SEMT

Society of Electron Microscope Technology

Affiliated to the Royal Microscopical Society

2.2.89

Dear Member,

You are cordially invited to a half-day meeting of SEMT on Friday, Feb. 24th. at the Imperial Cancer Research Fund, Lincpln's Inn Fields, on the theme -

"THE E.M. LABORATORY : DESIGN AND SAFETY"

Programme

2.00	Chairman's Introduction.
2.10	Electron Microscope Laboratory Design. Mr. Chris. Walker (Philips Analytical, Cambridge)
2.40	Instrumental Hazards in the E.M. Laboratory. Mr. Steve Chapman (Protrain, Oxford)
3.10	Tea.
3.30	Biological and Chemical Safety Problems in the E.M.Laboratory. Mr. Tony Hardiman (St. Bartholomews' Hospital)
4.00	Open Forum - Free Discussion on design and safety matters.
4.30	Chairman's Summing-up.

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Please complete the slip below and send it as soon as possible to

Dr. Jill Lewis, EMU, St. Bartholomews' Medical College, Charterhouse Square, London EC1M 6BQ (01 253 0661 x 120)

SEMT 24.2.89

The E.M. Laboratory : Design and Safety

E.M. Laboratory Design - Mr. Chris Walker (Philpps Analytical)

Two essentials to set up at an early stage in planning are good lines of communication with the architect, and a clear idea of the required functions of the unit. The latter point could determine the balance between Teaching/Research, Trouble-shooting on quality control, Demonstrations, Research & Development, Routine & Repetitive examinations e.g. for diagnosis. Then decide the various rooms: E.M., specimen preparation, section cutting, vacuum preparation, darkroom, storage and office. You need plenty of office space for spreading out photos, layouts for demonstrations etc.

It is highly advisable to have an independent adviser at the architect stage, before building has begun (e.g. Chapman of Protrain - this is not just the Old Boy network, he really does know his stuff). Plans of the floors above and below should be considered too; Any points raised at meetings with the consultant and architect should be put in writing afterwards ! - and check continually that subcontractors are working to contract and not cutting corners.

It is important to avoid vibration - from roads, lifts, service equipment (the latter on the top of a building may cause vibrations many floors away), and other large equipment nearby. Also electrical fields e.g. from large cables (subcontractors may run these across a floor instead of around the room) water pipes can take an electrical field too. It may perhaps be best to add an equal and opposite field rather than try to eliminate an existing one: but check all equipment on the field wire first. The Pre-installation manual for the E.M. will give detailed specifications. It will help to reduce problems from vibration if the E.M. can be on its own separate foundations.

For most E.M.s, cooling water will be necessary; some local water authorities now require a closed-circuit cooling system. Check the mains delivery pressure and temperature - there may be problems if the temperature is below 18'C. Heating and ventilation should also be considered. If a new E.M. is being put into an existing EM laboratory, you should still do a site check; the laboratory and its surroundings may have changed since the original installation, especially with regard to electrical and vibration problems.

Other points with regard to the EM room: access - doors must be wide enough and lifts strong enough. Many of the newer instruments now have a separate power pack again. N.B. height of room - with some of the taller new EMs in rooms with a low suspended ceiling, it may be necessary to make a "box" in the ceiling so that the gun can be lifted in order to change the filament. Storage space is needed for notebooks, specimens, accessories etc· movable wall-hung units are best.

The specimen preparation area should be as large as possible· it is advisable not to put the ultramicrotome against a south-facing outside wall. If freeze-slamming or other techniques involving liquid nitrogen are used, it is essential to have good access and good ventilation. Trimming blocks produces hazardous dust. There should be an area to contain chemical spillage. It is possible to have a mobile fume cupboard. Separate darkrooms should be provided for negative development and for printing; and an area for dedicated maicroanalysis, if used.

Design of the Electron Microscope Laboratory

Today, Electron microscopy is a mature and established technique utilizing an extensive range of commercial instrumentation, in an advanced state of development.
In a time of rationalisation of resources, research funds and public money, many microscopists are being called upon to re-evaluate their existing facilities. This may be in terms of column replacement or the centralisation of Electron microscopy instrumentation to establish a generic service to a college or institution.
The task of designing and planning a new environment for either a replacement microscope or a completely new laboratory requires a great deal of careful thought and discussion to evolve the correct department which will satisfy the scientific needs for the years ahead.
The purpose of this talk is to utilise the application of good laboratory practice, common sense and to highlight some of the less obvious aspects concerning the installation requirements of modern Electron microscopy instrumentation.

Topics: Basic requirements for electron microscopy.
- Microscope rooms-correct siting, services.
- Specimen preparation areas.
- Darkrooms-photomicroscopy, image analysis.

Refererences: Horne, R.W, HillsG.W, and A.Paine(1972) The design and construction of a laboratory for ultrastructural studies, JEOL News 9.4.

Agar, A.W, R.H.Alderson and D.Chescoe(1974) Principles and practice of electron microscope operation, in Practical methods in electron microscopy, Vol.2 A.M.Glauert, ed

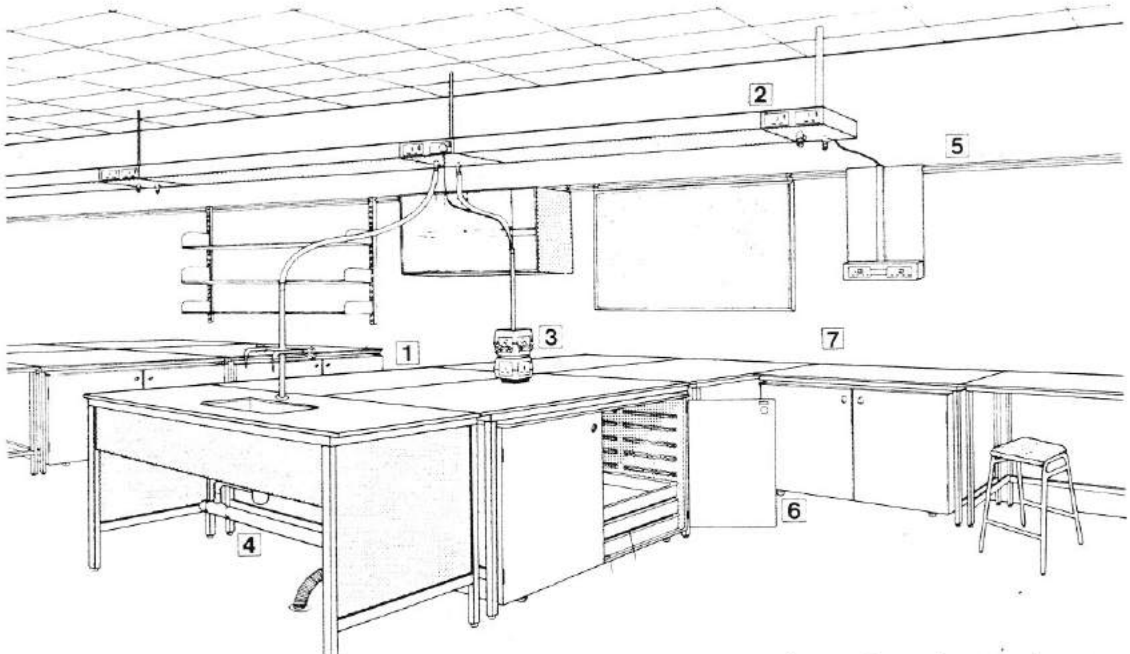
J.C.H.Spence Experimental High resolution Electron Microscopy, Monographs on the Physics and Chemistry of Materials. Oxford Science Publications.

R.H.Alderson(1975) Design of the electron microscope laboratory, Practical methods in electron microscopy, A.M.Glauert, ed.

Laboratory Design—Traditional or Flexible?

by A H RABY FIMLS

The problems of planning medical laboratories are many and varied. Many errors are made in this process and examples of new laboratories that could not be occupied without major alterations are often cited. Many of the errors committed in the National Health Service are the result of inappropriate consultation—not at regional level, but mainly at district level, where management roles are ill-defined. This frequently results in decisions being made by individuals who may not understand the factors or the technical innovations that should influence space allocation and by those who, in many cases, will not work in the environment they create by their planning activities.



See text for explanation of numbers

Another of the major problems in the NHS is the length of the planning process: even minor projects can take many months or, more often, years to complete. This is a far cry from a situation the author witnessed recently in Australia where the laboratory manager (Chief Technologist) discussed with a consultant obstetrician the latter's needs with respect to space he proposed to rent for use as consulting rooms from a private laboratory complex. Rough drawings were made on the spot by a local building contractor; approximate costing was given, and a completion date of three weeks from that date was agreed—a process which, sadly, in our health service could have taken many months and would have been arrived at only after many phases and committee discussions.

In the rapidly changing world of medical laboratory sciences these significant delays, which can occur in the best laboratory planning, frequently result in laboratories that are out of date before they are occupied.

Mr Raby is Principal Medical Laboratory Scientific Officer in the Pathology Department of Wexham Park Hospital, Slough.

Flexible Planning

It is only in the past few years that the opportunity to build laboratories with a high degree of flexibility has been available: laboratories where the conformation of benches can be changed with absolute ease and without the expensive and often time-consuming dealings with plumbers, carpenters, builders and electricians (and the planning process!).

The system described here uses laboratory furnishings manufactured by Sintacel, which based its designs on work done a few years ago by the Laboratory Investigation Unit of the Department of Education and Science.

To achieve almost total flexibility, benches are, in essence, free standing tables [1]. They possess, however, a high degree of stability, which is further improved by clamping benches together when they are in their required position. These clamps are then removed when the benches need to be re-sited.

Gas and electricity for the benches are provided by overhead booms [2]. These booms carry the various services across the laboratory and regular node service points allow individual benches to receive the gas and electricity they require from service bollards [3], which bolt to the bench at any one of a number of possible locations. The bollards carry the gas taps and electrical points needed to allow easy access to the main services at bench level.

Changing the conformation of the laboratory is a simple matter of disconnecting the overhead services, moving the bench to its new location and re-connecting the services to the nearest overhead boom. The connection between boom and bollard is by means of the appropriate cable or pressure tube. Although some visitors to the unit have remarked on this feature, we have had no problem whatsoever with this arrangement either hindering or interfering with work.

Water Installations

Water and de-ionised water are provided from appropriate pressure tubing and water taps at all sinks. Wexham Park Hospital laboratory uses a two-bed de-ioniser followed by a polishing process capable of producing very high quality water which, by means of a ring main system and the overhead boom, is delivered to individual rooms and benches. In the system used, water can be "tailored" to the individual needs of a particular bench or a particular process.

This would probably be best achieved by using the two-bed de-ioniser and then reverse osmosis prior to circulation by the ring main, with polishing to the specifically-needed quality using locally installed mixed bed cartridges. By these means washing-up areas, for instance, where "less pure" water is needed for washing machines, would use a lower quality product.

Water produced by distillation is significantly more expensive than that produced by de-ionisation and reverse osmosis; in an age of high-cost energy and energy conservation, "still rooms" could well become a thing of the past.

To achieve greater flexibility, special consideration must be given to drainage. The system used in the author's laboratory in Wexham Park Hospital was a glass drainage system [4].

Although a specific area may be envisaged for a particular use when planning laboratories, there is frequently a need to make major changes in the function of any nominated area. Glass has the advantage of allowing the disposal of a wider range of materials through the drain; this, together with the fact that at the original planning stage it was anticipated that the drains at Wexham Park Hospital would operate on a "zero fall", suggested glass drainage would be the most appropriate to meet the hospital's needs. The fairly sophisticated drainage system offers many drainage points in each working area that are easily connected to the under-bench glass drainage system, thus allowing the movement of benches with sinks.

Flexibility of wall-mounted furnishings—bookcases, shelves, notice boards, and so on—is achieved by using a top track system for hanging these items [5]. Once again items are simply locked into position when in their appointed site and, as with the benches, the clamps can be undone easily when necessary.

Clearly, therefore, wall-mounted furniture not required in one laboratory can be transferred easily to another; this is the case also with under-bench units [6]. Built in three sizes, these units are mounted on castors for easy mobility and, because they have "safe" laminate tops, can be used as extra work tops [7]. Baskets and trays from these under-bench units are all interchangeable, fitting also into units in hot and cold rooms and into trolleys for transporting stored items around the working area.

Safety Cabinets

Limitations on flexibility are inevitable in areas around safety cabinets. Regulations affecting safety cabinets and areas in which they are mandatory in the United Kingdom are contained in the Howie *Code of practice for the prevention of infection in clinical laboratories and post-mortem rooms* (HMSO, 1978). The factor restricting flexibility here is the exhaust ducting for these cabinets, the path of which has to be predicted at the design stage. (A similar point is made about fume cupboards.)

The correct siting of safety cabinets is important and, although this is dealt with in the Howie Code, these recommendations may not be easy to implement if laboratories are small or perhaps narrow. Manufacturers should be consulted at an early stage about the siting of these vitally important items so that maximum flexibility is preserved in areas where safety cabinets and fume cupboards are to be installed.

Movable Walls

So far this article has dealt with the separation of services from work benches in order to create flexibility and it has been suggested that these services be kept independent also of the walls by their provision overhead. This allows further opportunity for flexibility by designing working areas separated by movable dividing walls and, although Wexham Park has not, as yet, made full use of this facility, it has the potential to create a range of laboratory working spaces within several larger areas.

Laboratory design and planning is, without doubt, one of the most expensive processes in which medical laboratory scientists participate. Certainly it is they who, unfortunately, have to come to terms with any errors made in the planning of their working environment. The system described here presents an opportunity to design laboratories with the flexibility to meet the demands of the future; a system which, at introduction, costs no more to install and yet costs nothing to alter as needs demand. It is a system that can be expanded easily with only capital cost for the furniture; there is no extra cost for installation and fitting.

It is pleasing to see several other regions adopting the policy of flexible design and thus, hopefully, they will avoid many pitfalls inherent in planning working areas on traditional lines and provide laboratories that can cope with the changes inevitable in the future.

E.M. Instrumental Hazards - Mr. Steve Chapman (Protrain)

1. Electron gun - shock, heat, radiation.

It is essential to switch off the kV before opening the gun chamber; don't just trust the safety devices, and make sure that the safety bar falls so that it earths the residual charge on the gun. It there is a smell of ozone when you open the gun chamber, the kV HT is still on !!

Chamois leather gloves will protect the hands from a hot gun. Have a step-ladder for access to the gun, don't just stand on a chair.

You should have a radiation check after each service, and call the engineer back if there are leaks: these can happen simply if something hasn't settled exactly as it was before, and often it is enough just to lift the bit of column and re-settle it. Also if ports have been left in the shielding for accessories which have not subsequently been fitted. The cladding around the manifold may also have some shielding effect, and the E.M. should not be run at the highest kV without the cladding in place. Film badges are not much use, as any leakage may be very narrow beam.

(Dave Gunner, Barts, uses the Mini Monitor type 5.10: cost c. £200)

2. Column - radiation.

As for the gun; there is a little less hazard in the SEM because the kV used is lower. But remember that the secondary electron detector in the SEM carries 10,000 V !! Again, this should switch off when the chamber is opened; but again, if there is a smell of ozone, there is HV still around - call the engineer.

3. X-ray microanalysis - so placed as to give engineers and operators a nasty bump on the head; should be padded with foam. Also uses liquid nitrogen; Edwards do a good booklet on this. There is not much danger to hands, but eye protection should be worn; and there is considerable danger to the lead glass window of the microscope when filling the XRD. Do not pour from a container which has been sitting around for a while, because of the presence of ice which will upset the readings. Also top up at the end of the day so that it can stabilise overnight; if you top up first thing in the morning, there will be bubbling and instability for several hours.

4. Rotary pump. The hot oil is a carcinogen; the exhaust should be pumped to the outside, or filtered - Japanese instruments have only a crude filter fitted, but the Philips one is O.K. Other vacuum instruments e.g. sputter coaters, should also have filters. The oil should be changed regularly.

5. Electrical system. Only do work on this is you are absolutely sure what you are doing ! Switch off before starting - N.B. if the room lights are wired to the E.M., it may still be live after the E.M. mains is off. Use bare hands only, remove jewellery and metal watch.
Stay within shouting distance of the service engineer.

When checking radiation the monitor must have the right "window", which is different from that used for measuring isotopes. Check the E.M. under its normal working conditions· and check under the sitting position as well as up and down the column.

Protrain

Safety in the Electron Microscope Room

by S.K.Chapman Protrain,
16 Hedgerley, Chinnor, Oxon OX9 4TN.

Whilst the actual electron microscope room is probably one of the safest areas in an electron microscope laboratory, it contains a number of hazards that demand attention. This talk is intended to identify some of the dangers that operators may face. Some of these are present on all occasions. Whilst others only present themselves when some form of maintenance or other routine procedure is being performed. Perhaps at this stage we should set a standard for any action toward, or performed around, the microscope. "Never attempt any routine maintenance task without thinking of the possible hazards involved with that task!"

Most manufacturers include a "shorting bar" which falls across the cathode assembly as the gun is opened. This bar under normal circumstances should never pass a current. Only if things go wrong could it save your life. Now for that hot cathode. Wear gloves that are thick enough to protect you from the hot metal. The electron gun is also a source of x-rays. If the gun or condenser area is disassembled for any reason, you must ensure that it is checked for radiation leaks prior to using the instrument.

The specimen chamber of the scanning electron microscope is also an area where you may inadvertently come into contact with a high voltage. When admitting air to the chamber the detector high voltage should be automatically switched off, if not - sparks!

Many instruments use liquid nitrogen as a coolant for an anticontamination device or an energy dispersive x-ray detector. When handling any very cold liquid great care must be taken. The EDX detector also becomes a hazard in itself. The position of the detector, particularly on a transmission electron microscope, makes it a prime cause of cracked heads.

The rotary pump seems to be an innocuous part of the electron microscope laboratory, but it contains hidden dangers! The fumes from any hot oil are said to be carcinogenic, contaminated vapours even more so. A most recent problem for me is the sensitising of this electron microscope operator by cleaning media. Even that most user friendly media, Duraglit, will start to affect you if you use it for year after year.

Safety in the electron microscope room as I have already said is probably considered to be the lesser of your laboratories evils. Do not fall into the trap of becoming careless because that is when the electron microscope is at its most dangerous!

Biological and chemical safety problems in the EM laboratory.

Mr. Tony Hardiman - Virology Dept., St. Bartholomews' Hospital.

Chemical - Relevant chemicals used in EM suites in conjunction with other pathology departments - Fixatives- including heavy metals (predominantly aldehydes and metal salts), resins (epoxy), stains (metal salts) and relevant disinfectants (their uses and disadvantages). Hazards - fire, skin contamination, eye and nose damage, poison, radiation, carcinogens, mutagenic com pounds- the monitoring tests available. β-propiolactone, its uses, its problems- does one outweigh the other - why it is so good.

Biological - predominantly microbiological - Guidelines from 1978 Howie report through to 1989, Draft HSAC Report - the progression, slow steps forward. Categorization of pathogens, groups 1 to 4, which type of facilities are required for safe working conditions when dealing with common organisms. Clarification for each group and its members, the implication for EM operators and their relevant safety. General safety rules (common sense - do we all use it ?) applying to all laboratories. Practical safety guidelines - how to treat all general specimens whether tissue/blood or any other body fluids safely. - to avoid contamination from all pathogenic sources. Distinction of high and low risk - a poor classification system - what does it mean - is HIV positivity a higher risk state than Rubella positivity ? What are we not detecting at present ? The unknown is often the biggest danger to safe working conditions. Inactivation procedures for HIV, comparison with sterilization of prions (Creutzfelt-Jakob) - a major factor in EM sectioning of neurological tissue. Do we take enough care ? The case of Creutzfelt-Jakob disease in histopathology technicians - transmission 11-16 years previous - can it be avoided ?

Biological and Chemical Safety ~~Hazards~~ Problems in the E.M. Laboratory

- Mr. Tony Hardiman (St.Bartholomew's Hospital) Dept. Virology

Fixatives: formaldehyde - dermatitis

glutaraldehyde - skin sensitising

acetaldehyde - very low boiling point

possible associations with skin cancer

Heavy metals: osmium - fixes surface tissue, e.g. cornea, olfactory mucosa

mercuric chloride - cumulative poison; ? skin cancer

uranium - low level radiation; affinity with DNA.

Resins: epoxy - mutagenic, carcinogenic

Stains: lead - very poisonous

uranyl - radiation (as above). Preferably use in fume cupboard, with lead shielding

Decontaminants: glutaraldehyde - skin irritation

hypochlorite - skin irritation; corrosive to fume cupboard!

ethanol - fire hazard

phenol - damages skin/mucous membranes

B-propiolactone - very effective but a Very Nasty Chemical, can be fatal. Inactivates vaccines; can inactivate Lassa fever virus in 20 minutes. Make safe by adding water.

Safety guidelines

1978 Howie code
1980 Hepatitis B
1984 Categories of pathogens DHSS
1986 HTLV III guidelines
1986 WHO safety measures for use in outbreaks
1988 HSE Control Of Substances Hazardous to Health
1989 "son of Howie"

Categories of pathogens

1. Unlikely to cause human disease
2. Unlikely to spread usually treatable e.g flu, Legionella
3. Serious laboratory hazard, possible spread· e.g. TB, HIV, Hep B.
4. Severe disease, ~~xxxxx~~ serious risk, high spread, no treatment· e.g. Creuzefelt-Jacob

Safety measures

Protective clothing - and plastic aprons

No smoking/eating in laboratory

Kepp office and laboratory work separate

Radiation protection

Disposable gloves - check that will not be damaged by chemicals used

Eye protection

Fume cupboard

Frech hypochlorite

Plastic rather than glassware where possible

Inactivate sera at 56'C for 30 minutes, if possible for the test

Centrifuge in sealed buckets

Handle Category 3 materials only in Category 3 containment facility

Dispose infected material in hypochlorite or by autoclaving

It is not practicable to handle grids in a microbiological safety cabinet unles switched off !! - because strength of suction would pull grids up into the filter !

HIV - drying for 10 minutes will inactivate it: or use use glutaraldehyde, hypochlorite, sun etc.

Prions - autoclave for 4½ hours !!. Many fixatives don't work on them: glutaraldehyde, formaldehyde and hypochorite don't affect them. Many neurological sections could contain prions which could be infectious; many other specimens could contain them.

For most viruses, drying and negative staining will generally inactivate them.

Fume cupboard cannot be sealed and disinfected; only by fumigating the whole room.

In the SEM, a lower kV will be more affected by the environment.

If the EM is on the ground, vibrations will be damped by the ground; if on a separate pad, can become sensitive to sonic waves.

The Department should have its own safety policy; a "Near Miss" book is also a good idea.

Spurr resin causes much contamination of the column, & is drawn out by the rotary pump.

Vinyl gloves are soluble in resin & in acetone: latex gloves have holes!

Osmium can be reduce with stannous chloride. Johnson Matthey will take waste osmium free of charge if you buy from them.