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DESIGN AND OPERATION OF A LAMINAR FLOW CLEAN-ROOM FOR TRACE METAL ANALYSIS IN MARINE CHEMISTRY

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Abstract

Contamination, one of the biggest problems a marine chemist has to overcome when investigating trace metal distributions, can be minimized if a laboratory is converted into a laminar flow clean-room according to analytical, technical and industrial hygiene requirements. The design and construction of such a clean-room is described. Procedures for its optimum use in conjunction with oceanic or estuarine field work are also discussed.

INTRODUCTION

Marine trace-metal chemists have entered an age when most instrumental techniques available give detection limits well below those required for the levels of metals naturally present in their samples. However, the art of presenting to the instrument a trace-metal at the same concentration as it was just prior to sampling is extremely difficult (La Fleur 1976). Changes in concentration can be positive or negative. Atmospheric particles or vapour, dirty containers, impure reagents, bad sampling and handling techniques can randomly contaminate the sample, giving positive errors, while adsorption and volatilization cause negative ones. The problems associated with sampling and storage of water samples for trace metal analysis have been recently reviewed by Batley and Gardner (1978a) and Robertson (1976).

The results from interlaboratory comparisons of the trace-metal concentrations in standard seawater samples (Brewer and Spencer 1970; Van Loon 1974; Anon 1974; Major and Pettis 1978) and fish samples (Anon. 1977) indicate the seriousness of gross random and systematic errors introduced during handling and analysis.

Patterson and Settle (1976) have shown that if great care is taken at all stages of sample handling and analysis most of these problems can be overcome. The use of a dust free working area is essential. This paper describes how a general laboratory was modified for use as a laminar flow clean-room for trace metal analysis. Procedures for its operation in conjunction with field work are described. Although the layout of the clean-room is convenient for the space available at our Cronulla laboratory, the general requirements and layout can be adapted and modified to suit any group of marine or trace metal chemists who need such a laboratory conversion.

GENERAL REQUIREMENTS

The plans for a clean-room for trace metal analysis of seawater were drawn up to overcome the inadequacies of a dangerously overcrowded

6 m x 4 m laboratory which housed three or four scientists. Unsuccessful attempts to do cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) analyses by anodic stripping voltametry (ASV) were made alongside Cd-Cu reduction columns used for nitrate analyses. Mercury (Hg) analyses were attempted alongside the ASV instrumentation, which uses and emits mercury (Hg) (Gardner 1976). Pb- and Zn-based paint flakes fell from the ceiling where possums had nested in the roof and had caused dampness to penetrate the ceiling panels. Green copper hydroxycarbonate and hydroxysulfate and rust covered all brass and steel taps, fittings and equipment. Talcum powder (Zn) was liberally sprinkled into gloves used when acid washing equipment. Dust, probably containing Al, Ca, Cu, Fe, Mg, Mn, Ni (Robertson 1972), and glassware that would not fit into cupboard space, lay everywhere. Cleaners proudly kept the brass door knob shining.

We therefore gave up trying to do Cd, Cu, Pb and Zn analyses in our laboratory and did these in a cleaner laboratory set up for trace analysis elsewhere in Sydney (Batley and Gardner 1978b). We continued with Hg analyses by taking extreme care and by using analytical methods especially designed for determining Hg in contaminated working areas (Gardner and Riley 1974; Gardner 1977; Gardner and Dal Pont 1979).

The chemistry section was provided with a complex of five small laboratories. One was dedicated to nutrient analyses, one for analysis of organic matter and one for general purposes and office space. The remaining two were set aside to be converted into a clean-room and semi-clean-room.

These were designed on the basis of the following requirements:

1. All possible metal and metal impregnated materials to be replaced with non-metal alternatives or covered with a non-metal skin.

2. The laboratory to be sealed, airconditioned and all dust filtered from the air through high efficiency particulate air (HEPA) filters.

3. The laboratory to be free of mercury vapour.

4. All service facilities to be controlled from within the clean-room.

5. Design to include safety factors, fume ventilation, emergency exits, good lighting and low noise, etc.

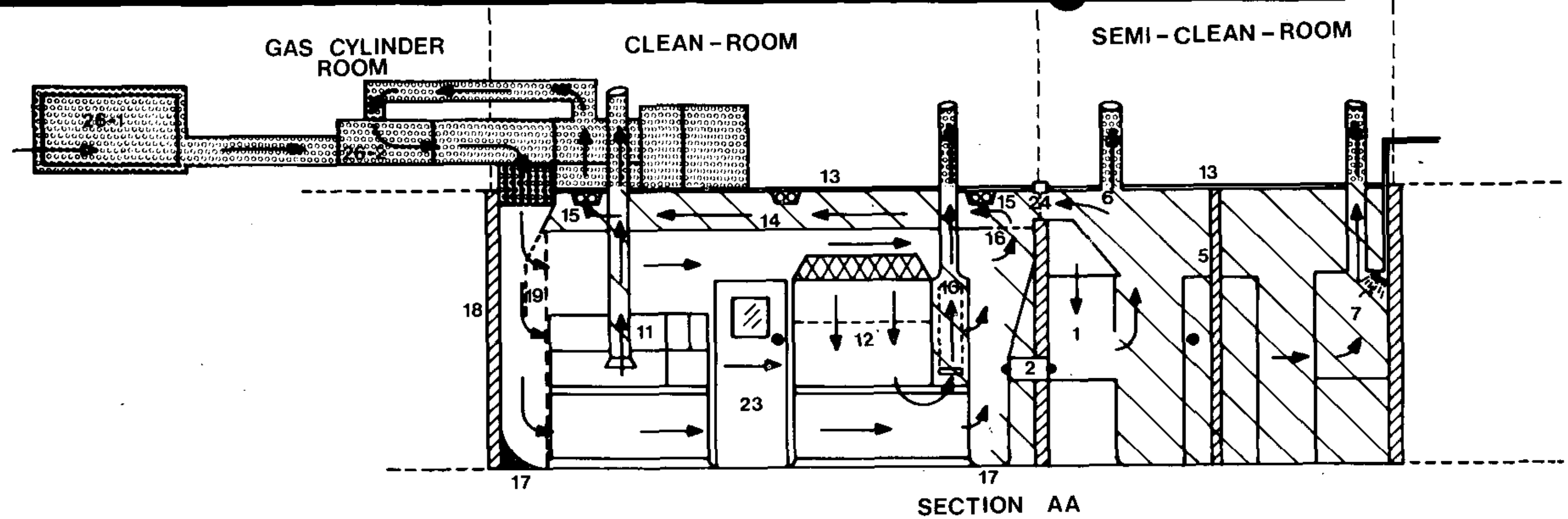
CLEAN-ROOM DESIGN - MAJOR CONSIDERATIONS

Clean Air

Laboratory air can only be cleaned and kept clean if a room is designed correctly, if the operators are robed correctly and if they use and maintain the room according to a set of strict principles (Zeif and Mitchell 1976). The U.S. Federal Standard 209a (1966) specifies classes of air cleanliness. Part of the laboratory was required to be "class 100", i.e. less than 100 particles 0.5 μm and larger and less than 10 particles of 5 μm and larger in each cubic foot of air.

Laminar Flow

"Creating the right airflow is as important, in a clean room, as having efficient filters" (Hughes 1974). Laminar air flow is a condition in which all the air in the room is made to flow at uniform velocity. The flow can be from wall to wall or ceiling to floor. The latter requires a grill floor and there is less chance of cross contamination between people or material within the clean-room. Due to financial considerations, wall to wall flow was selected (Figs.1 and 2)



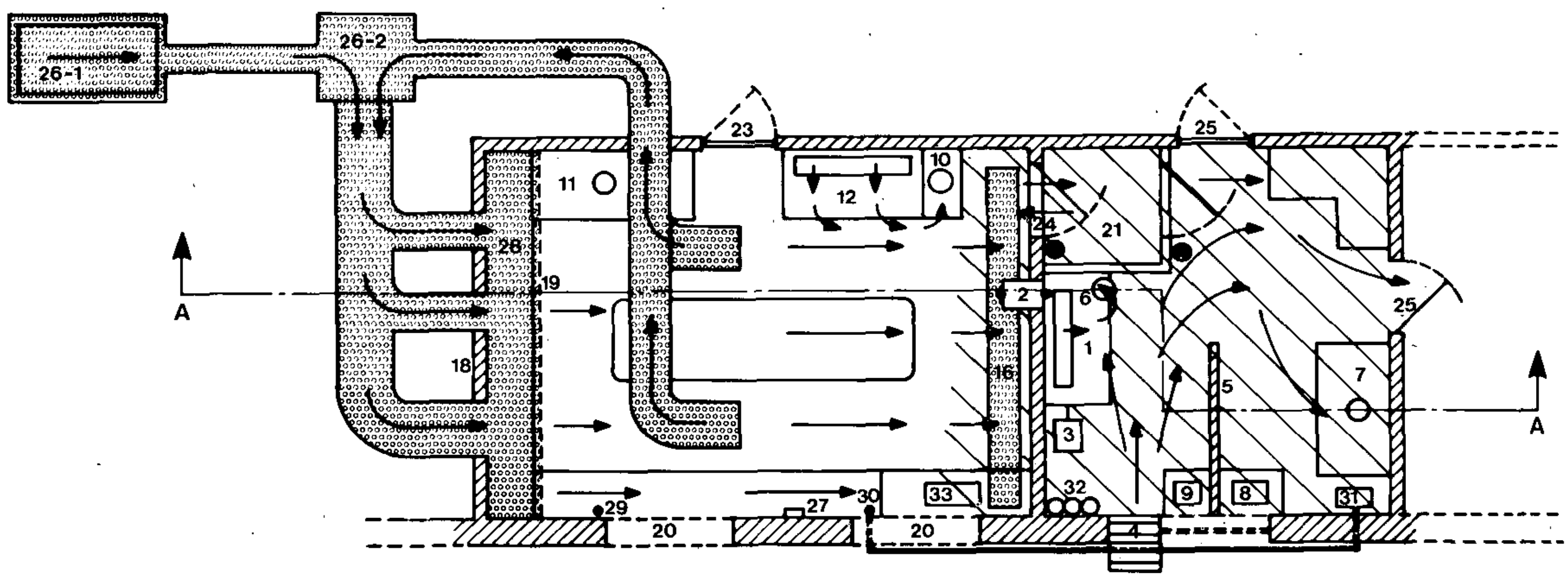


Fig. 1 (lower). An aerial view of the clean-room complex with clean and less clean laminar air flows.

Fig. 2 (upper). A sectional view of the clean-room complex with clean and less clean laminar air flows.

and the benches and equipment were arranged so as to give the least amount of cross contamination between workers, or disturbance to the flow. Disturbance of laminar air flow causes turbulence and subsequent spots of dirt accumulation.

In the following description of the rooms, bracketed numbers refer to items of apparatus shown in Figs 1-4.

Elimination of mercury vapour and acid fumes

It was necessary to eliminate the Hg vapour which is emitted from the ASV instrumentation so that samples, collected for Hg analyses, could be handled and analyzed in the clean-room without fear of cross contamination within the room. An adjoining semi-clean-room was therefore incorporated into the laboratory design, to house the ASV instrumentation.

To prevent contamination of samples which have been prepared in the clean-room for analysis by ASV, the instrument is placed in a laminar flow clean air cabinet (1). The cabinet backs onto the clean-room and a small air lock (2) separates the two clean environments. The air-lock is large enough to hold a large plastic tote box to contain samples. Positive air pressure in the clean-room ensures that there is no back flow of mercury contaminated air from the ASV instrumentation when either of the air-lock doors is opened. The sample air-lock can also be used to transfer equipment that has been cleaned in acid or in an ultrasonic bath in the clean-air cabinet.

The recorder (3) for the ASV instrumentation is housed on a bench alongside the clean-air cabinet and the temperature kept at 25 ± 0.5°C by means of an air conditioner (4). A ceiling height, half glass panel (5) divides the two sections of the semi-clean-room, allowing good temperature control. Hg vapours are exhausted from the area through a vent (6) and the perchloric acid fume cupboard (7). The glass panel also keeps any acid and dirt present in the fumehood-glassware sink (8) away from the scientists' scrubbing up area (9).

The 122 cm perchloric acid fume cupboard, which can also handle radio-tracers, is housed in the semi-clean-room instead of the clean-room because it would have been too costly to install a sufficiently large air conditioning unit to service the clean-room if the clean, conditioned air was continually being exhausted. Instead, the fume cupboard (10) in the clean-air laboratory has only a 45.7 cm frontage. Air can enter this fume cupboard in two ways. If the atomic absorption spectrophotometer (AAS) (11) is in use, particles will contaminate the air upstream of the cupboard; to ensure only clean air enters the fume cupboard, air is drawn from the adjacent laminar flow clean-air cabinet (12), while the front of the fume cupboard is sealed. If the AAS is not in use, air can be drawn directly into the fume cupboard from the room through the small slot openings on the front. The exhaust is only weak, so to maintain a high air velocity into the cupboard a narrow inlet is used, and manipulations are done in the cupboard through elbow length plastic gloves. When not in use cover slides drop into place over the glove inlet.

Industrial Hygiene and Safety Factors

When designing a working area it is necessary to consider the requirements of the occupants in order to maximize their efficiency. Light intensity and machinery noise levels, temperature and humidity control, pleasant decor and view, safety (Muir 1977; Everett and Hughes 1975; Ferguson 1973) and ease of room operation were incorporated into the clean-room plans.

Figures 1 and 2 show the aerial and floor plans of the clean- and semi-clean rooms designed from the lists

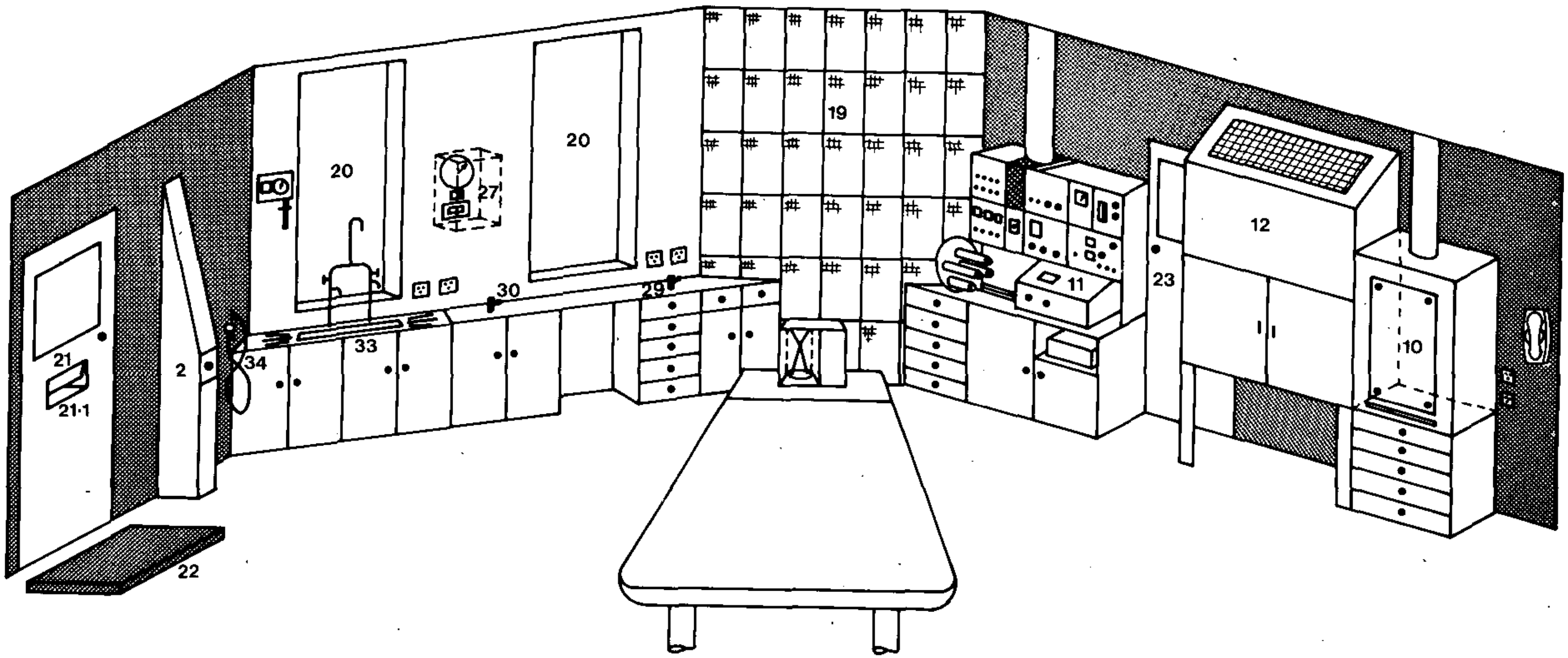


Fig. 3. An artist's impression of the clean-room.

of requirements and major considerations, and Figs. 3 and 4 show artist's impressions of the finished rooms. The size of the air-conditioners and trunking, the number and type of filters, the clean-air blower and exhaust fan sizes, as well as the temperature and pressure regulation system were specified by a clean-room engineer who also supplied the contractors with requirements for noise levels, wiring, lighting and floor quality.

CONSTRUCTION OF CLEAN-ROOMS

Preparation

The two adjoining rooms were stripped of all existing fixtures, floor coverings, ceiling and wall paint, window frames, doors, skirting boards, plumbing and light fittings. A floor (13) was built in the roof cavity to support the air-conditioning units, filters, fans and exhaust systems. Above this the ceiling joists were covered with a tarpaulin to prevent dust and leaves dropping onto the motors, fans and trunkings.

Ceiling

Strong, plastic-finished paper is firmly bonded to the ceiling panels in both laboratories. In the clean-room, an epoxy coated frame, 48 cm from the ceiling, supports a false ceiling made from opaque plastic sheets (14). These sheets absorb some of the light so, to ensure good illumination, twice the normal number of fluorescent tubes are fitted above the false ceiling (15). The illumination is sufficient that if, between annual maintenance and cleaning schedules, several tubes malfunction their loss should not be noticed. However, if necessary, tubes can be rolled along a track to a sealed double trapdoor in the roof cavity and changed without entering the clean-room. The opaque sheets and the tubes are lifted out and cleaned yearly. An air grill (16) stretches the full length of the ceiling at the "return air" end of the room.

Walls

The walls are panelled with laminated white sheets joined by plastic strip. At the floor a deep coving† (17) is moulded onto the laminate. The exterior wall (18), where the clean-air enters, is insulated to prevent heat change of the conditioned air. In front of this, a frame supports a wall of perforated epoxy-coated, air-supply grills (19) which are coved to the floor, but can be removed for maintenance.

Windows

The windows (20) are double glazed to maintain a good temperature balance and are connected flush to the wall panels with plastic strips.

Airlock and Doors

The clean-room is under positive pressure in order to keep out dust and Hg vapour from the semi-clean room, and the corridor. To maintain the pressure, an airlock (21) is situated between the two rooms. The two doors of the airlock are never opened together. Glass observation windows make it possible to see if anyone else is using the airlock.

The airlock has pegs and a shoe rack on which are kept the clean-room smocks and overshoes. Just inside the clean room is a 'tacky' mat (22). Any grit and dust which may have been deposited on the floor of the airlock by outdoor shoes, and then transferred onto the soles of the overshoes, is removed while stepping on this mat.

† Coving is a moulded curve between floors and walls or ceilings and walls which eliminates the right angled joint where dirt collects.

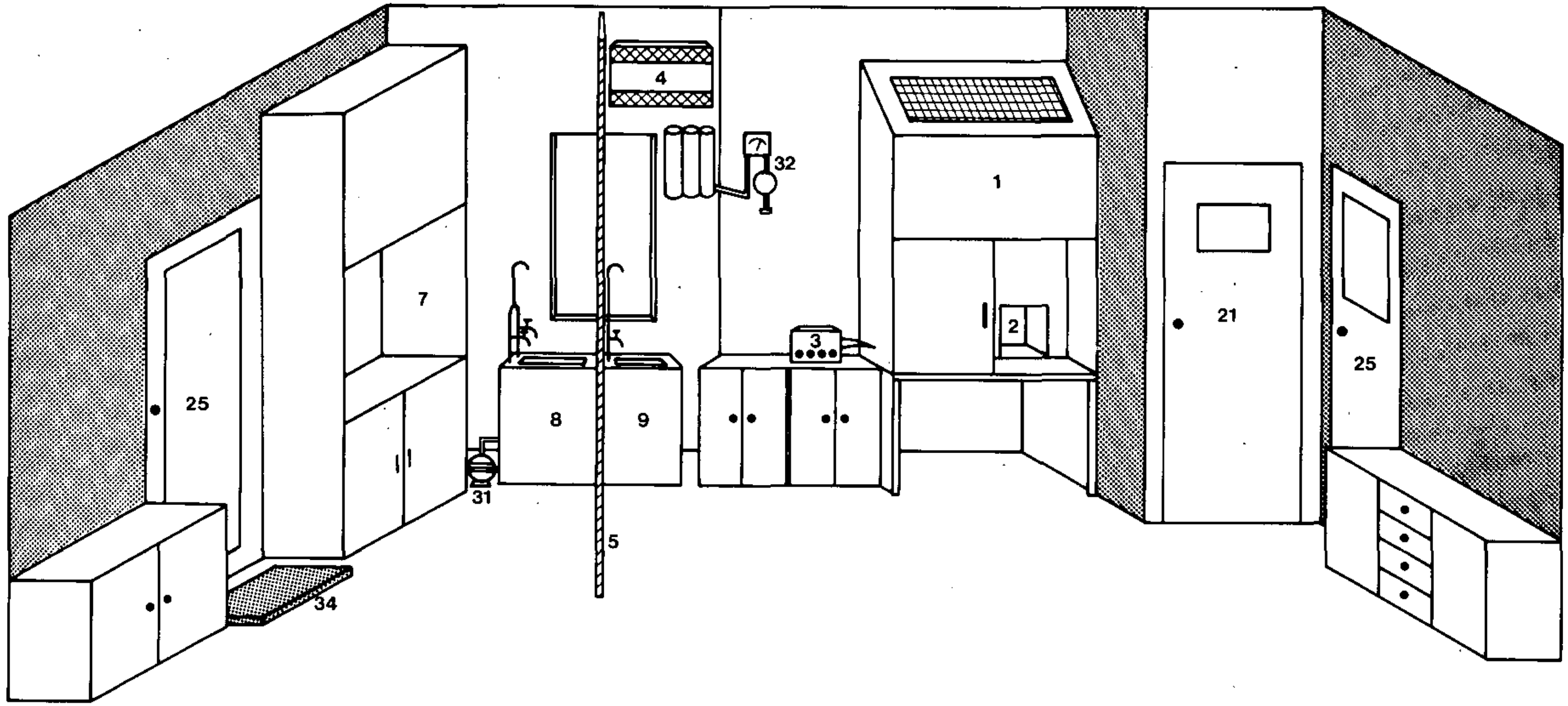


Fig. 4. An artist's impression of the semi-clean room.

There is also an emergency exit door (23) which only opens into the corridor from inside the clean-room. When this opens, an alarm sounds which indicates that a scientist is in need of help, and that the room needs to be closed while it re-establishes its dust-free state. This door has a double glazed observation hatch through which the room can be viewed.

All doors are constructed without ledges which would collect dust. The use of door frames which are flush with the wall panels prevents the laminar air becoming turbulent. They have seals around the jambs and the floor. The door which opens from the clean-room has a pillar box vent (21.1) to prevent excessive build up of pressure and to allow air to cleanse the airlock. Some of this air returns into the false ceiling via a grill (24).

The semi-clean-room door (25) was also designed for exit only and, for safety, opens outwards. All doors "en route" to the clean-lab have wooden handles. The semi-clean-room door and the outer airlock door have locks to prevent unauthorized persons entering the clean areas. They too open outwards for safety.

Benches

To maintain a non-turbulent laminar air flow, work benches are arranged along the length of the laboratory under the windows, along the corridor wall and down the centre of the laboratory. All wooden benches are coated with polyurethane varnish inside and out, are topped with formica laminate and are coved to the floor. The centre bench top is supported by four round legs that are again coved to the floor. At the cleanest end of this centre bench is a heavy marble balance table which is installed so that the air flows through the supports which are coved to the floor. The leg space is at right angles to the flow of air. The marble is covered with epoxy varnish and the top with a teflon sheet. The window bench has cupboards, drawers, leg space and supports all the service facilities. On the corridor wall one bench supports the AAS (11) and houses all the AAS gas supply taps.

Floor

Once all the work in the clean-room airlock and semi-clean-room had been completed the doors were sealed and self-levelling epoxy floors were poured in. The finish is smooth, non-skid and pale gray. Gray was chosen rather than white to eliminate glare and to disguise accidental chemical stains.

Airconditioning

Laboratory air is supplied through a reverse cycle split system air-conditioning unit (26). The outside unit (26.1) has a condensing unit, filtered air inlet grill and is installed well away from the laboratory to keep the noise level low. This is connected to an indoor fan-coil unit (26.2) which is housed in an acoustic chamber in the roof enclosure. The system has two Viledon type sectional pre-filters in the return-air ducts and an air supply fan unit. Room pressure, air flow, and temperature controls can be operated from the laboratory (27).

Air filters

The conditioned air enters HEPA absolute filters installed in a wall-mounted, acoustically-lined plenum box (28). To ensure a positive room air pressure, an imbalance has been made between the clean-air inlet through the perforated wall and the discharge grills at the return-air end of the room. The volume of air which enters can manually be increased, using the damper control, if the exhaust systems are operated in the clean-room, or if the prefilters become clogged with dust particles and require extra pressure to maintain adequate air flow through the laboratory. A pressure gauge in the laboratory indicates when a manual adjustment to the air flow is required.

Service facilities

Adequate numbers of electrical sockets are placed around the benches. To prevent metal dust, released during on-off arcing from entering the room, all sockets, switches and unused plugs are covered in plastic. A (0.2 μm) filtered nitrogen supply for pressure filtration is positioned on the window bench (29). The gas bottle for this system and those for the AAS are kept in an adjoining room. On the same bench is a vacuum line (30) and switch to operate the vacuum pump which is housed under the fume cupboard in the semi-clean area (31). Distilled and metal-free water (32) enter the clean-room above the poly-propylene sink unit (33). The still, water purifiers and pump are in the semi-clean area. However, there is an on-off switch, conductivity meter and tap in both rooms so that pure water can be drawn in both laboratories.

To further assist the room operators, a portable two-way-dictating intercom system connects the clean-room to the nearest occupied laboratory. This helps scientists who require technical assistance to get help without unrobing and leaving the room. This is especially useful when samples have to be passed through the sample airlock.

General requirements to eliminate metal-containing dust

All pipework is made of non-metal material or is covered with plastic sheathing. All brass hinges have been replaced by the nylon type and metal handles by a plastic variety. All large instruments, stools and cleaning equipment have had their metal parts replaced or covered. Corrosion proof, covered stainless steel (chromium Cr, nickel Ni) gas lines connect the gas bottles to the AAS. The gases will be filtered before use should our future program include Cr and Ni analyses. A special heating plate for use in the fume cupboard ensures none of the air passing the heating filament is mixed with the air circulating in the fume cupboard. A battery-operated digital clock is housed in a plastic casing. All controls for the airconditioner, pressure regulation and filters are mounted in a plastic case. The telephone is installed at the return-air end of the laboratory. In fact, the only metallic item that has not been coated, removed or replaced with a non-metal substitute is the airconditioning thermostat. Working areas down wind of the thermostat are protected by a clean-air cabinet.

Safety Factors

The emergency exit has been discussed. Hand operated face safety showers are installed near the sinks of both laboratories. The metal parts in the spray are covered with epoxy varnish. In case of fire and the need to leave the laboratory quickly through the airlock, all the doors in both rooms open outwards. The corridor door in the semi-clean-room cannot block the way as it opens outwards and can only be used as an exit. Fire extin-guishers are available.

Air tests for leaks, particulates and mercury

When the construction of the room was complete, the filters and flashings were tested for leaks, the air passing through the supply grill tested for uniformity of velocity and laminar flow, then the particles in the air throughout the room were counted and the room pressure adjusted. The room passed as "class 100" when at rest (nobody working in the room). To demonstrate what would happen if an ungloved person moved around the room too energetically, the clean-room certifier clicked his fingers down-stream of the particle detector. The meter went off scale indicating that this slug of air was now contaminated until recycled and filtered.

Mercury vapour levels were checked using Gardner's technique (1976) and found to be 0-6 ng m^{-3}, the same as background levels outside, while laboratories nearby had levels of up to 136 ng m^{-3}.

Maintenance schedule

A set of clean tools are kept in the clean areas for maintenance workers to use. The room is closed yearly to renew overloaded filters, maintain fans and motors, clean and replace light tubes, clean the false ceiling and, after a settling down period, the air is again tested for particles. For the rest of the year the fans run continuously. Only the airconditioning or heating, and fume ventilators can be switched on as required.

Emergency shut-down procedure

Should the air pressure fan fail the integrity of the room can be maintained for a period of 24 hours by evacuating all personnel, running the air-conditioner and sealing the outer airlock door.

CLEAN-ROOM ENTRY PROCEDURES AND GOOD HOUSEKEEPING

Actions such as foot stamping, head scratching and finger clicking raise many millions of particles of >0.5 µm into the surrounding air. Even walking, coughing and writing produce particles. To use and maintain the room's cleanliness, therefore, one must imagine that every action one takes produces a puff of smoke similar to that of a cigarette burning.

Housekeeping

The act of cleaning a clean-room increases the number of particles in the air and time is required before the air has been recycled and repurified. Therefore the room is always cleaned in the evening after work has finished.

To clean up, all unused glassware is replaced in cupboards and the bench tops are swabbed down with pure water on a covered cellulose sponge. The floors are swabbed with pure water, from a high grade, non-flaking, colourless, polypropylene bucket, which is pushed to the sink end of the laboratory with a teflon rake (Fig. 5). A vacuum line then sucks up the water and dust particles into a buchner flask. Cleaning materials are kept in the small cupboard under the sample airlock.

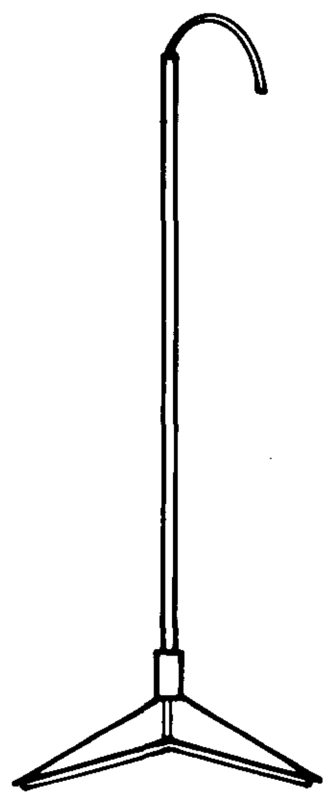


Fig. 5. The rake used to clean water from the floor.

Detergents are not used unless trace metal levels present in any residues that may be formed have been examined. Waxes, cleaning fluids, aerosol and spray type containers are not used as they all put particles into the air.

Vacuuming dry particles is done using a long lead from the vacuum supply line into a water trap. (If portable vacuums are used they must have an exhaust filtration system as efficient as the HEPA system cleaning the room).

All items are thoroughly vacuumed outside the laboratory if they are to be taken into the room. Some small items need to be cleaned in an ultrasonic bath to remove adhering particles. This is done in the laminar flow cabinet in the semi-clean-room. The cleaned item is then passed through the airlock.

Garments

Clothing fibers and skin are shed continuously from everyone. Clean-room overalls and caps made of non-shedding material and fastened with nylon buttons or tapes are needed to cover all parts of the body except hands and face to contain loose fibers and skin. For bearded persons or those suffering from dermatitis of the face (flaking skin has a large percentage of Zn and Cu) head attire is also available which has a mouth cover. Overshoes made of similar material are also worn. Hands are covered with disposable plastic or white nylon gloves. So as not to transfer unnecessary dust into gloves and shoecovers, shoes are cleaned and hands and face washed in the designated areas provided before entering the airlock. All garments are sent to a clean-room laundry each week. They are washed with filtered water, dried and packed into sealed plastic bags in a clean-air room.

Entry procedures

A detailed list of procedures appears in Appendix I (F. Kish personal communication). Briefly, outdoor clothing is left outside the semi-clean-room and shoes are brushed free of dust or mud. All metalwear such as watches, rings, keys and coins are placed in a polythene bag and put in a person's pocket or in a second polythene bag if they are to be put in a lab coat pocket. Next, hands and faces are washed in the scrubbing up area. After this the airlock is entered, the outer door closed and overshoes, clean-room lab coat and head covering put on. The clean-room is entered via the tacky mat set just inside the room.

Signs

To remind scientists, maintenance workers and visitors of the entry procedures and behaviour required to keep a "clean-room" clean, we have installed small signs summarizing the most important points taken from a long list of procedures that are hung under glass by the clean-room observation hatch, e.g. "put all metalwear in bags here"; "wash hands and face here"; "have you read clean room entry procedures?" (see Appendix I); "no smoking, scratching, jumping around, eating"; "only ball point pens and plastic paper"; "only open one airlock door at a time".

How and where to work

To get the maximum benefit from the clean air, the work requiring the cleanest conditions is done at the air intake end of the room, where subsamples are taken, ultra-pure reagents weighed out and samples analysed on the AAS. The benches are so arranged that clean air passes the work before reaching the operator. The only part of the operator upwind is his hand which is enclosed in a polythene glove. The scientist is reminded that he is the dirtiest thing in the room.

Apart from the chart paper on the AAS recorder, all paper used in the clean-room is plastic and only ball point pens are used (with the metal ends painted with epoxy varnish). Manuals are covered in plastic film.

In exceptional cases where ordinary paper must be brought into the room, a plastic bag holds it and it is only used at the return-air end of the room. Most paperwork is completed in the chief technician's office space in the semi-clean-room.

THE USE OF THE CLEAN-ROOM AND FIELD WORK

The sampling methods used in this laboratory have been described by Batley and Gardner (1978a and b). This section is an account of how the sampling gear is cleaned, kept clean, and used in the field so that an uncontaminated sample can be taken to a clean air environment in a clean container where it can be handled before storage or analysis. Patterson and Settle (1976) have described their techniques for ensuring samples for lead analysis are returned to the lab in an uncontaminated form; our procedures are not too different.

Procedures for obtaining pure reagents for the analytical methods which are used are not covered in this paper. Zief and Mitchell (1976) have covered the topic thoroughly.

Equipment

The equipment used for field sampling water includes, Nalgene hand operated vacuum pump, polypropylene tubing, "Millipore" filtration assemblies and "Sartorius" polycarbonate filtration units, polypropylene jerry cans with two tap openings, plastic bags and sheets, polystyrene cooling boxes and refrigerators, National Institute of Oceanography (NIO) all-plastic water samplers, Niskin "Go-flow" samplers, and a dust-free glove box or laminar flow cabinet.

Cleaning equipment

Cleaning all containers, caps and tubing is basically the same. New equipment is soaked in detergent, then rinsed in dilute nitric and sulphuric acids, rinsed, and soaked in 6M HCl until required, when it is rinsed in copious volumes of metal-free water. The containers are placed in three layers of similarly treated polythene bags. Caps are lined with the treated polythene. Filtration equipment, be it glass or polycarbonate, is treated similarly, and kept in cleaned polythene bags. The polythene bags are labelled so that they can be replaced in the same order if they are removed.

Procedure for Filtering

Filters are soaked in redistilled 2 M HNO_3, then rinsed with metal-free water, but are retreated just before filtering by drawing 1 M HCl through them into a polycarbonate unit for waste acid. The acid cleaned filtration head is then switched to another collection unit which is rinsed with several portions of sample. Finally the sample is collected, and several aliquots of the filtrate are used to rinse out the storage container.

Field work

Samples collected locally in estuaries or on cruises are brought back to the clean laboratory for filtration the same day.

On longer cruises, water is fed directly from plastic water sampler bottles into the acid cleaned plastic sealed filtration units, from which the filtrate is transferred to the storage bottles by allowing filtered air to enter the chamber while pouring. They are then kept at 4°C until they are transported back to the clean-room for analysis.

Later, it is planned that all equipment, including a single rack for the NIO bottle, from which water is about to be drawn, are to be kept and used in a laminar flow clean-air cabinet. The outer travel-stained covering will be removed before placing each item in the cabinet. The equipment will not be removed

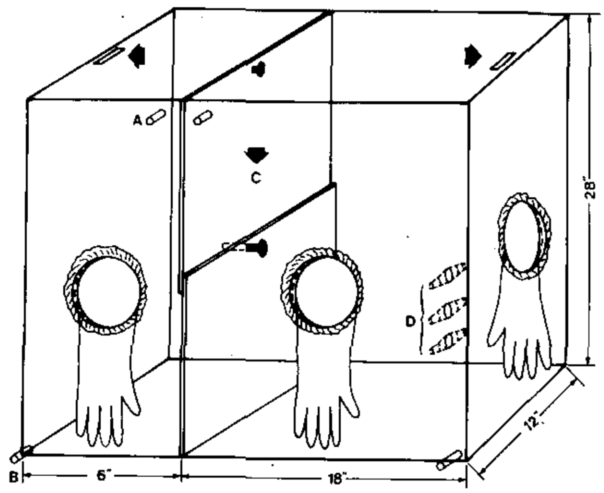


Fig. 6. The dust-free glove box for handling samples in the field.

from its other coverings until the cabinet has had time to generate its dust-free condition.

Alternatively, a dust-free glove box can be used with either pressure or vacuum filtration. Figure 6 shows the design of such a box. It has three elbow length disposable plastic gloves fastened as shown, is made from perspex and has two compartments with corner drains. The box is cleaned and filtration equipment placed into it while it is in the clean-room. Plastic clips can prevent movement of equipment, especially for cruise work. In the field, sample bottles can be put into the smaller compartment without letting air into the filtration side. Clean air is then sucked in through a 0.2 µm filter on "A" by exhausting through "B". The centre division "C" can then be lowered and the sample filtered. "Nalgene" hand pumps are connected to outlets "D" for filtering, and air is replaced through a 0.2 µm syringe filter connected to "E". Alternatively, a small nitrogen bottle and a 0.2 µm filter can be used for pressure filtration. Any outlet or inlet not in use is protected by a 0.2 µm filter. The filtration procedure is as described above.

Solid samples

Particulate matter, marine plants, fish and sediments are brought to the laboratory in contact with "Nasco" whirlpak bags which in turn are placed in two metal-free outer bags. Samples are deep frozen immediately or freeze dried then frozen until required. The outer bag is removed when the sample is placed in the semi-clean laminar flow hood before they are passed into the room through the sample airlock.

DISCUSSION

Since the room was completed there have been some minor problems:

1. The single fan exhaust system, connected by a T-piece to the AAS and fume cupboard, was insufficient to pull fuming nitric acid out of the fume cupboard when the front panel was removed for manipulations. To solve the problem we had two options. The first was to give the AAS and the fume cupboard their own exhaust fans. However, only one or the other could be used at one time in order to maintain a positive pressure, i.e. the volume of air being exhausted is less than that entering through the air filters, which prevents dirty air being sucked in from surrounding rooms and corridors. The second option was to install two elbow length polyethylene gloves with cover slips for when not in use which enables the manipulations to be done with a very narrow air inlet through which the air velocity is sufficiently high to prevent negative pressures and backflow through the opening and into the laboratory.

2. When using a carbon rod, the airflow was so great that the furnace and electrodes quickly burnt out. A silica baffle (Fig. 7) had to be made to ensure the air was still around the furnace. We found that air flows can be made visible quickly when such problems arise by allowing liquid nitrogen to vaporize from a beaker in the areas under investigation.

Analytical, technical and industrial hygiene requirements necessary in laboratories for trace metal analysis of environmental samples were used to design and build this clean-air-room. One must remember that in order to get samples to such a clean-air-room in an uncontaminated condition, a series of strict sampling and handling procedures have to be used. Similarly, strict entry and behavioural procedures are required by all personnel who use clean-rooms.

Further information on clean-rooms can be found in Austin (1967) and Whitfield (1962).

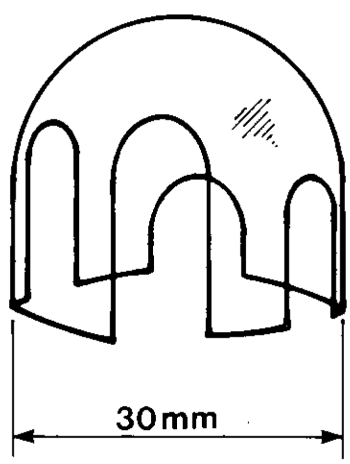


Fig. 7. The silica baffle used over the carbon rod on the AAS to prevent laminar flow air from excessively burning the electrodes.

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APPENDIX 1

CLEAN-ROOM PROCEDURES

Behaviour

The following general rules should be enforced to assist in the successful operation of the clean-room:

1. Keep hands, fingernails, and face clean.
2. Never comb your hair in the clean-room.
3. Do not wear fingernail polish.
4. Never wear or apply cosmetics in the clean-room (lipstick may be worn). Skin blemish, dandruff must be treated.
5. Avoid wearing jewelry (i.e. large rings, necklaces, ear-rings, lockets, watches, bracelets, etc.).
6. Valuable items such as wallets may be carried into the clean-room in street clothes pockets, provided that they are not removed inside the clean-room.
7. Personal items such as keys, coins, cigarettes, matches, pencils, handkerchiefs, watches, tissues, and combs should not be carried into the clean-room unless in polythene bags.
8. No eating food, chewing gum, or smoking in the clean-room.
9. Nervous relief type mannerisms such as scratching head, rubbing hands or parts of the body, or similar type actions are consciously avoided.
10. Avoid wearing soiled or dirty street clothes in the clean-room.
11. Always wear the specified protective clothing in the specified manner.
12. Wear plastic gloves, if required.
13. Keep equipment at the work station as clean and orderly as possible.
14. Never leave unused equipment and chemicals on the work bench.
15. Keep surplus equipment in appropriate containers or cupboards.
16. Make certain that equipment is clean before use.
17. Work on a clean surface; never on cloth or paper towels which can transfer contaminants to the work piece.
18. Don't walk around unnecessarily. Be cautious in approaching another's work area.
19. Report adverse changes in environmental conditions (dust generation or accumulation, marked changes in humidity or temperature) to your supervisor.
20. Pencils and erasers must not be used.
21. Paper must be plastic-covered or of special Limited-Linting type.
22. Tools brought in must be cleaned and protected by a plastic cover.
23. When in doubt, contact your supervisor.
24. Personal hygiene is of great importance. People must not enter clean-room with the following symptoms:
    - cold, coughing and sneezing.
    - severe case of sunburn.
    - skin disorder.
    - gloves must be worn by people with a high amount of acid in the moisture of the hand.

General

1. The Clean-Room No. 9 is a restricted area. Entry will be allowed only to personnel assigned to the area.
2. Specified protective clothing will be worn at all times within the clean-room in such a manner that only the face is uncovered. Such clothing will not be worn out of the clean-room.
3. Routine cleaning of the clean-room will be performed by assigned operating personnel on a scheduled basis.
4. Personnel assigned to the area will go through the complete personnel entry procedure each time they enter the area. Fire door shall not be used, unless in emergency.

5. Normal floor and wall cleaning will be performed by personnel specifically assigned to this task. These people will observe the personnel entry procedure for entering the area.
6. Visitors or maintenance personnel will enter the area only with the approval of the Scientist-in-Charge.
7. Visitors and maintenance personnel, if allowed access, will follow the personnel entry procedure when entering the area.
8. No smoking or eating will be allowed in the area.
9. No personal effects will be carried into the area.
10. All paper must be laminated in plastic or sealed in a reversible plastic bag prior to entry. Under no conditions will paper be exposed in open area within the clean-room.
11. Personal tools will not be brought into the area.
12. No materials will be left on open work benches at the end of the shift with the exception of cleaning fluids. These will be covered.
13. Protective clothing will be changed once weekly.
14. All tools will be cleaned with sonic energy, if possible, prior to entering the area.
15. All hand tools will be scheduled for return to the cleaning room for periodic cleaning.
16. All writing will be performed with ball point pens.
17. Work benches, cabinets, and other furniture will not be moved except under instructions from the Scientist-in-Charge. Should such items require moving, they should be lifted and carried clear of the floor. Scraping of walls and painted surfaces will be avoided.
18. All blow-off or purging operations should be avoided. Where such activity is unavoidable, dry nitrogen gas should be used in the cleaning area.
19. Internal communications will be accomplished with minimum possible movement in the area.
20. Communications from outside the area will be accomplished through the Pass Box, telephone or two-way intercom system.
21. No open or metal waste baskets will be allowed in the area.
22. Central air handling unit (and filtering) will operate 24 hours per day, seven days a week. Filter changes and similar maintenance will be performed when the room is empty of personnel.
23. Special work stations using exhausters will operate with a maximum exhaust air flow.
24. No grinding, chipping, sanding or drilling operations will be performed in the area.

Procedures

1. Only persons assigned to the area may enter the clean-room. An up-to-date list of assigned personnel will be maintained outside the entrance to the clean-room.
2. Before entering the area immediately outside the semi-clean-room, leave hat, coat, umbrella and personal effects in locker provided.
3. Remove shoes or put on soft shoes provided. Step onto nylon mat at semi-clean-room entrance.
4. Persons intending to enter the clean-room will first thoroughly wash their face, comb hair, then scrub hands thoroughly and clean finger nails. Shake hands and dry with warm forced air dryers if possible, or by using lint-free towel.
5. Proceed to the air lock.
6. Take uniform, overshoes (and gloves, if required) assigned for personal use (or for visitors), inside the air lock and wear them in the clean-room only.
7. Proceed into the clean-room, then tread on tacky mat.
8. One of the two doors to the air lock will be shut at all times, while the other door will be open temporarily for the purpose of

entry or exit only. Both doors must not be open simultaneously, unless in emergency.

9. The fire door in the clean-room will be used only for emergency exit and it cannot be opened from the outside. This door is sealed and an alarm will sound if it is opened, until the alarm bell is muted from the outside. If for any reason this door is opened, operation in the clean-room must stop, to allow the room to 're-generate' its designed cleanliness level.
10. All writing will be done near an exhaust air outlet. Paper will be removed from plastic bags only at these writing stations. Use ball point pens only. After completion of note taking put all writing materials back into the plastic bag and seal it as it was brought into the clean-room.
11. All tools and materials will be brought into and removed from the clean-room in sealed plastic bags.
12. No materials of any kind will be subjected to excessive handling, shuffling, bending or rolling in the clean-room.
13. No materials of any kind will be stored in the clean-room without a sealed plastic bag.
14. Bench tops will be damp cleaned daily with a cellulose sponge.
15. Exit procedure from the clean-room requires no additional requirement to those described above.
16. The air lock door that is furthest from the clean-room will be locked at all times, either on the air lock side when someone is in the clean-room, or from the outside when the clean-room is not in use. The Scientist-in-Charge will have the key and a duplicate is deposited with the Officer-in-Charge of the Division.
17. If for any reason contamination of the room may have occurred (e.g. some of the above rules were not observed), operation will stop to allow the room to settle, or, if so judged by the Scientist-in-Charge, decontamination of the room may be required.

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