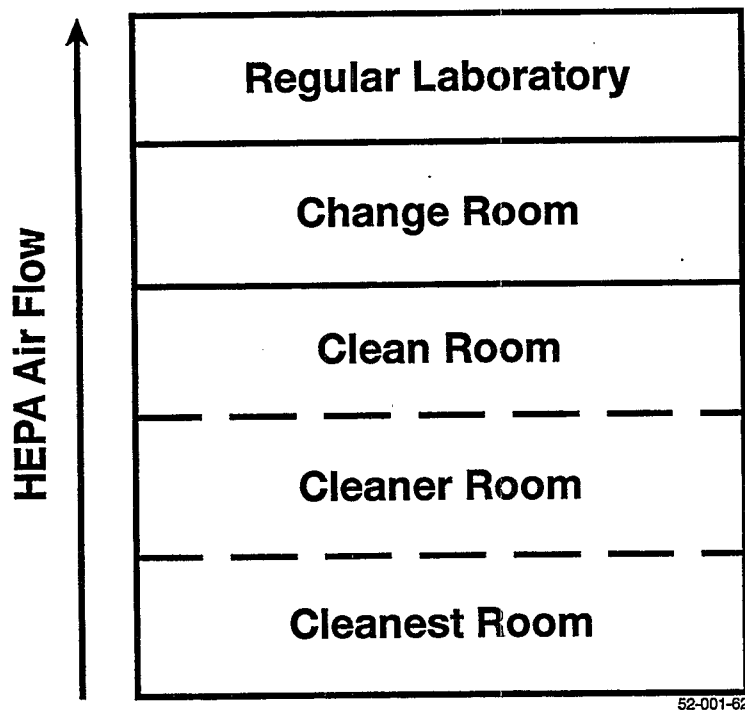
This guidance document provides a mix of what is optimal and what is acceptable in establishing trace metal clean laboratories within existing facilities. It is based on experience rather than specific engineering designs. This experience includes work in Patterson's, Martin's, and Bruland's laboratories, as well as in Sid Niemeier's laboratory at Lawrence Livermore National Laboratory and M. Tatsumoto's laboratory at the U.S. Geological Survey (USGS). This experience also includes constructing three "temporary" (one is now ten years old) trace metal clean laboratories in existing facilities and designing a trace metal clean laboratory in a new building. The design of each laboratory was based on numerous discussions of the advantages and disadvantages of different designs with numerous other users of trace metal clean laboratories. In almost every case, the designs of those other laboratories were constrained by existing facilities, size, and cost.

Figure 1 illustrates the general design of a trace element clean laboratory, and shows the hierarchy of cleanliness within rooms and associated flow of HEPA-filtered air. Solid lines between the regular laboratory, change room, and clean rooms indicate their physical isolation, preferably by solid doors. Hatched lines between the "clean," "cleaner," and "cleanest" rooms indicate their relative isolation, which may be achieved with physical barriers (e.g., solid doors or plastic sheets) or with defined laboratory practices. For example, the "clean" room could be for instrumental analyses, the "cleaner" room could be for sediment analyses, and the "cleanest" room could be for water analyses. Conversely, all three clean areas could be within a single laminar flow work station, where the cleanest materials are placed at the back. In either case, the arrangement is designed to establish a hierarchy of cleanliness that minimizes the potential for contamination within the facility.

This arrangement does not need to be linear, as depicted. Such linearity is difficult to achieve in the design of many new buildings, and it is even more difficult to achieve when retrofitting an old building. Fortunately, the primary criterion is that the arrangement of clean facilities is hierarchical, with HEPA airflow conforming to that hierarchy within those facilities.

The other important criterion is that health and safety features are not compromised. There must be visibility into the clean-room areas from outside areas, and there must be reasonable access and egress. Also, individual rooms should be equipped to meet all fire and safety codes. This equipment would include metal sprinkler heads installed in the ceilings of the otherwise trace metal-free laboratories. As described later in this document, however, minor actions can be taken to minimize the potential for contamination from these sprinkler heads.

FIGURE 1.





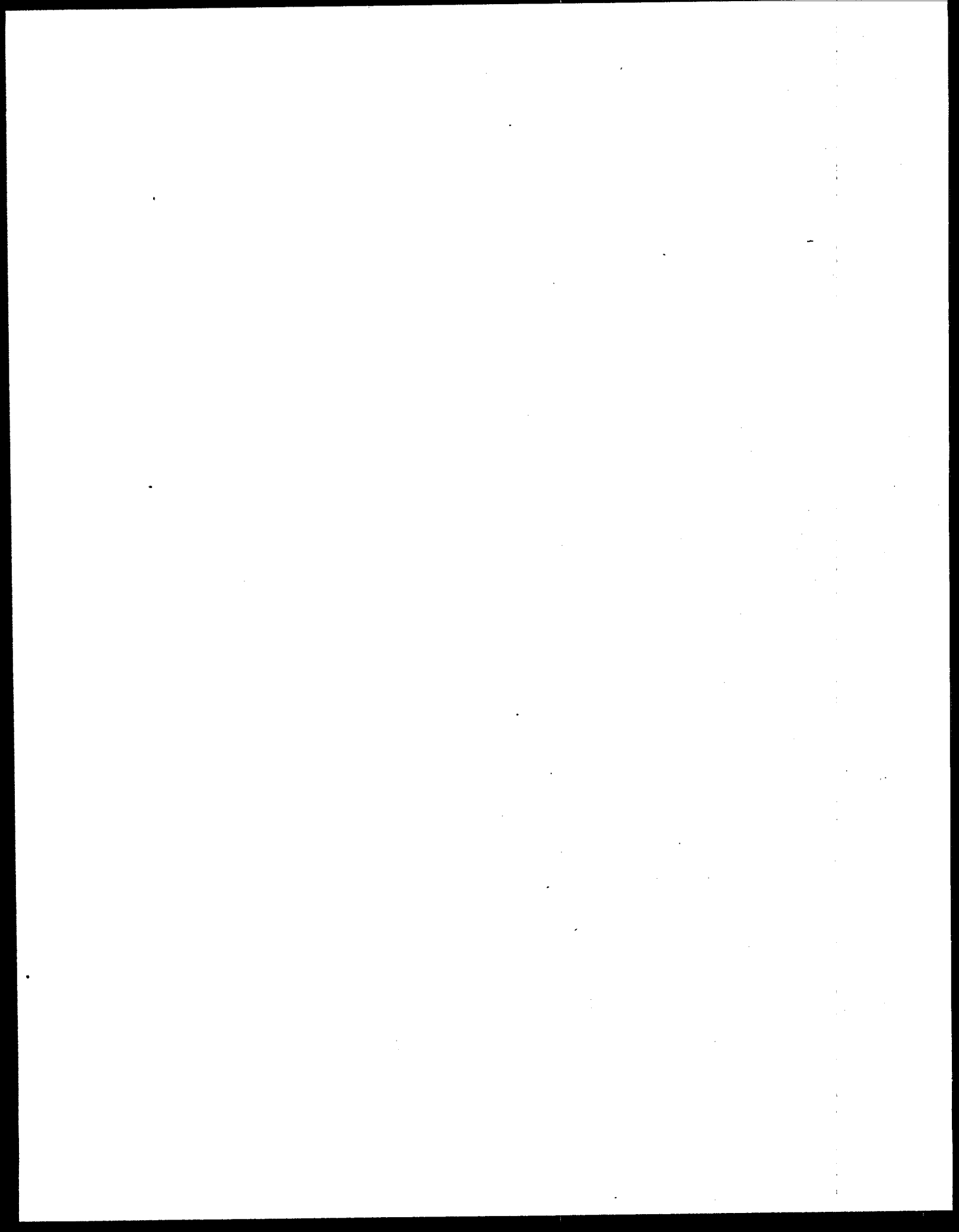
## Section 3 Change Room

With the preceding caveats, a separate change room should be constructed whenever feasible. The room should be located between the general laboratory and the trace metal clean room(s) to provide both the physical and psychological barriers to the transport of contaminants between the other two areas. These barriers are partially created by constructing the change room with the same materials as those of the clean room and by maintaining a positive-pressure flow of HEPA-filtered air from the clean room(s) through the change room and into the regular laboratory to preclude most atmospheric transport of contaminants into the clean areas. These barriers also are created by minimizing the movement of individuals into the clean area, and by requiring them to change into proper garments before entering that area. Changing into proper garments includes (1) removing street shoes and replacing them with clean-room shoes that are never worn outside the clean areas, or covering street shoes with booties; (2) donning clean-room hats and gowns, which are never worn outside the clean areas; and (3) wearing plastic gloves, which are replaced regularly and whenever they come in contact with a potential source of contamination.

Change rooms provide a transitional place for moving samples and other materials from dirty to clean environments. Those materials are commonly enclosed in a series of protective coverings that may range from highly contaminated shipping boxes on the outside to acid-cleaned polyethylene bags on the inside. Since this requirement for existing materials is similar to the preceding requirement for changing from street shoes to clean-room shoes within the change room, an ideal change room should have its own highly defined areas of cleanliness. Notably, a relatively dirty area, where contaminated materials (e.g., shoes and boxes) from the outside are discarded, and a relatively clean area, where noncontaminated materials (e.g., clean-room attire and clean sample containers) are maintained for use in the clean room(s). These areas should be further demarcated by tacky mats at the entrance and exit of the change room.

The hierarchy of cleanliness within the change area may be improved with a wash area. Before putting on clean-room attire, individuals may be required to wash their hands in this area. Containers for sample and clean-room supplies may also be rinsed off in this area before they are moved into the clean room. Since these are some of the primary sources of contamination in many clean laboratories, the installation of a wash area within the change area is strongly recommended whenever feasible.

As an example, the cleanliness hierarchy used at the University of California, Santa Cruz ranges from (1) a large (8 ft x 20 ft) room with a sink in the middle for a stable isotope laboratory, (2) a smaller (4 ft x 10 ft) room without a sink for water and tissue laboratories, and (3) a small area (2 ft x 3 ft) within an existing sediment laboratory. (The small area is essentially defined by the size of the tacky mat immediately inside the entrance. Clean-room shoes and laboratory coats are hung on the inside wall of the entry. Immediately outside the entrance, street shoes are discarded on a mat, and general laboratory coats are discarded on a coat rack. Clean laboratory shoes are kept on the tacky mat, and street shoes are discarded on a mat outside the sediment laboratory.) Each change area should provide a sufficient amount of cleanliness for the transition from a regular laboratory to a clean laboratory.



## Section 4 Clean Rooms

### 4.1 HEPA-Filtered Air

A positive flow of HEPA-filtered air is of paramount importance, as previously emphasized. For example, all work in Patterson's laboratory was ceased when his clean air supply had to be shut down over a weekend. Work was not resumed until the air supply was running again, even though no one had entered the laboratory during that period. Every surface in the laboratory was thoroughly cleaned before any new analyses were initiated, and the first set of analyses consisted of blank analyses to determine potential lead contamination in every reagent and sample container that had been in the laboratory when the clean air supply had shut down.

Again, the concept of airflow in a trace metal clean laboratory is simple. HEPA-filtered air should flow from the cleanest part of the facility to the dirtiest part. The flow should be steady and uninterrupted, with sufficient positive pressure to preclude the atmospheric transport of contaminants into the clean area. There are engineering arguments for the location of the HEPA filters above the work surface (National Science Foundation design) and behind the work surface (i.e., blowing over the sample and toward the analyst). Empirical observations (and numerous blank measurements) show that either orientation is sufficient.

The location of the intake for the filtered air supply is very important. The intake should be at the opposite end of the room from the place where the filtered air enters the room. If more than one room is in the sequence, the intake should be at the opposite end of the farthest room from the filtered air outlet. This configuration ensures a positive flow of clean air throughout the system. Other intake configurations may establish cells of airflow within the clean room and may even draw unfiltered air into that area.

### 4.2 Ultrapure Water Supply

Ultrapure (18.3 M $\Omega$ /cm) water needs to be plumbed into the clean laboratory. An outlet should be located near the work surface to minimize movement within the area during chemical processing. Movement may be minimized by locating the systems near the entrance to the clean room or within the change room, and by plumbing the water to a carboy with a spigot near the back of the laboratory. This location of systems minimizes the potential for contaminating the work area with metal or paint flakes from the system or while replacing cartridges within the system. It also minimizes the potential to contaminate the systems by maintaining them in a clean environment.

### 4.3 Materials

Trace metal clean laboratories must be physically isolated from the sources of contamination that are so common in regular laboratories. These include cements, paints, metals, and plastics that contain relatively high concentrations of metals that are mobilized by degradation of those materials. Surfaces that may contaminate a sample should be constructed of relatively clean, inert materials such as polypropylene, polyethylene, and Teflon. In addition to counter tops, these surfaces include regular and laminar flow acid hoods, which are now commercially made of plastics. All other materials in the laboratory should either be constructed of comparable materials or covered with a relatively clean, inert epoxy paint. This includes all handles, hinges, and electrical outlets, which are commonly made of metal, but are also made of plastics to prevent corrosion in kitchens, bathrooms, and marine and other outdoor fixtures. It also includes the walls, ceiling, and floor. The latter should be covered with a non-skid epoxy paint.

### 4.4 Walls and Ceilings

Additional care must be taken to prevent the transport of contaminants through the walls and ceiling of a trace metal clean room constructed within an existing room. As an example, one existing facility that was converted to a clean room contained a source of sawdust that fell through the plastic subceiling. The problem was remedied by sealing every joint in the plastic ceiling with silicone, vacuuming the structure supporting the subceiling, and covering it with sheets of plastic. These sheets are now inspected for integrity whenever the HEPA filters on the subceiling are replaced.

### 4.5 Windows

Access to the clean-room areas may be further controlled with windows that minimize traffic and maximize communication between adjacent areas. Large windows in both the walls and doors facilitate communication between individuals in adjacent areas. The windows may also be used to check on the status of the clean areas without entering them or to show the facilities to visitors without taking them into the clean areas. Ideally, windows should be installed on each wall that connects to adjacent clean rooms or changing areas.

Pass-through windows facilitate the transfer of materials between the clean room with minimum movement, but they may be an additional route of contamination. The principal problem is their disruption of positive-pressure airflow out of the clean room. Therefore, placement of pass-through windows should be limited to areas within a clean-room facility (e.g., between a "clean" room and a "cleaner" room).

## 4.6 Doors

While doors serve as barriers to the transfer of contaminants into clean rooms, they also serve as the major routes of those contaminants into the clean rooms. This is due to the movement of individuals and materials through the doors. It is also due to the disruption in positive-pressure airflow that occurs when they are opened and closed.

Flexible sheets of plastic may be preferable to solid doors within some internal areas. These doors are akin to the strips of plastic that are used in grocery stores to insulate frozen food areas, while allowing customers to take foods from those areas. These plastic strips can be placed between areas with different levels of cleanliness in the clean labs. They can also be placed between an instrument room and a regular laboratory. The latter application can help maintain cleanliness within an instrument, in spite of the often continuous flow of individuals in and out of that area. Another advantage of using plastic strips in doorways is that they do not take up as much space as hinged doors.

Sliding doors are another option that have been used with some degree of success in clean laboratories. This includes both sliding glass doors, which provide optimal visibility, and pocket doors, which require the least amount of space. However, these doors are not generally recommended for most clean-room facilities because they tend to be left partially open.

## 4.7 Safety Features

It should be emphasized that visibility and access into the clean-room areas are important health and safety features. The use of acids and heat (hot plates, stills, and ovens) in those rooms, combined with the extensive use of highly flammable materials in the construction of those rooms, makes them dangerous. For example, one clean room essentially melted when a plastic fume hood was overheated by a hot plate that was inadvertently left on overnight.

Consequently, it is essential for clean rooms to be designed with the involvement of fire marshals, as well as health and safety officers. The small amounts of metal introduced for safety features are inconsequential in terms of contamination. Therefore, the use of fire retardants, additional doors for emergency exits, metal sprinkler heads in the ceilings, eye wash stations, fire extinguishers, and other standard laboratory safety materials (e.g., acid spill kits) should be incorporated into in every clean room.

## 4.8 Commercial Options

It should be noted that there are commercial options that, in many cases, may be more cost-effective than those described in this document. These include cases where (1) laboratory personnel are not in a position to cease their regular activities in order to construct a clean laboratory, (2) laboratory personnel are not familiar with clean-room materials and design, and

(3) time is limited. In those cases, it may be most appropriate to have commercial clean laboratories installed. Some of those laboratories are constructed with flexible plastic ceilings and walls and with rigid frames that may be placed in any configuration. This enables them to be installed, essentially, overnight.

## Section 9 Illustrations

While there are numerous clean-room facilities that are superior in scope and design to those shown in the following illustrations, it has been found that the facilities described and illustrated in this document are sufficient for trace element analyses at any existing or theoretical (i.e., pristine) level. Moreover, many of the clean-room facilities shown in the following illustrations were constructed within existing facilities and at minimal cost. This includes one change room and three clean rooms that were constructed in the basement of a twenty-five year old building at the University of California at Santa Cruz. The materials for constructing those retrofitted facilities only cost a few thousand dollars. (Students, technicians, and teaching staff did most of the construction; university contractors retrofitted the electrical wiring, plumbing, sprinkler system, and acid hood connections.)

Figure 2 shows the WIGS trace metal clean laboratory for water chemistry, which was installed within an existing laboratory at the University of California at Santa Cruz. The figure illustrates the extraction counter at the rear of the laboratory and the adjacent laminar flow exhausting acid hood. The counter is abutted to a HEPA air system at the back of the laboratory. The laminar flow exhausting hood has a HEPA air system within the roof of that hood. There is also has a HEPA air system installed in the ceiling at the back of the laboratory.

The placement of the counter top below the bottom of the HEPA system is a design error. This was due to the installation of a standing HEPA system, which had been acquired as surplus from a computer company, in the laboratory without changing the height of the system. Theoretically, this displacement creates small turbulent airflows across the back of the counter. Since there is no evidence of the advection of contaminant air toward the back of the counter, this design error has not been corrected.

The counters are covered with polypropylene. The tops and the sides of the extraction counter and acid hood are constructed of plexiglass. There are two movable plexiglass sheets on the front acid hood, so that samples may be processed with only one side of the hood opened. The hinge for the plexiglass is plastic with plastic screws. When opened, the plexiglass is held up with velcro strips, which appear as black stripes in the photograph. The frame of the work area is constructed of plastics; the frame of the acid hood is constructed of metal that is coated with epoxy paint because it must hold the HEPA system on the ceiling.

The counter in the work area has a small plastic sink, and the bottom of the perforated counter in the acid hood has a collection trough. Other sinks in the clean-room areas have plastic fixtures. These provide water from clean water systems, which are plumbed with plastic. Additionally, the alcove to the right of the work area contains a carboy that receives water from a high purity system located in an adjacent clean (albeit less clean) room. The water is also fed into three sub-boiling quartz stills, which are aligned in sequence and mounted on the wall of that alcove.

The metal bases on the hot plates in the acid hood have been modified for the laboratory.

Specifically, the metal bases have been replaced with pyrex bases that have been sealed with silicone, the electrical cords have been encased in clean plastic tubing, and the controls for the hot plates have been placed within plastic boxes located beneath the acid hood.

The laboratory cabinets and furniture are constructed of wood and painted with epoxy. All metal handles and hinges in those cabinets have been replaced with plastic fixtures. Since the drying oven below the work area is metal, all materials placed in the oven are enclosed in plastic containers.

The light fixtures within the acid hood and on the outside of the ceiling in the work area are constructed of plastic materials. The fixtures in the acid hood are mounted to the frame with metal hardware that has been painted with epoxy. All other light fixtures in the clean room, as well as all other clean-room facilities, are also constructed of plastic and mounted in a similar manner.

Figure 2.

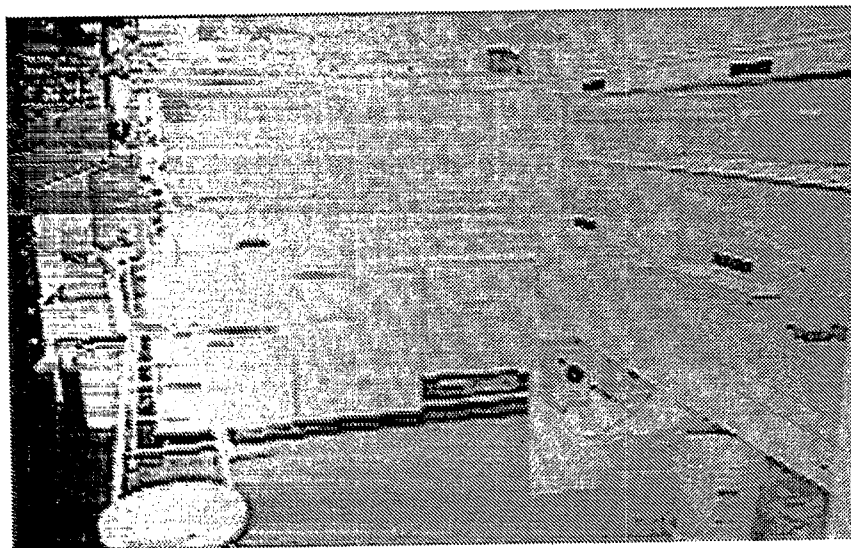




Figure 3 provides another view of the laminar flow exhausting acid hood in the WIGS water chemistry room, and shows both the floor and ceiling within the laboratory. The existing concrete floor was covered with a non-skid epoxy paint. The ceiling within the laboratory was constructed with solid plastic sheets, which were glued and screwed into a wooden frame. The white spots in the ceiling are plastic caps for the crews. The screws were sealed with silicone, as were the edges of the plastic sheets and the openings in the ceiling for the plastic ducts to the acid hood. The outer sides of the wood frame were also covered with plastic sheets. Internal walls that were built for the clean rooms were constructed in the same manner. Existing outer walls constructed of concrete, including the wall at the rear of the laminar flow exhausting acid hood, were covered with an epoxy paint.

Figure 3.

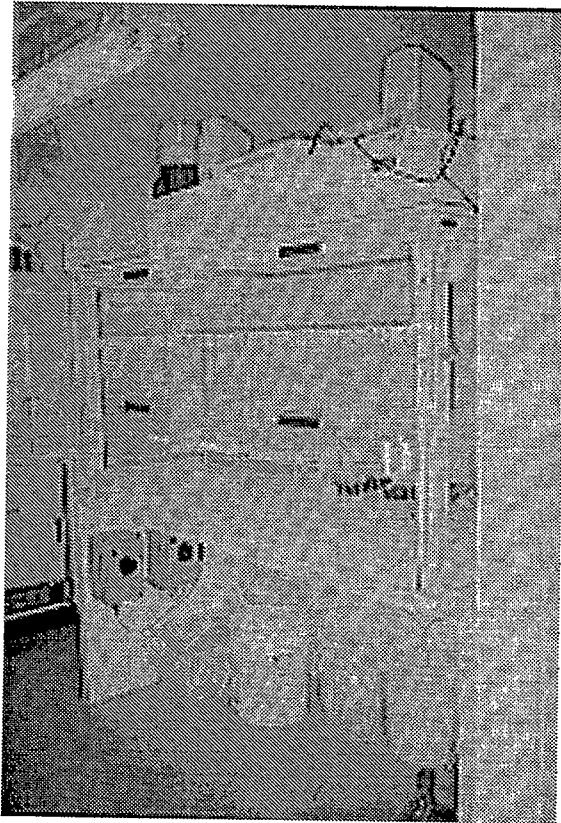


Figure 4 illustrates the interior entrance from the WIGS general laboratory to the change room for the water chemistry laboratory. Windows in the doors and adjacent walls provide views into the clean room from the general laboratory. Variable transformers mounted near the ceiling in the change room control heating units within the clean room. This placement allows the units to be adjusted without going into the clean room and keeps the metal transformers of the clean room. A tacky mat is located at the entrance to the change room and at the entrance to the clean room.

Figure 4.

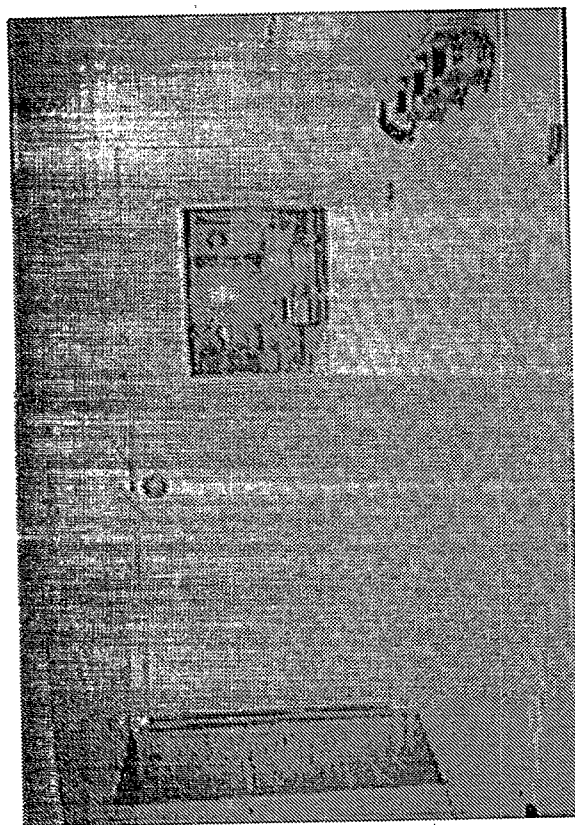


Figure 5 shows the external entrance to the WIGS change room. Because of space limitations, street shoes and regular laboratory jackets are discarded before entering the change room. Clean-room booties, laboratory coats, and hats for the clean room are stored on non-metal hooks in the interior of the entrance to the change room. Materials from dirty containers are transferred to clean containers within the change room. Only clean materials are taken into the clean room. Only clean-room materials are stored in the closet at the rear of the change room. A window in the interior door between the change room and the clean room is aligned with a window in another door on the other side of the change room to provide visibility into the clean room from the adjacent instrument room. That instrument room is to the right of the entrance to the change room, and is entered through hanging plastic sheets. The light fixture installed in the change room is constructed of plastic. The handles on the door from the change room to the clean room are plastic. A metal fire sprinkler head is extended from the main ceiling down through the ceiling in the change room, where non-operative parts of the sprinkler system are painted with epoxy.

Figure 5.

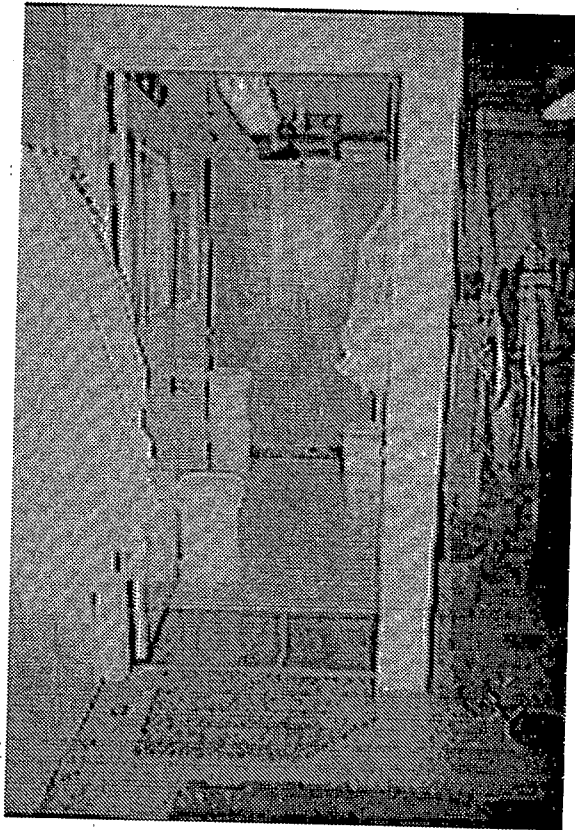


Figure 6 depicts the entrances to the WIGS change room (on the left) and the instrument room (on the right). The change room and connecting clean rooms have solid doors, walls, and ceilings. Their wood doors and frames are covered with epoxy paint. The walls and ceilings are constructed of wood frames and covered with solid plastic sheets on both sides. Windows in the doors and walls in those rooms are plexiglass. The ceiling of the instrument room is constructed of wood and covered with flexible sheets of plastic, because ducting for the acid hood located in that room precluded the use of solid plastic sheets. Individual areas within the instrument room are separated by wood frames with sheets of transparent plexiglass. The entrance to the latter room is through a plastic sheet, which facilitates movement into that room while maintaining positive-pressure flows from HEPA air supply systems located within the room. HEPA work stations are located within the instrument room.

Figure 6.

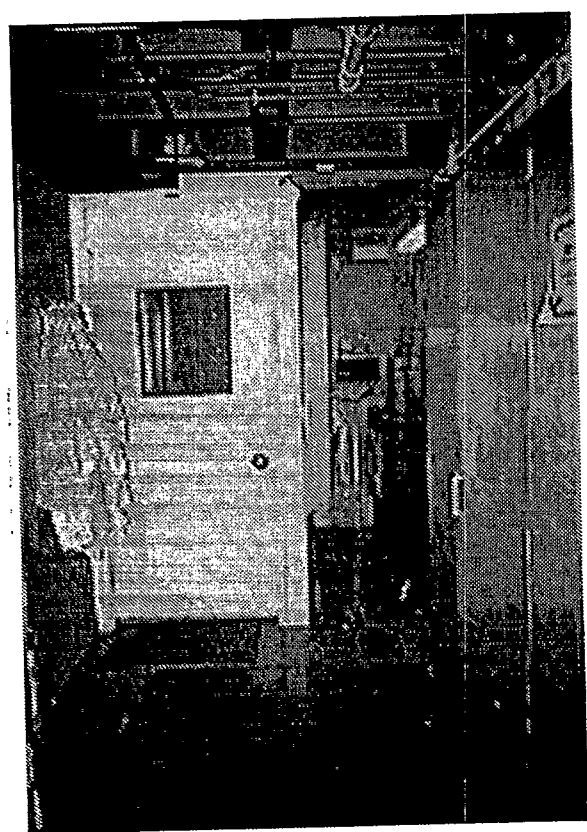
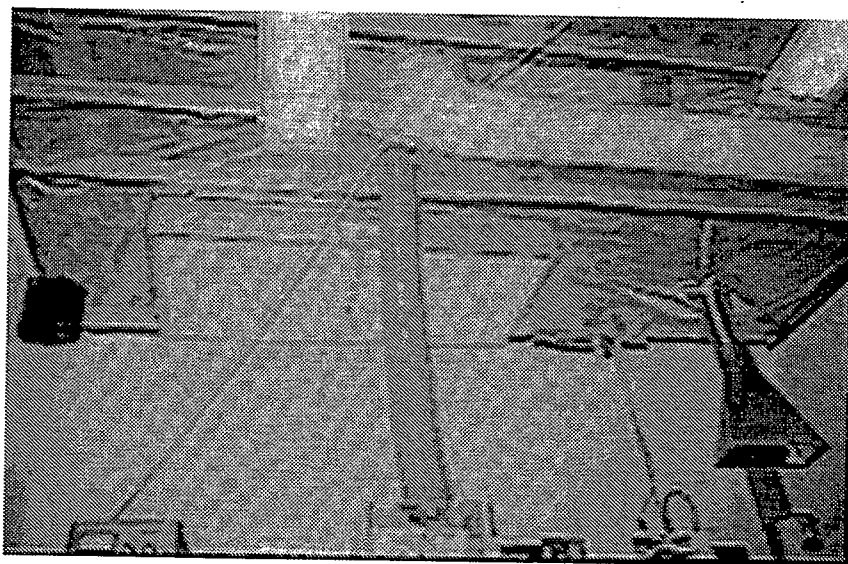


Figure 7 shows the ceiling in the WIGS instrument room. The room was framed in wood and covered with plastic sheets. The sheets were stapled to the frames and covered with duct tape. HEPA air systems were mounted to the wood frames, directly above each instrument. One section of plastic sheeting was lowered to enclose the acid hood exhaust duct (white plastic). The height requirements of that duct precluded constructing a solid plastic ceiling within the instrument room. Exhaust ducts (blue plastic) of the atomic absorption spectrometer were vented through the plastic roof. (Only one exhaust duct is visible in the photograph.) Other electrical wires and plumbing were encased in plastic and extended through the plastic ceiling. Existing light fixtures in the original ceiling were left in place, and supplemented with plastic light fixtures that were mounted beneath the plastic ceiling. The concrete block walls were covered with epoxy paint.

Figure 7.



## Section 10 Summary

The basic requirements for a trace metal clean room are minimal. They include metal free work surfaces and hoods, positive pressure with HEPA-filtered air, and clean (18.3 MΩ/cm) water. Each of those requirements may be readily achieved with commercially available materials, and they may be easily installed within existing facilities at relatively little expense. For example, the clean facilities illustrated in Figures 2-7 enclose approximately 1,000 ft<sup>2</sup> of trace metal clean rooms within the basement of an old building, and were initially designed as temporary facilities. These "temporary" facilities have been proven to be sufficient for the past decade, as evidenced by the recent attainment of a procedural lead blank of 30 picograms (Flegal and Smith, 1995). Costs were minimized by doing the labor in-house, but it is now possible to purchase relatively inexpensive "portable" clean rooms. Those rooms are designed to be placed within existing rooms, and they have proven to be sufficient for trace element analyses. Therefore, trace metal clean facilities are now readily available for any laboratory.

It should be noted, however, that the availability of those facilities does not ensure the validity of data generated within them. Moreover, the quality of those data may not improve with the establishment of elaborate trace metal clean facilities. That quality may be achieved only by competent analysts using trace metal clean techniques within a trace metal clean laboratory.

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