Fort Detrick study evaluates relative infectious hazards of microbiological laboratory techniques.

Microbiological Safety

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LABORATORY workers often have acquired infections during microbiological investigations. A 1951 survey of 1,342 laboratory-acquired infections in the United States revealed 39 deaths, a case fatality rate of about 3 percent (*1*). In all, recognizable accidents in the laboratory accounted for only 215, or 16 percent, of the infections. The source of most of the illnesses was unknown.

Although literature on industrial and chemical hazards is abundant, little has been published on microbiological hazards until recently (*2–9*). The Fort Detrick Laboratory Hazards Section, necessarily concerned with the prevention of laboratory infections, has attempted to determine their causes. From observations and from experimentation on infectious laboratory hazards, safety ideas have been developed which may be helpful in decreasing the number of laboratory-acquired illnesses.

Aerosol Determination

Studies of the potential sources of infection have centered on the hazards associated with common laboratory techniques. The experimental method used has been described previously (*5, 7*). Essentially, this method involves sampling air with the sieve-type air sampler during standard bacteriological operations such as pipetting, centrifuging, inoculating and lyophilizing cultures, and autopsy of animals. The operational area is surrounded by samplers, each of which draws air at the rate of 1 cu. ft. per minute through 340 small openings, thereby impinging organisms on the surface of a petri dish agar plate 1–2 mm. below the openings. After a suitable incubation period (36–48 hours at 30° C. for bacteria and 4–16 hours at 30° C. for bacteriophage) colonies or plaques are counted in a Quebec colony counter.

Contamination of the environment is determined also by swabbing surfaces with cotton moistened with nutrient broth. The swabs are streaked on agar plates, which are then incubated as are the air-sampler plates.

Three easily identified organisms were used in these studies: (*a*) *Serratia indica*, a red pigmented vegetative rod; (*b*) *Bacillus subtilis* var. *niger*, designated *B. globigii* in Fort Detrick laboratories; and (*c*) coliphage T_3. *S. indica* and *B. subtilis* spores were sampled on cornsteep, molasses agar, and coliphage T_3 on tryptose phosphate glucose agar (*7*).

Area Contamination Found

Wide variations from average determinations of contamination hazards associated with laboratory procedures are possible (see table). These variations often seem to depend on minor changes in technique peculiar to the individual testing a particular procedure. A reported count of two, for example, means that two colonies grew on the agar sampling plates. It has been reported that most bacteria in the air occur in clumps (*10*). Also, the efficiency of the sieve

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Aerosols produced by common bacteriological techniques

Technique	Number of colonies appearing on sampler plates			
	Number of operations	Average	Minimum	Maximum
Agglutination, slide drop technique (one slide)	60	0. 3	0	0. 66
Animal injections (*Serratia indica*):				
1. 10 shaved guinea pigs injected intraperitoneally with 0.5 ml. culture, no disinfectant	3	15	15	16
2. Same as (1) but injection site disinfected before and after injection with 1 percent tincture of iodine	3	0	0	0
Autopsy, guinea pig:				
1. Immediately after 1 ml. *S. indica* culture injected intraperitoneally	2	4. 5	3	6
2. Immediately after 10 ml. culture injected intracardially	6	3	1	6
3. Grinding tissue 2 minutes in mortar and pestle with 2 ml. sterile broth:				
Guinea pig liver as in (1), 10 ml. inoculum	10	1. 8	0	8
Guinea pig heart as in (2)	6	19. 5	0	103
Centrifuging:				
1. Pipetting 10 ml. *S. indica* culture into 50 ml. tube	100	. 6	0. 1	1. 2
2. Pipetting 30 ml. culture into 50 ml. tube	100	1. 2	0	5. 5
3. Removal of one cotton plug after centrifuging	100	2. 3	. 8	5. 0
4. Removal of one rubber cap after centrifuging	80	. 025	0	. 25
5. Decanting supernatant into flask	10	17. 6	0	115
6. Siphoning supernatant from 10 tubes, each containing 30 ml. centrifuged culture	100	3	0	24
7. Adding 30 ml. saline to one tube of packed centrifuged cells and resuspending by mixing by alternate sucking and blowing with a pipette	100	4. 5	. 7	12. 8
8. One 50 ml. tube breaking in centrifuge but all 30 ml. culture staying in trunnion cup	10	4	0	20
9. As in (8) but culture splashing on side of centrifuge	10	1, 183	80	1, 800
10. Swabbing outside of centrifuge tubes after filling, centrifuging, taking off supernatant, and resuspending	10	(1)		
One drop of *S. indica* culture falling 3 inches onto:				
1. Steel surface	200	1. 3	. 02	4. 7
2. Painted wood	200	. 3	. 01	. 6
3. Kem-rock	100	. 04	. 00	. 05
4. Dry hand towel	100	. 16	. 00	. 35
5. Dry paper towel	200	. 11	. 00	. 45
6. Dry wrapping towel	100	. 02	. 00	. 05
7. Towel wet with 5 percent phenol	100	. 02	. 00	. 05
8. Pan of 5 percent phenol	100	. 00	. 00	. 00
Inoculating loop:				
1. Streaking one agar plate with one loopful of *S. indica* broth culture	10	. 6 4. 6 [2]	0	20
2. Streaking one agar plate with one loopful of agar culture	15	. 26	0	. 7
3. Loopful of broth culture striking edge of tube	15	. 60	0	2. 3
4. Inserting one hot loop into 100 ml. culture in a 250 ml. Erlenmeyer flask	550	8. 7	. 68	25
5. Inserting one cold inoculating loop into 100 ml. culture in a 250 ml. Erlenmeyer flask	250	. 08	0	. 22

sampler in recovering aerosolized particles of heterogeneous size and composition under varying humidities is not easily nor precisely determinable. In the presence of bacterial aerosols of known concentrations, efficiencies have varied from 43 to 73 percent. Therefore, the reported number of colonies is significantly smaller than the actual number of bacteria.

It is evident that certain procedures create larger amounts of aerosols than others. Grinding tissue with mortar and pestle, decanting the supernatant after centrifugation, resuspending packed cells, inserting a hot loop in a culture, withdrawing a culture sample from a vaccine bottle, opening a lyophile tube, streaking an inoculum on a rough agar surface, and shaking and blending cultures in high-speed mixers appear to be potentially dangerous to the tech-

Aerosols produced by common bacteriological techniques—Continued

Technique	Number of colonies appearing on sampler plates			
	Number of operations	Average	Minimum	Maximum
Hypodermic syringe and needle [withdrawing 1 ml. phage suspension from rubber-capped vaccine bottle and making tenfold dilutions in rubber-capped vaccine bottles (10^{-1} to 10^{-9}), pledget does not always protect fingers against contamination]:				
1. Cotton pledget around needle	90	2.3	0	10
2. Ethanol soaked cotton pledget	90	0	0	0
Lyophilization:				
1. Breaking one ampule containing 2 ml. of lyophilized *S. indica* culture in milk plus broth menstruum by dropping on the floor, first 10 minutes	10	2,029	1,939	2,040
2. Same as (1), 50–60 minutes after breakage	10	741	162	1,447
3. Opening one lyophile tube by filing and breaking tip	20	86	4	256
4. Same as (3), but wrapped in 70 percent ethanol soaked cotton pledget	50	.08	0	.8
5. Transferring one dry inoculum from one lyophile tube by wire loop	50	1.0	0	5
6. Same as (5), but shaking powder into broth tubes	20	5.4	0	30
7. Same as (5), but wet inoculum transferred by syringe and needle after reconstituting with one ml. broth	10	4.4	0	17
Petri dish plates:				
1. Preparation of pour plate, pipetting one ml. inoculum of *S. indica* into plate without blowing, and adding melted agar and mixing	15	2.6	.2	5
2. Streaking one smooth agar plate with 0.1 ml.; spread with glass rod	50	.06	0	.4
3. Streaking one rough agar plate with one loopful of broth culture	10	25.1	7	73
4. Same as (3), but using 0.1 ml. and glass rod	50	8.7	2	25
Pipettes (also see centrifuging.):				
1. Inoculating 50 ml. broth in 125 ml. Erlenmeyer flask with 1 ml. culture (*S. indica*)	5	1.2	0	2
2. Mixing 7 ml. broth culture by alternate suction and blowing, without forming bubbles	5	.2	0	1
Plug, stopper, or cap removed from culture container of 1–10 dilution of 24-hour broth culture of *S. indica:*				
1. Escher rubber stopper removed from 5-oz. square dilution bottle immediately after shaking up and down	15	5.0	0	20
2. Same as (1), stopper removed after 30 seconds wait	15	2.5	0	12
3. Plastic screw cap removed from 8-oz. prescription bottle immediately after shaking	15	4.0	0	13
4. Cotton plug removed from 250 ml. Erlenmeyer flask immediately after rotary shaking (dry plug)	15	5.0	0	16
5. Same as (4), but wet plug	5	10.2	0	35
High speed blendor, *S. indica* culture mixed 2 minutes:				
1. Screw-capped, no rubber gasket (1 minute)	10	8.7	0	31
2. Screw-capped, rubber gasket, worn bearing	10	61.0	12	126
3. Loose fitting plastic cover	15	518	77	>1,246
4. Removing tight cover immediately after mixing	15	[1]	[1]	[1]
5. Removing tight cover 1 hour after mixing	15	8.2	5	33

[1] Colonies too numerous to count. [2] Two technicians.

nician if the micro-organisms are infectious. Accidents during centrifugation or handling of dried cultures caused extensive contamination of the laboratory. Practically every manipulation in the microbiological laboratory creates aerosols, and these aerosols are probably the source of many laboratory infections.

Corrective Measures

Bacteriological Cabinets

Protection from infectious particles disseminated into the environment surrounding their source may be afforded by a bacteriological safety cabinet, a modification of the hood or

Figure 1. This cabinet may be used without the glove-port panel when small amounts of aerosol are expected.

dry box used for chemical and radiological studies. All potentially infectious operations are thus carried out behind a plane of glass with ventilation sweeping contaminated air away from the technician. When the cabinet is to be used will depend upon the organism, the technique, the skill and immunity of the technician, the seriousness of the possible illness and its posssible sequelae, and the relative isolation of the laboratory. A cabinet system is also advisable when there is potential repeated inhalation of large volumes of nonpathogenic micro-organisms. These occasionally cause hypersensitivity (*11*). Hypersensitivity is also known to develop during the repeated preparation of tuberculin and brucellergen. Nonpathogens have been reported to cause infection in man (*12*).

The bacteriological safety cabinet (*13*, *14*) may be simple (fig. 1) or elaborate (fig. 2), depending on the need. When there is a large volume of work, special cabinets are justified for particularly hazardous equipment such as the centrifuge and shaking machines (figs. 3 and 4). Ultraviolet light provides partial disinfection of the cabinet interior; sterilization is best accomplished with steam formaldehyde.

Contaminated cabinet air may be filtered through a bacterial filter or piped directly to some sort of air incinerator. An electric grid

Figure 2. Modular gas-tight cabinet system—animal holding, all-purpose, and autopsy cabinets.

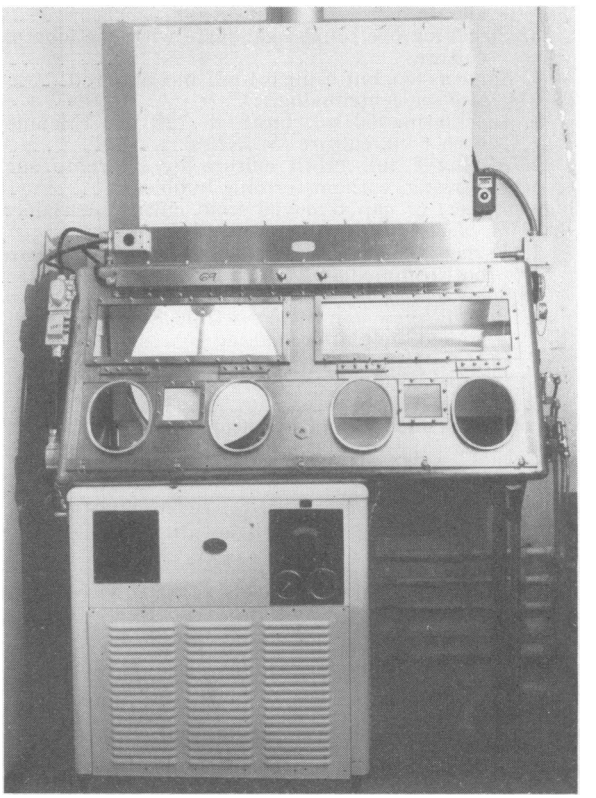

Figure 3. This centrifuge cabinet may alternatively be used as a bacteriological work cabinet.

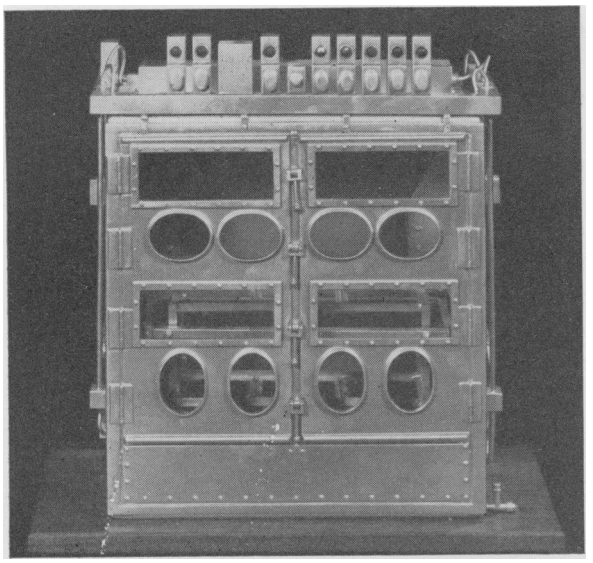

Figure 4. Model of shaking machine-incubator cabinet which may be used to house various types of apparatus.

incinerator (*A*) has been in use at the National Institutes of Health, Public Health Service, for some time. The efficiency of this incinerator has been reported (*15*). Individually designed gas or oil fired incinerators are useful for larger volumes of air. Sterilization before renewal or examination of the filter may be accomplished by incorporated electric strip heaters.

The Centrifuge

For most laboratories, adequate centrifuge safety is possible by use of commercially available screw-capped, safety cups for the swinging head. The angle head in a refrigerated or nonrefrigerated centrifuge has required special attention; a new head has been designed with enlarged recesses to hold the safety cups and will soon be available (*B*). These cups should be filled and opened in a safety cabinet.

Animals and Cages

Experimentally infected animals can be a source of infectious aerosols (*16*). Such animals are more safely housed in cages with solid bottoms and sides than in wire cages. A simple method for minimizing dissemination of infectious material is equipping cage racks with ultraviolet fixtures (fig. 5). It has been found that when these are adjusted so as to bathe the area immediately above the cage tops with continuous ultraviolet radiation at an intensity of about 250 microwatts per sq. cm. no viable vegetative organisms escape from the cages. A more expensive method uses ventilated cages in which all input and exhaust air is filtered (fig. 6).

Depending upon the animal, route of inoculation, cage, bedding, and the micro-organism, the accidental aerosol may not only be a hazard to the technician but may peril the validity of an experiment through cross inoculation of ani-

Figure 5. Ultraviolet screen across cage tops prevents escape of viable vegetative cells.

Figure 6. Filtered air is supplied to individual cages connected to negative pressure manifold.

mals or augmentation of the test inoculation (*16*). In some instances, it has become necessary to cage animals individually to insure the validity of an experiment. Before disturbing the debris or cleaning, cages should be disinfected or sterilized, depending upon the infectiousness of the organism.

The Pipette

The pipette is a significant source of infection, more because of aspiration of liquid than because of inhalation of aerosol. To eliminate the possibility of aspiration and oral contamination, a pipetting device of some kind is desirable (*17*). There are many pipetting devices which are commercially available, for instance, the aba (*C*), Adams (*D*), and Kadavy (*E*) micropipettors and the aba (*C*), Caulfield (*F*), and Fisher (*G*) pipettors. A new pipetting device which has found favor with the Fort Detrick personnel is the Propipette (*H*). A simple pipettor can be constructed from a short piece of rubber tubing, or a rubber bulb, such as those used to operate medicine droppers, may be used.

For a pipetting device to be suitable for handling infectious materials outside a safety cabinet, it should not deliver its liquid by forceful ejection, which may produce aerosols. Gravity flow delivery is given by the Caulfied pipettor and the Propipette but not by the other devices mentioned. Contaminated pipettes should be placed in a tray large enough to allow their complete horizontal immersion under a layer of germicidal solution, and the tray should be autoclaved before removing the pipettes.

Other Equipment

The infectious hazards associated with the use of high-speed mixing bowls have been investigated (*5*). It was found that bacterial aerosols may be set free by (*a*) a loose fitting cover, (*b*) lack of a gasket in a tight fitting cover, and (*c*) a worn bearing or loosely fastened drive shaft. Aerosols are also liberated during removal of blended materials from the bowls. A leakproof blendor bowl has been developed and may be commercially available in the future (*5*, *16*).

Aerosols formed by the process of decavitation during operation of the sonic oscillator may escape through a loosely fitting cover or when the contents are removed from the sonic cup. Use of a larger size O-ring (No. 24) will generally give adequate closure, while modification of the cover to allow for insertion of a rubber diaphragm so that the contents can be removed by syringe and needle, provides for safe removal of contents. Due to the hazard involved, however, it is best to use a blender or a bacterial sonic disintegrator in a safety cabinet.

Clothing

It is advisable to wear suitable laboratory clothing in infectious disease laboratories. The long-sleeve, operating gown which ties in the back and is worn over duck trousers is suitable for men, while a smock may be worn by women. A pair of shoes should be reserved for use in the laboratory only. Wearing a surgical cap may be desirable in animal rooms, when showers do not include the hair or when respiratory protective apparatus is used. Preferably, street clothing should not be worn beneath laboratory clothing, since the former may become contaminated if a spill occurs. Infection of laundry workers has proved that it is necessary to autoclave laboratory clothing of personnel working with some infectious agents (*19*).

Personal cleanliness is an important barrier to infection. Locker rooms should be equipped so that showers may be taken in case of accidental exposure to infectious materials or at the end of the working day. A germicidal soap containing Hexachlorophene is recommended.

Ventilation

Except in congested areas and with highly infectious agents, sterilization of building exhaust air is of slight importance to the nearby nonbacteriological areas if air from the bacteriological safety cabinets is sterilized. Long experience with this installation has shown (*a*) that during weeks and months when the exhaust air sterilization system of the general laboratory building was accidentally inoperative (but the cabinet exhaust air was being sterilized), there was no infection of the laboratory personnel or of passers-by and (*b*) that it is extremely difficult to maintain consistently an air-flow

control such that no potentially contaminated room air escapes into an adjoining "clean area." Proper control of air at the immediate work site may reduce or eliminate the need for exhaust air filtration of the whole building. It should be pointed out that sometimes treatment of air is necessary for public relations or legal reasons. But for the technician, the most important source of infection is within 12 inches of his nose.

Education

Equipping a laboratory with the finest safety devices does not insure against all possible laboratory infections. Equipment is no substitute for safe technique, which is based upon active participation by the worker at the laboratory bench in the process of establishing safe practices. All employees, new and old, should receive safety orientation and training. Supervisors and senior personnel must accept the responsibility for training new personnel and for insisting upon safe practices. A safety manual is an excellent medium for disseminating pertinent information. In our laboratories we have found that the establishment of a laboratory safety council has increased cooperation and facilitated an exchange of ideas. A tangible public award for a good safety record is always an incentive.

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EQUIPMENT REFERENCES

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(*B*) Centrifuge angle head, International Equipment Co., Boston, Mass.

(*C*) Micropipettor and pipettor, Alfred Bicknell Associates, Cambridge, Mass.

(*D*) Micropipettor, Clay-Adams Co., New York, N. Y.

(*E*) Micropipettor, A. S. Aloe Co., St. Louis, Mo.

(*F*) Pipettor, Caulfield Safety Devices, Philadelphia, Pa.

(*G*) Pipettor, Fisher-Scientific Co., Pittsburg, Pa.

(*H*) Propipette, Schaar and Co., Chicago, Ill.