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Biological Safety Cabinetry

RICHARD H. KRUSE,[1]* WILLIAM H. PUCKETT,[2] AND JOHN H. RICHARDSON[3]

MEDI, Inc., P.O. Box 11486, Lexington, Kentucky 40576-1486[1]; Department of Pharmacy, St. Luke's Episcopal Hospital and Texas Heart Institute, Houston, Texas 77030[2]; and Office of Occupational Environmental Safety and Health, Emory University, Atlanta, Georgia 30322[3]

INTRODUCTION

Concerted attempts to discover how infections are transmitted have existed since the origin of humans. Diseases were believed to originate supernaturally, and sacrifices to the gods were made to prevent the spread of diseases. The Egyptians believed that disease was spread by touch, while the Hebrews in Moses' time believed that disease was created de novo or could be contracted by contact with patients' clothes, as illustrated in Chapter 12 of the *Book of Numbers* and Chapters 13 and 14 of the *Book of Leviticus*, respectively. Hippocrates believed that two factors, miasma and malaria, meaning noxious vapor and bad air, respectively, not divine origin, induced disease. In 1546, Fracastorius described the agent of communicable disease as a living contagium vivum that spread by direct contact, by intermediary fomites, or through the air and postulated that these living seeds, passed from one infected animal, produced the same disease in an animal that received them. In 1676, Leeuwenhoek discovered and described bacteria, but bacteriology as a science dates from the middle of the 19th century, a result of the scientific studies of Louis Pasteur. Microbiologists are cognizant of his study disproving the theory of spontaneous generation and his work with cultures and the propagation of bacteria; however, because all of the media were fluid, these methods were not applicable for isolating a single microorganism from mixed cultures. Robert Koch's studies with nutrient gelatin and, later, solid

* Corresponding author.

TABLE 1. Early laboratory-acquired infections[a]

Date of event or laboratory infection	Occurrence
1676	Leeuwenhoek described bacteria
1857	Pasteur wrote a paper on lactic fermentation
1866	Koch found the first purposive pure culture
1881–1884	Isolation and culturing of diphtheria bacilli
1898	**Diphtheria laboratory-acquired infection, by pipette**
1882	Koch isolated tubercle bacilli
1883	Koch isolated cholera vibrios
1894	**Cholera laboratory-acquired infection, by pipette**
1884	Gaffky isolated typhoid bacilli
1885	**Typhoid laboratory-acquired infection, unknown mode**
1887	Bruce isolated *Brucella melitensis*
1887	**Brucella laboratory-acquired infection, by syringe**
1889	Kitasato isolated tetanus bacilli
1893	**Tetanus laboratory-acquired infection, by syringe**
1896[b]	Gilchrist described *Blastomyces dermatitidis*
1903[b]	**Laboratory-acquired blastomycosis, by needlestick**
1896[b]	Schenck isolated *Sporothrix schenkii*
1904[b]	**Laboratory-acquired sporotrichosis, by spray from syringe**

[a] Data are from Wedum (218). Boldfacing indicates data relating to laboratory-acquired infections.
[b] As reported by Hanel and Kruse (72).

nutrient agar with sterile techniques paved the way for the isolation, cultivation, and identification of causative organisms of disease. A complete and concise history of bacteriology (20) with theories and techniques of the past illustrates the advances made in bacteriology with today's maze of electronic, automated, and computerized laboratory equipment.

LABORATORY-ACQUIRED INFECTIONS

Historical Perspective

Although efforts to secure and maintain pure cultures of bacteria had begun, it was not until human laboratory-acquired infections appeared that attention was given to the protection of personnel. As a general rule, the isolation and identification of an infectious microorganism that caused human disease were followed in less than 15 years by a laboratory-acquired infection (Table 1). The first reported laboratory-acquired infection was published in 1893 (152) in France, when an accidental inoculation resulted in a tetanus infection. Five years later, two cases of diphtheria, one of which was caused by oral aspiration while mouth pipetting, were reported. In 1903, Evans (50) reported the first American laboratory-acquired infection when a physician stuck himself with a needle while performing an autopsy on a patient who had died of systemic blastomycosis.

The first survey was published in 1915, when Kisskalt (101) presented data on 50 cases of laboratory-acquired typhoid fever that occurred in Germany between 1885 and 1915. Of the 50 cases reported, the mode of infection was known in 23 cases. Pipetting was the caustative factor in 16 cases, and there were six deaths. In 1929, Kisskalt (102) published a second survey, in which there were 59 cases of laboratory-acquired typhoid fever occurring between 1915 and 1929, and reviewed 24 other laboratory-acquired infections that resulted in five deaths. Other German surveys were reported in 1939 (49) and 1950 (181).

In 1930, McCoy (126) published details of a psittacosis outbreak at the National Hygienic Laboratory in Washington, D.C. In 1940, Huddleson and Munger (86) described a brucellosis outbreak among laboratory personnel and students at Michigan State University. Hornibrook and Nelson (84) described a Q fever outbreak that infected 15 people in a laboratory building at the National Institutes of Health (NIH). In 1941, Meyer and Eddie (131) published a survey regarding 74 laboratory-acquired *Brucella* infections. In 1947, Huebner (87) reported 47 infections with Q fever at NIH. Many of the infected personnel were visitors who had been in the building only for a brief duration. In 1949, Nauck and Weyer (148) described 13 laboratory-acquired infections with Q fever, and when the rickettsial organism was transferred to a neighboring laboratory, 20 additional laboratory-acquired infections occurred. In 1949, Sulkin and Pike (199) published their first survey of 227 laboratory-acquired viral infections. Only 27 of these infections were the result of known accidents, the most common of which was splashing infectious material in the face or eyes.

At the Annual Meeting of the American Public Health Association in St. Louis, Mo., on 2 November 1950, Sulkin and Pike reported (and later published [200]) their summary of data received from a questionnaire sent to 5,000 laboratories in the United States. There were 1,342 laboratory-acquired infections, resulting in 39 deaths. Only 467 cases had been published, and 1,175 cases had occurred since 1930. Recognizable accidents accounted for 215 (16%) of the infections, and the probable cause of the remaining 84% was believed to have been exposure to or ingestion of accidentally formed microbial aerosols. In 1965, Pike et al. (165) updated their 1951 report with data accumulated from countries outside the United States and added 641 new or previously unreported laboratory-acquired infections. In 1967, Hanson et al. (73) reported 428 laboratory-acquired infections with arboviruses, with the causal factor of most of the infections believed to have been exposure to infectious aerosols.

In 1967, Hanel and Kruse (72) analyzed laboratory-acquired mycoses published in the literature and tabulated 288 clinical and seroconversion cases. They did not include 204 possible cases of coccidioidomycosis because they believed that the seroconversions did not represent occupational infections. They discovered that needles and syringes used to administer the coccidioidin skin test reagent had been used for other skin tests, including tuberculin. Biological agents adsorbed on syringes or needles could result in false-positive tests, as reflected in inconsistent yearly skin test readings on the same patient: for example, positive first-year, negative second- and third-year, positive fourth-year, and negative fifth-year readings. Of the 288 laboratory-acquired mycoses described, known accidents or incidents accounted for only 13% of the infections and most of the infections resulted from exposure to accidentally created mycotic aerosols.

In 1969, Wedum and Kruse (220) provided four "indicators of risk" to serve as guidelines for the safe handling of microorganisms to protect laboratory personnel. Each indicator hypothesized the presence of aerosolized infectious microorganisms. The first indicator of risk was "number of laboratory infections." They listed 2,912 laboratory-acquired infections caused by 111 microbial agents. The sec-

ond indicator of risk was "infectious human dose." A working assumption used in deciding whether a microorganism might present a hazard to humans was that the minimal human and animal infective doses were approximately the same. However, among viruses there was tremendous variation in the number of organisms that constituted an infective dose. The third indicator of risk was "infection of uninoculated control animals caged with or near inoculated cagemates." When an uninoculated control animal became infected while caged with an inoculated animal, infectious microorganisms that could infect the animal handler were present. The fourth indicator of risk was "presence of microorganisms in urine and/or feces of inoculated animal." Far greater danger results when microorganisms are found in the urine and/or feces of infected animals. Multiple laboratory-acquired infections have been due to microorganisms aerosolized from dried urine and/or feces during research on Soviet hemorrhagic fever (113), Venezuelan equine encephalitis virus (119, 189), and *Chlamydia psittaci* (126). The indicators of risk have been used in other publications to alert the scientific community to inherent aerosol hazards (16, 217, 219).

At the Center(s) for Disease Control (CDC) (179) and at the National Animal Disease Center (202), 109 and 18 laboratory-acquired infections were reported, respectively. At the 18th Biological Safety Conference, Pike reported (and later published [162]) a summary of 3,921 laboratory-acquired infections with 164 deaths. Analysis of these worldwide cases revealed that 2,465 occurred in the United States, 1,456 were foreign, and only 18% were due to known accidents. There was a decrease in infections between 1955 and 1964 from the previous decade and an even greater decrease between 1965 and 1974, despite the discovery of new microorganisms and a great increase in the number of personnel working with infectious materials. Analysis of those years indicated that the five most frequent recognized causes of laboratory-acquired infections were (i) spills, sprays, and spattering of infectious material (26.7); (ii) accidents involving needles and syringes (25.2%); (iii) injuries with broken glass or other sharp objects (15.9%); (iv) bites of animals or ectoparasites (13.5%); and (v) pipetting accidents (13.1%).

Pike (163) updated the total to 4,079 laboratory-associated infections with 168 deaths in 1978 and, in the following year (164), summarized laboratory-acquired infections, provided data on institutional and common source outbreaks, reviewed 173 fatalities from laboratory-acquired infections, and discussed methods to prevent further infections. He stated, "Knowledge, proper techniques, and the equipment to prevent most laboratory infections are available today," but laboratory-acquired infections still occur (164).

In 1980, the Subcommittee on Arbovirus Laboratory Safety (198) published data compiled from two surveys sent to 585 laboratories throughout the world. The questionnaire (182) requested (i) the frequency of handling viruses; (ii) the number of people at risk; (iii) the number of overt and subclinical laboratory-acquired infections; and (iv) the probable route of infection. Response to the 1976 survey was 32%, and that to the 1978 survey was 25%. This subcommittee reported 818 arbovirus laboratory-acquired infections, 17 of which resulted in death. The subcommittee further recommended four levels of practice and containment.

In 1987, Miller et al. (133) updated the previous study (202) at the National Animal Disease Center and included data for the preceding 25 years on laboratory-acquired infections. Vesley and Hartmann (212) sent questionnaires to 54 state and territorial health departments in the United States and to 165 clinical laboratories in Minnesota, with response rates of 79.6% from health departments and 90.3% from state clinical laboratories. The health departments reported no laboratory-acquired infections, and the clinical laboratories reported an incidence of 0.5 infection per 1,000 employees who worked directly with microorganisms. Although Pike (164) reported that brucellosis and Q fever were the most common laboratory-acquired infections, today more emphasis has been placed on tuberculosis, hepatitis B, and AIDS.

Tuberculosis

Tuberculosis is extremely difficult to identify as a laboratory-acquired infection. Numerous publications have described cases of tuberculosis in medical schools and laboratory personnel in England (75, 190), in Sweden (81), in Canada (130), and in the United States (129, 134). Reid (171) surveyed 368 medical laboratories in England and reported that active pulmonary tuberculosis was three times more frequent in laboratory personnel exposed to infectious material than in laboratory personnel not exposed to infectious material. Kaufmann and Anderson (96) reported that tuberculin conversion was 25 times higher in animal handlers than in the general population. Laboratory workers have increased their manipulations with fluid tubercle bacillus suspensions, and more tubercle bacilli have become drug resistant, adding to the risk. In an investigation of 13 incidents of laboratory-acquired tuberculosis (110), 92% of infected staff personnel were not aware of directional airflow and did not know proper maintenance of laboratory equipment.

The AIDS epidemic has increased work with mycobacteria in the laboratory, and the incidence of tuberculosis, especially in correctional institutions (191), also has increased. Vandiviere and Melvin (211) reported that cross-reacting and false-positive tuberculin tests are common and that 8% of bacteriologically confirmed cases of tuberculosis give false-negative skin tests. They stated that the diagnosis of tuberculosis in laboratory personnel has become more difficult because of the imperfect "witch's brew" of tuberculosis: purified protein derivative.

Hepatitis B

All laboratory personnel are concerned with hepatitis B virus (HBV). In 1951, Sulkin and Pike (200) reported 99 laboratory-acquired infections; that number increased to 268 infections in 1978 (163). Skinhoj (187) reported that the incidence was seven times greater in clinical chemistry laboratory personnel than in the general population. Higher incidences in clinical laboratory personnel were corroborated by Levy et al. (121) and Maynard (125). In 1981, Skinhoj and Soeby (188) reported a fivefold increase in hepatitis in physicians, surgeons, and laboratory personnel. Peterson et al. (159) attempted unsuccessfully to recover hepatitis B surface antigen from 60 air samples, but they recovered the surface antigen in 15% of surface samples. However, Lauer et al. (118) demonstrated antigen on 34% of laboratory surfaces sampled. HBV infection is one of the most frequently reported laboratory-acquired infections (53). Recent data from CDC (29) estimate that 18,000 cases of hepatitis B occur annually among the nation's 5 million health workers. Furthermore, 12,000 of these cases are thought to be occupationally acquired, and 10% of these patients become long-term carriers, with an estimated 250 hepatitis B-associated deaths occurring each year. HBV is

found in blood, body fluids, and tissues and can be transmitted directly by parenteral inoculation and by exposure of mucous membranes and/or broken skin. The virus can be transmitted by indirect means on the surfaces of test tubes, laboratory surfaces, and other surfaces contaminated with infective blood, serum, secretions, and excretions. Universal precautions (25) should be used for handling all specimens of human origin, because blood-borne pathogens cannot be reliably ruled out. Favero et al. (54) state that the following specific precautions should be used to reduce infection risk: (i) strict hand washing; (ii) wearing gloves and protective clothing; (iii) use of safety equipment and procedures; and (iv) excellent housekeeping. All laboratory personnel at risk for HBV infections should be vaccinated.

AIDS

AIDS has created more attention, not only in the scientific community but also in public and political sectors throughout the world. Severin (184) chronicled episodes of pandemic infectious diseases in history: plagues in Europe in medieval times and in Asia in modern times; influenza in the early 20th century; and recent outbreaks of polio and hepatitis B. He stated, "We are now experiencing an infectious disease pandemic that manifests both a high mortality rate and economic devastation. In its brief history, this communicable disease has already changed the legal and social relationships that health care workers depend on for the delivery of their professional services to those seeking aid."

Since June of 1981, when AIDS was first reported (22), research workers have, as a result of massive efforts (i) defined the disease parameters (23); (ii) identified at-risk populations (26); (iii) identified various methods by which the virus is spread (24); (iv) isolated the causative virus (52); (v) developed screening procedures for detecting antibody to human immunodeficiency virus (HIV) in body fluids (27); (vi) diagnosed HIV antigen in body fluids (99); (vii) developed guidelines for health care workers (28); and (viii) educated at-risk populations of certain dangers (58, 93). The Surgeon General issued a report on the status of the disease, the method of spread, and precautions (208). The limited success of therapeutic agents has been reported (60). Guidelines (29, 140) describing procedures and practices to protect laboratory personnel working with HIV and HBV have been established.

HIV is spread by blood, semen, vaginal secretions, and breast milk (56, 57). As with HBV, universal precautions (25) should be the rule when working with human blood and body fluids. With all the precautions and guidelines, the old nemesis throughout the history of laboratory-acquired infections, the needle, has been instrumental in 16 of the 25 documented worldwide occupationally acquired infections (28, 123). Not all of these infections occurred in the laboratory. Analysis of the occupationally acquired infections showed that (i) six were in nurses (five had needle punctures and one had blood from an HIV patient splash on her hands and eyes); (ii) ten were in health care workers (eight had needle punctures while collecting blood or fluids, one had blood splash on her face when the top of a blood-collecting tube blew off, and one had blood from an HIV patient on her hands after applying pressure to an arterial catheter); and (iii) three were in a mother who had frequent contact with her child's infected blood and body fluids, a dentist who worked with high-risk patients, and a woman who provided health care services to a patient who died of AIDS.

Six laboratory workers became HIV positive, and investigation revealed the following. (i) One worked with concentrated HIV type 1. A virus-positive culture fluid had leaked from equipment and contaminated centrifuge rotors. The worker did not follow level 3 precautions all the time, and he reported that there were incidences in which there were pin holes or tears in gloves but that, when he discovered them, he changed the gloves immediately. (ii) A worker sustained a cut when a vial of HIV-infected blood broke in his hand. (iii) A worker handled large volumes of HIV in a high-containment laboratory. It is unknown how he was infected, but he had cut his finger with a blunt needle while cleaning contaminated equipment. (iv) A worker had two parenteral exposures by sustaining a needle puncture and a cut on his hand while processing blood 8 and 16 months, respectively, before he tested positive. (v) A worker reported having many accidental punctures and blood contact and recalled receiving a deep cut in his right hand during an accident while working with contaminated plasma. (vi) A medical technologist was exposed to a blood spill that covered her hands and arms while operating an apheresis machine. She was not wearing gloves, nor did she report any open wounds on her hands. She had dermatitis on her ear and might have touched that ear.

Reporting Laboratory-Acquired Infections

The laboratory-acquired infections most frequently reported are summarized in Table 2. Why are there discrepancies in the numbers of laboratory-acquired infections reported in different surveys? One important aspect limiting our knowledge of infections is the absence of a universal requirement for reporting infections. Concerted efforts have been made to make reporting mandatory, without success. Many infections have never been reported, and even questionnaires sent to various laboratories have gone unanswered. Furthermore, with many diseases, only overt cases (those with a clinical illness) are reported, while other investigators report a change in agglutination titer, skin test, or serologic conversion as a laboratory-acquired infection on the premise that seroconversion verifies that a person has been infected with infectious material. As far as identifying a hazardous operation, however, it makes no difference whether the person is ill or converts serologically. Listed in this category are work with tuberculosis, histoplasmosis, coccidioidomycosis, and many viral and rickettsial diseases.

Laboratory-acquired infections still occur. In Great Britain, annual surveys (68) have reported laboratory-acquired infections since 1976. In the United States, cases of laboratory-acquired infections have been published (66, 91, 116) or reported (64, 69), but usually they were from individual laboratories. Some infections are never reported because such reports might reflect unfavorably on the laboratory. Documentation of the numbers of laboratory-acquired infections that occur annually in the United States is virtually impossible, although once it had been planned to conduct a surveillance program, summarize the data, and make the listing available to interested individuals on an annual basis (162). Individual cases have been published in CDC's *Morbidity and Mortality Weekly Report* or listed in *Index Medicus* under the subject listing "Laboratory Infections." Sullivan (201) stated, "There are few who dispute the need for improved recording and reporting of job-associated injuries or illnesses. Mandatory reporting of a defined type of job-associated injuries or illnesses provides a much broader data base, which is needed to identify, assess, and control or eliminate hazards found in the workplace." Some method or

TABLE 2. Most frequently reported laboratory-acquired infections

Laboratory-acquired infection	No. of cases reported by:				
	Sulkin and Pike in 1951 (200) (1,342 cases)	Wedum and Kruse in 1969 (220) (2,912 cases)	NIH in 1974 (142)	Pike in 1976 (162) (3,921 cases)	Pike in 1978 (163) (4,079 cases)
Brucellosis	224	274	276	423	426
Q fever	104	184	214	278	280
Typhoid	58	292	293	256	258
Hepatitis	95	126	182	234	268
Tularemia	65	129	133	225	225
Tuberculosis	153	174	217	176	194
Dermatophytosis	14	84	40	161	162
Venezuelan equine encephalitis	11	118	107	141	146
Typhus (endemic and epidemic)	64	82	82	124	
Psittacosis	44	70	70	116	116
Coccidioidomycosis	49	108	57	93	93
Streptococcosis	53	67	67	78	—[a]
Histoplasmosis		81	46	71	
Leptospirosis		43	45	87	
Kyasanur Forest fever		65	65	67	
Soviet hemorrhagic fever		113	113	—[a]	
Salmonellosis		54	54	48	
Shigellosis	31	54	54	58	

[a] —, Pike did not include these, although he listed 113 cases.

system should be instituted for voluntarily reporting every laboratory-associated infection with the inherent cause to a special committee established at, perhaps, NIH, CDC, or the American Biological Safety Association.

LABORATORY PROCEDURES ANALYZED

Recognition of the extent of unknown causes of laboratory-acquired infections emphasized the need to develop equipment and procedures that would prevent exposure to infectious microorganisms. It was reported at the 49th Annual Meeting of the Society of American Bacteriologists in 1949 (194) that aerosols were created during the performance of common laboratory procedures. In 1952, Anderson et al. (7) arranged sieve-type air samplers (224) around a test area to approximate the position of a person's hands and nose and analyzed quantitatively the aerosol released while (i) using a high-speed blender; (ii) dropping a liquid culture on various surfaces; (iii) removing rubber stoppers, screw caps, and cotton plugs from dilution bottles and test tubes; (iv) removing culture fluid with a hypodermic syringe and needle from vaccine bottles; (v) flaming an inoculating loop; and (vi) inoculating cultures with a pipette and inoculating loop. In succeeding years, the above-described procedures plus additional laboratory procedures and equipment were examined by investigators who often recommended solutions to eliminate or decrease aerosol formation.

One piece of equipment that produced aerosols of great magnitude was the high-speed blender. When two types of blending bowls (plastic cap versus screw cap) for homogenizing cultures were compared, the plastic-cap bowls generated 28 times more aerosol than did the screw-cap bowl. Removing the caps immediately after blending released a large aerosol regardless of the cap used. When either cap was removed 5 min to 1.5 h after blending, an aerosol was still produced. Evidence that leaking gaskets and worn bearings contributed to aerosol formation led to the design of a new blender (Waring Products Division, New Hartford, Conn.) that contained the aerosol generated (82, 173).

The amount of aerosol created by 1 drop of culture falling 3 in. (ca. 7.6 cm) and 12 in. (ca. 30.5 cm) was influenced by the nature of the surface (7). Even when the surface (cloth towel) was moistened with a disinfectant, an aerosol was formed (42). Careful manipulations learned with training and experience should eliminate the hazard of falling drops from needles and pipettes.

No microbial aerosol was released when dry cotton plugs were removed from test tubes containing liquid cultures of *Serratia indica* (7). However, when the cultures were shaken and bubbles formed and when the cotton plugs were wet, either by accident or vigorous shaking, organisms were released. When dry cotton plugs were removed from test tubes containing 3-week-old *Coccidioides immitis* cultures, air samplers arranged around the work area (7) recovered from zero to three arthroconidia (105). The older the culture, the more arthroconidia released.

Hanel and Alg (70) reported the use of the syringe and needle to be one of the most hazardous laboratory procedures. Capillary action contaminated the user's hands, injection sites on animals were contaminated, and withdrawing the needle from a rubber-stoppered test tube or vaccine bottle caused the needle to vibrate. All of these factors contributed to aerosol formation. Closer examination and testing of syringe and needle fit resolved capillary action leakage. Surrounding the needle with a disinfectant-soaked cotton pledget and disinfecting the injection site reduced microbial skin contamination of the animal by 70%. When a syringe and a needle were used to prepare 10-fold bacteriophage dilutions, a disinfectant-soaked pledget surrounding the rubber stopper and needle curtailed aerosol formation (172). Expulsion or separation of the needle from the syringe created hazardous conditions, such as splashing of the culture in the face, augmentation of the animal inoculating dose, and creation of an aerosol in the room that persisted even when the ventilation system was operational. Needle-locking syringes alleviated this hazard.

Needles and syringes create one final hazard. They must be properly and safely disposed of. There are devices to break needles, but an aerosol results from this operation. Needles should not be broken, bent, or recapped by hand. Needlesticks continue to be a laboratory worker's nemesis,

as they are the second leading cause of laboratory-acquired infections (162). Needles and syringes should be placed in labeled, leak- and puncture-resistant containers for autoclaving or disposal by incineration.

Transferring a liquid culture to an agar plate for isolating microorganisms requires many steps, and analysis revealed that aerosols were created during each step (7). A cool, not hot, inoculating loop inserted into a liquid culture produced less aerosol. Streaking a culture on a rough-surface agar plate created an aerosol 95% larger than did streaking a culture on a smooth-surface agar plate. Substituting a sterile glass spreader for the loop further reduced the aerosol (175, 214). Many microbiologists continue to flame inoculating loops. This procedure creates an aerosol, but it is of a small size, as illustrated by the recovery of 0 to 0.3 organism per test (161). However, when this procedure is performed in a class II safety cabinet, too large a flame can disrupt the airflow pattern and damage the filter. A microburner or electric incinerator (Bacti-Cinerator; American Scientific Products, McGaw Park, Ill.) should be used.

The pipette has been associated with laboratory-acquired infections for years. Hanel and Halbert (71) thoroughly studied the pipette, describing history, mixing, transferring, mouth pipetting, last-drop expulsion, plugging versus not plugging, injuries, breakage, and safe disposal. They listed examples of micro- and macropipetting devices that should be used for safe pipetting manipulations.

Other hazardous procedures have also been examined. Opening an ampoule containing a lyophilized culture created an aerosol that was reduced by wrapping the neck of the ampoule with a disinfectant-soaked pledget and breaking the ampoule with the pledget in place (174, 174a). When an ampoule dropped and broke on the floor, an aerosol of a large magnitude resulted (177). Experience and good training should eliminate this hazard.

The centrifuge is another source of laboratory-acquired infections. Only a few individual laboratory-associated infections have been reported, because centrifuge accidents typically result in multiple cases (86, 160). Leaking or broken centrifuge tubes release infectious particles (98, 176). Enclosing the centrifuge tubes in safety centrifuge cups eliminated aerosols created by broken centrifuge tubes, as the generated aerosols were contained within the safety cups (177). A symposium (136) assessed centrifuge biohazards, examined bench-top centrifuges, zonal centrifuges, and ultracentrifuges, and described numerous procedures to eliminate hazards.

With the increased work involving hepatitis B and AIDS, pathologists have become concerned with biohazards arising from autopsies. The first reported laboratory-acquired infection in the United States was acquired during an autopsy (50). In one study, six slit-type air samplers (224) were used to survey the microbial flora present in the air while autopsies were performed. Many procedures used by pathologists created aerosols. When a lung was excised to ascertain pulmonary involvement (later found to be caused by *Staphylococcus aureus*), three samplers recovered the same organism approximately 1 min after excision and for an additional 45 min. Visible aerosols of bone dust and fluid were observed when the Stryker saw was used, and over 500 colonies of a tracer organism were recovered by three samplers. Fluid was removed from the chest cavity with a hose connected to a water aspirator located in the sink. During this procedure, fluid spattering occurred and tracer organisms were recovered throughout the room. In one study at NIH (222), Andersen six-stage impactor samplers (224) were used to determine the microbial characteristics of aerosols created while autopsies were performed. The number of organisms recovered was directly proportional to the number of people in the room. When the Stryker saw was used, there was a large increase in the number of organisms. Risks for hepatitis have been reported (67), and it has been recommended that all autopsies be performed with the same precautions as if the individuals were known hepatitis patients (54).

Studies focusing on viral (44, 172) and fungal (105) hazards, cross-infection experiments (106, 109), and simulated accidents in the laboratory (12, 98) have been reported in scientific journals. Numerous comprehensive reviews on the hazards in the laboratory have been published in journals (32, 175, 177, 195, 216), as chapters in books (3, 15, 33), as government studies or guidelines (136, 156, 160), and as entire books dealing solely with laboratory safety (36, 61, 132, 143).

Several laboratory procedures and the amounts of microbial aerosols liberated during them are presented in Table 3. These data emphasize that education, training, and inspections are but a few concepts that should be instituted to make protective practices part of every laboratory worker's activity (215). In addition, they emphasize the importance of a safety cabinet to separate the laboratory worker from infectious microorganisms liberated during common laboratory procedures.

EARLY SAFETY CABINETS

The microbiological safety cabinet had its inception in 1909, when the W. K. Mulford Pharmaceutical Co., Glenolden, Pa., designed a ventilated hood to prevent infection with *Mycobacterium tuberculosis* during the preparation of tuberculin (218). A vacuum pump drew air through a cotton filter into the chamber, maintained negative air pressure in the chamber, and exhausted the air through a flask containing a disinfectant. Arm-length rubber gloves were attached, and all manipulations were performed through these gloves. Various cabinets appeared in the following years, but the type and usage were at the discretion of the individual, who usually was the designer and user. The earliest publication describing microbiological cabinets was in 1943 by Van den Ende (210), who designed a cabinet using an electric furnace to create inward airflow and to incinerate the exhaust air. Shepard et al. (185) built a wooden cabinet that used a gas burner to incinerate the exhaust air and to provide inward airflow. Keeney (97) developed a cabinet of stainless steel with a glass front and top, but it had no ventilation. The first cabinet constructed of stainless steel with glass viewing panels, interior rear baffle, service piping, exhaust blower, and spun-glass fiber filters was fabricated in 1948 and described in the literature in 1953 (214). Microbiological cabinetry became sophisticated and attained its ultimate containment efficacy at the U.S. Army Biological Laboratories, Fort Detrick, Md. "As for means of preventing laboratory-acquired illness, the single most useful piece of equipment is the so-called bacteriological safety cabinet" (214).

CLASS I SAFETY CABINETS

The class I safety cabinet is a modification of the chemical fume hood. Figure 1 shows a class I cabinet constructed of stainless steel with glass viewing panels, an exhaust blower, lights, service piping, and a front opening through which the hands of the user are inserted to perform various technical

TABLE 3. Infectious hazards of common microbiological techniques

Procedures	CFU recovered/ft (ca. 30.5 cm) from air during the procedure			
	Bacteria		*Coccidioides immitis*	
	Range	Avg	Range	Avg
Opening and closing culture containers				
Glass petri dish		0	6–20	12.2
Plastic petri dish		0	6–19	10.4
Test tube with:				
Cotton plug		0	0–3	1.1
Screw cap	0–15	4.0	2–5	3.4
Rubber plug		1.0	0–3	0.9
Inoculating loop manipulations				
Picking colony from test tube with:				
Cool wire	0–0.22	0.08[a]	2–38	15.2
Hot wire	0.68–25	8.7	13–40	25.3
Picking colony from petri dish with:				
Cool wire		0.005	17–39	33.1
Hot wire		0.008	18–62	44.6
Streaking culture on:				
Smooth-surface agar plate		0.26	14–42	25.6
Rough-surface agar plate	7–73	25.1	17–39	32.6
Inserting wire into flame	0–0.3	0.1	7–16	11.1
Pipette manipulations				
Inoculating flask or test tube	0–2	1.2	0–9	4.9
Mixing suspension in test tube				
Bubbles formed	0.3–3	0.8	2–14	10.7
No bubbles formed	0–1	0.2	0–5	3.3
Hypodermic syringe and needle manipulations				
Withdrawing culture from rubber-cap vaccine bottle				
Disinfectant-soaked pledget used	0.8–16	5.3	0–1	0.1
No pledget used	4.4–28	16.0	9–19	13.1
Preparing dilutions in rubber-cap vaccine bottles				
Disinfectant-soaked pledget used	0	0	0	0
No pledget used	0–10	2.3	10–25	17.1
Injecting animals[b] intraperitoneally with culture				
Disinfection of area before and after inoculation	0	0	1–6	3.7
No disinfection	15–16	15.0	8–20	12.3

[a] Erlenmeyer flask.
[b] Guinea pigs for bacteria; mice for *C. immitis*.

procedures. Airflow is inward, across the work surface, through the chamber containing spun-glass fiber filters, and through the exhaust blower into the building exhaust or a duct leading to the outside. There is no recirculation of air. The first class I cabinet had an inward airflow of 50 ft (1 ft = 30.48 cm)/min (fpm) (214), which was later increased to 60 fpm (206); today, the recommendation is a minimum of 75 fpm (55). Spun-glass fiber filters have a nominal efficiency of 95% for removing particulates 1 to 5 μm in diameter (43). Because of advances in filter technology, spun-glass fiber filters are no longer advocated for class I safety cabinets and have been replaced with high-efficiency particulate air (HEPA) filters.

Figure 2 illustrates the CDC class I safety cabinet. Spun-glass fiber filters have been replaced with six or seven HEPA filters located in the filter chamber above the work area through which air is exhausted. Each HEPA filter is sealed in place to prevent exhaust air from bypassing it. Roughing filters are placed in front of the HEPA filters to extend filter usefulness. The cabinet is constructed of metal with a base of dried, tempered oak that is resistant to the usual laboratory chemicals. Four UV lamps are located inside the chamber above the HEPA filters. Published research on the effect of this UV irradiation on microorganisms contained in the airstream could not be located.

No product protection is afforded by the class I safety cabinet. Cross contamination may result from contaminated air flowing over the work area. It is recommended that work with biosafety level 1, 2, and 3 agents (207) (previously referred to as low- to moderate-risk infectious biological agents) be performed in the class I cabinet. Conditions that may draw contaminated air from the class I cabinet in the laboratory must be avoided. Examples are (i) people walking at a rapid pace in front of the cabinet and (ii) the user withdrawing his or her arms too rapidly upon completion of a certain task.

Operator protection can be increased if a metal glove panel is attached to the class I cabinet. Inward airflow through the open glove ports is increased to a minimum of 150 fpm.

Figure 3 shows a class I safety cabinet with and without the glove panel attached and with gloves attached to the glove panel, illustrating airflow direction. With the gloves attached, the exhaust system should maintain a cabinet pressure of −0.5 in. (ca. −1.3 cm) water gauge (205). This system affords the maximum protection attainable in a class I safety cabinet.

DEVELOPMENT OF THE HEPA FILTER

During World War II, a section of a German gas mask canister was sent to the U.S. Army Chemical Corps. A cooperative effort of the Army Chemical Corps, Naval Research Laboratory, National Defense Research Council, and later the Atomic Energy Commission culminated in the development of the HEPA filter. The grass and asbestos fibers were replaced with emulsified submicron glass fibers separated by crimped aluminum in a fire-resistant frame (59). Research instituted at Fort Detrick ascertained the particle size penetration and resistance of the HEPA filter and demonstrated that it filtered particles less than and greater than 0.3 μm with an efficiency of or greater than 99.97% (65).

Langmuir (115) stated that moving particles of >0.1 μm would be retained by interception and that moving particles of <0.1 μm would be retained by diffusion. He further recommended that filters be tested with 0.3-μm particles because interception and diffusion would be at a minimum with this size particle and the minimum retention efficiency would be determined. Particulates are not deposited on the filter surface but are deposited on fibers throughout the depth of the filter. There are several means by which particles are deposited on the filter media: (i) inertial effect; (ii) diffusion; (iii) electrostatic effect; (iv) direct interception; and (v) deposition. Decker et al. (43) published a monograph on the filtration of microbial particles and evaluated various filters.

Laboratory personnel often ask whether particles smaller than 0.3 μm will pass through the HEPA filter. According to First (59), this concern is unnecessary because (i) HEPA filter efficiency increases for particle sizes below 0.3 μm; (ii)

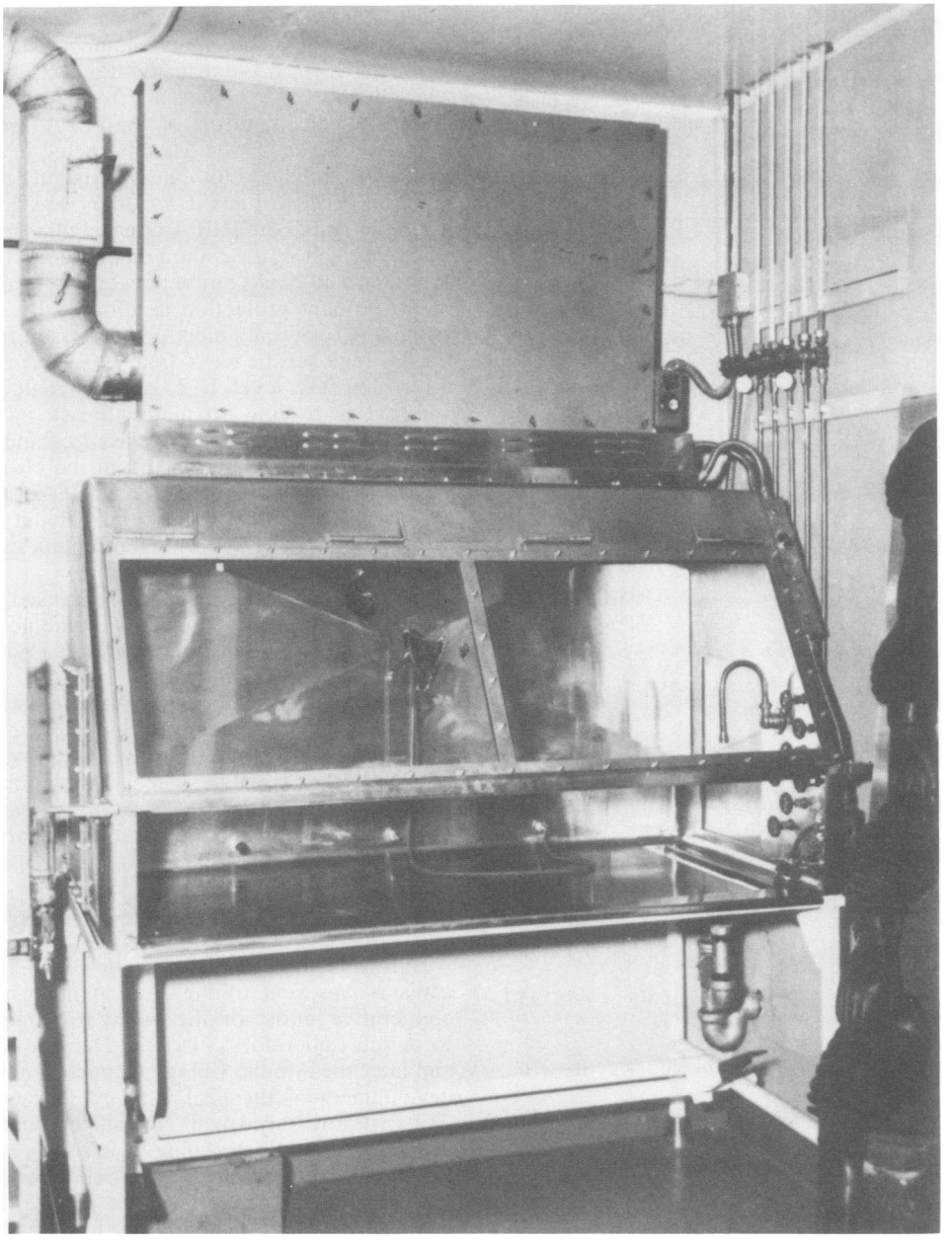


FIG. 1. Class I biological safety cabinet.

if a single virus were aerosolized, it is improbable that it would survive environmental stresses; (iii) viruses usually are encapsulated in mucus, dust, moisture, or culture media and become larger particles; and (iv) special orifices and high energy are required to generate such small particles. Harstad and Filler (79) described aerosol experiments with bacteria and T1 and T3 bacteriophages in which HEPA filters effectively removed particles less than and greater than 0.3 μm in diameter with an efficiency of 100%. However, the theory did not suffice even though research proved the hypothesis, because penetration data were evolved from filter testing but the HEPA filters were not installed in a biological safety cabinet.

In studies with bacteriophage R17, Kruse and Kruse (107) evaluated the efficacy of HEPA filters installed in a safety cabinet. Phage R17 met all test criteria: (i) size (it is an icosahedral particle 23 to 25 nm in diameter, among the smallest and genetically simple infectious, self-replicating organisms known); (ii) specificity; (iii) stability; and (iv) structure (it does not have a tail like T1 and T3 bacteriophages). With a Vaponefrin nebulizer (Ace Glass Inc., Vineland, N.J.), 5.6×10^8 R17 phage particles were aerosolized directly into the supply duct of a class II, type B2 (total-exhaust) safety cabinet. Airflow was such that it passed only once through the supply HEPA filter into the work area. The data obtained from replicate tests with filters from different companies substantiated that the efficacy of the HEPA filter is 99.99 to 100% and that work with viruses 23 to 25 nm in diameter can be done in biological safety cabinets with HEPA filters.

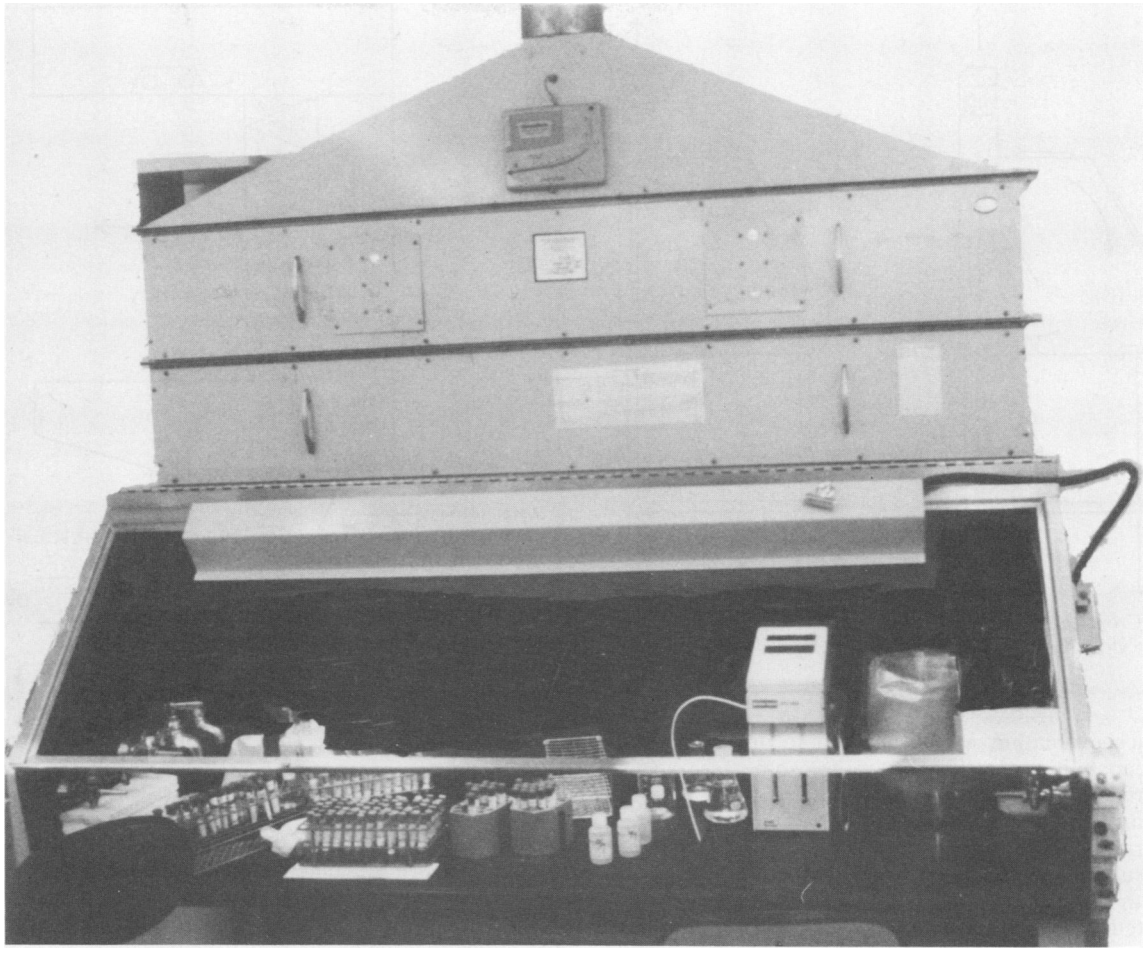


FIG. 2. CDC class I biological safety cabinet.

EVOLUTION OF CLASS II (LAMINAR FLOW) BIOLOGICAL SAFETY CABINETS

Clean Room

In 1962, Whitfield (221) stated that laminar flow occurred "when the entire body of air within a confined area moved with uniform velocity along parallel lines." This concept was later termed "mass airflow," but the term should have been "unidirectional airflow." Laminar airflow has a different, long-established meaning but, although a misnomer in biological safety cabinets, laminar airflow continues to be the term of choice; thus, we will use it throughout this paper. In the ensuing years, the laminar flow clean room, with either horizontal or vertical airflow, was developed. Aerospace and electronics industries used this clean room environment, as did the pharmaceutical industry and hospitals. A flow of filtered air passed by and over the worker and the work material, and any particles created were entrapped in the airstream and consequently trapped in the media of the HEPA filter. However, these clean rooms were very expensive to construct and costly to operate and maintain. Furthermore, once erected, most clean rooms could not be relocated.

Clean Bench

One way to describe the clean bench is to state it is a minaturized clean room. For construction of a clean bench (Fig. 4), the bank of HEPA filters is replaced with one or two HEPA filters [the work area is directly in front of the HEPA filter(s)], the number of motors is reduced, and a roughing filter is placed in front of air ingress to eliminate large particles.

The clean bench is easily constructed and can be moved to particular locations. Although the product (medium, tissue culture, or admixture) is protected in the clean bench, the clean bench is not suitable for hazardous chemical or infectious microbiological or sensitizing materials because the airflow is directed toward the user.

Class II Safety Cabinets

Protection for the user dictated a new cabinet design. Needed was a cabinet that used clean vertical airflow with auxiliary air entering the front of the cabinet to prevent the escape of aerosols across the front opening of the cabinet. Modifications such as tilted glass, two parallel glass panels, and various work areas ensued in attempts to fabricate a cabinet that provided product and personnel protection.

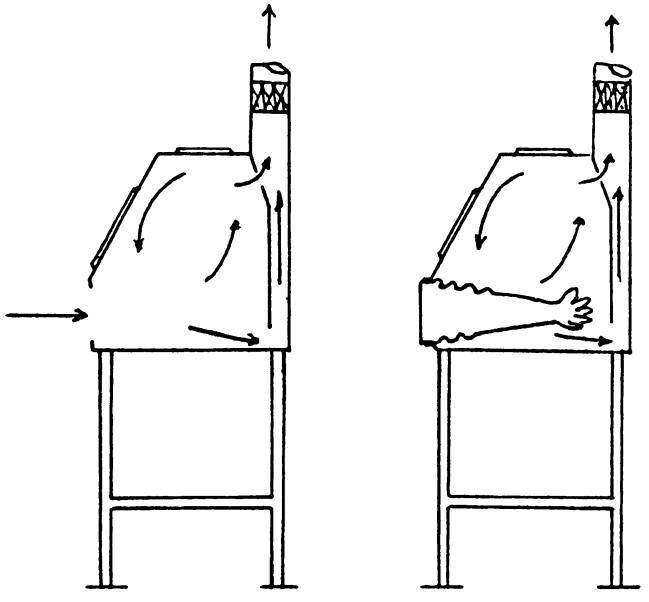


FIG. 3. Cross section of a class I biological safety cabinet with and without gloves attached.

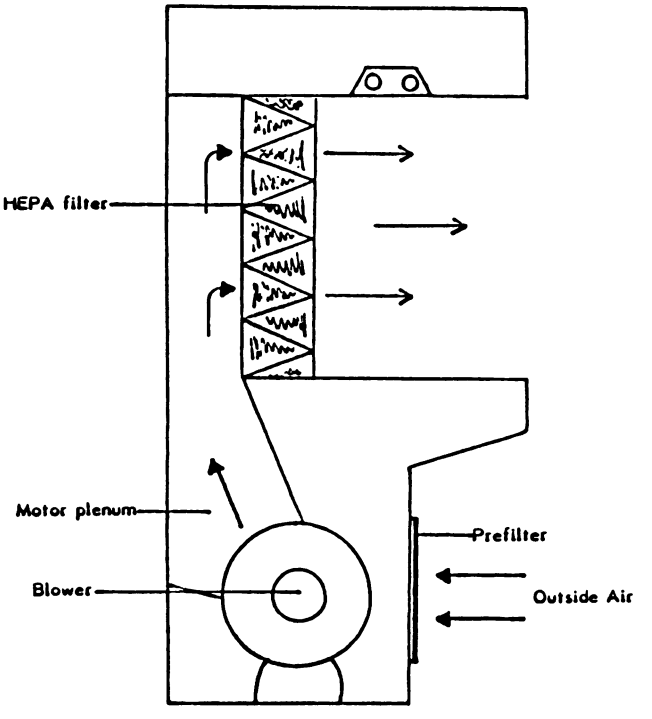


FIG. 4. Cross section of a horizontal airflow cabinet (clean bench).

Performance evaluation was conducted to determine containment capabilities (1, 38). One troublesome area was at the juncture of the vertical and incoming airflows. Excessive incoming airflow resulted in room contaminants traversing the inlet grill and entering the work area. Excessive vertical airflow resulted in air possibly laden with microorganisms escaping the confines of the cabinet. These data were corroborated by McDade et al. (128) with microbiological testing. These investigators also confirmed that the modifications were effective because the cabinet provided personnel, product, and environmental protection. Vertical airflow cabinets meeting these criteria were designated class II biological safety cabinets.

First (59) has summarized the early vertical airflow cabinets, describing fallacies, construction problems, airflow difficulties, and capabilities. The characteristics of vertical airflow cabinets have been described by Barkley (14), who thoroughly elucidated changes in performance capabilities, development of face velocities, vertical airflow, height of the front opening, percentage of air recirculation, and microbiological containment.

The principle purpose of the class II safety cabinet is to protect personnel, the environment, and the experiment. After the adoption of National Sanitation Foundation (NSF) standard number 49 (144), laminar flow biological cabinets designated class II, type 1 (141) and class II, type 2 (135) became class II, type A and class II, type B, respectively.

Figure 5 shows the major components of a typical class II, type A laminar flow biological safety cabinet. Air from the laboratory enters the cabinet at the front opening and, with vertical airflow, passes through the front air intake grill. The blower fan forces the air through the airflow plenum to the upper air plenum, where a certain percentage of air exits through the exhaust HEPA filter. Air is forced through the supply HEPA filter and enters the work area as clean filtered air. The clean air descends and, at the approximate center of the work surface, splits and passes through the air grills into the motor plenum, half through the front air intake grill and the other half through the rear air exhaust grill. Infectious particulates are prevented from escaping into the laboratory environment by the protective air curtain across the front opening (15) and the exhaust HEPA filter. Airflow is critical; too much positive pressure causes infectious particulates to escape into the laboratory environment through the work access opening. Conversely, too much negative pressure causes an inflow of room air over the air inlet grill into the work area that in all probability will contaminate the cabinet's work environment (122). Today, many class II cabinets

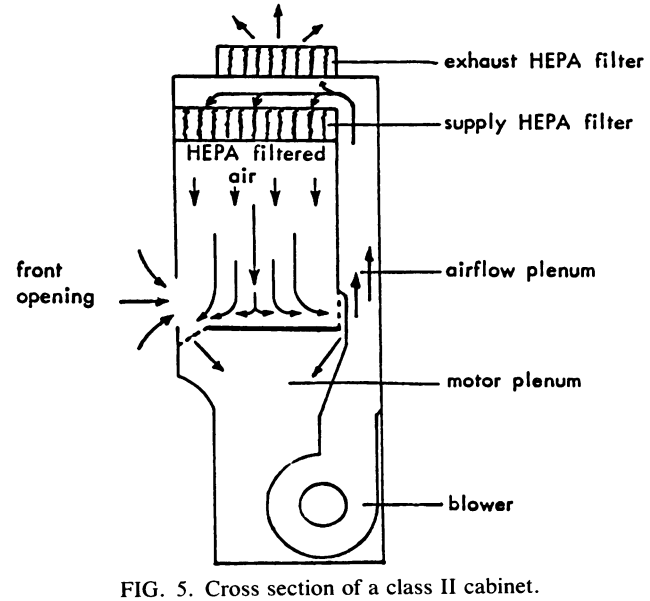


FIG. 5. Cross section of a class II cabinet.

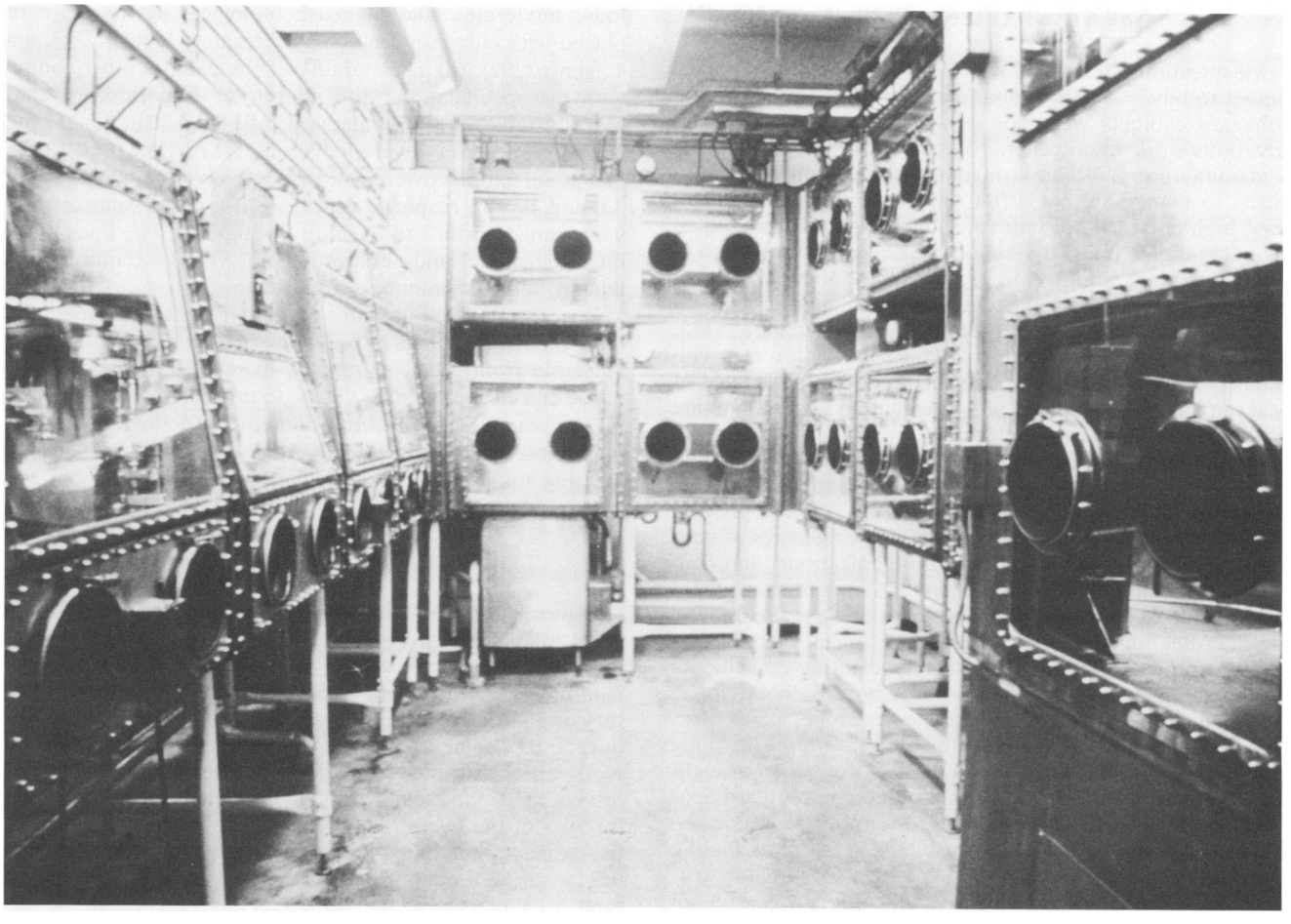


FIG. 6. Class III modular safety cabinets.

are bench-top models. The blower fan is located above the supply HEPA filter. The principle is the same, but air is drawn into the blower fan and all plenums are under negative pressure.

CLASS III SAFETY CABINETS

The class III cabinet (Fig. 6) is a closed-front, stainless steel, self-contained ventilated cabinet that is operated at a negative pressure (0.75 to 2 in. [ca. 1.9 to 5 cm] water gauge) in relation to the laboratory environment and that provides absolute containment of infectious materials. Arm-length rubber gloves are attached, and air enters the cabinet through HEPA filters and is exhausted either through two HEPA filters installed in series or through one HEPA filter and an incinerator to the outside environment. The entire system, including welds, gaskets, and duct lines, is tested by pressurizing (3 in. [ca. 7.6 cm] water gauge) with an air and halide gas mixture. No leaks greater than 10^{-6} ml/s are permitted (156). Work inside the class III cabinet is performed through arm-length rubber gloves, and clean equipment and supplies enter the class III cabinet through dunk tanks filled with a disinfectant, a double-door autoclave, and/or double-door air locks. Outgoing material is decontaminated in double-door autoclaves before removal from the cabinet line. All types of manipulations are possible in the class III cabinet because refrigerators, incubators, deep freezers, centrifuges, water baths, microscopes, and animal handling systems can be installed within the system; however, cross contamination between products is possible.

It is cumbersome and difficult to work in a class III system. There are a number of inconveniences in manipulating equipment, pipetting, and inoculating animals, etc., but the protection and sense of security afforded to the operator make the cabinet very useful. Furthermore, with proper training, most workers rapidly develop the facility to perform even the most delicate procedures through the attached rubber gloves. This equipment is very specialized. There are few class III systems in the world because the initial and maintenance costs are very high.

Research with highly infectious microorganisms, such as Machupo, Lassa, Marburg, and hemorrhagic fever viruses, and high-risk DNA research material (biosafety level 4 agents) should be performed in class III cabinets (111, 207). The alternative is the ventilated suit room. Chatigny described this as "a method wherein the operators are packaged, rather than the work or the work materials" (33). Class II cabinets are used. The operator wears a ventilated suit that is supplied with clean air maintaining a slightly positive pressure. The ventilated suit room is a biosafety level 4 facility (156).

NSF STANDARD NUMBER 49

One question often asked is, "How do I know what safety cabinet to buy?" Years ago, and all too often, safe, reliable biohazard cabinets were not available to users engaged in hazardous work when general specifications were submitted to manufacturers. When stringent specifications were submitted to manufacturers, often the responses were as follows: high cost, cannot innovate, too expensive for a small business, and no bid. Lowering the specifications resulted in users receiving an inferior product that often created a greater hazard for workers. Universities, industries, hospitals, and laboratories substituted federal specifications (135, 141) to obtain quality equipment. This substitution met with some form of nonadoption from manufacturers because of legal ramifications and lack of agreement and understanding between users and manufacturers.

The NSF, an independent, nonprofit organization devoted to research, education, and service and dedicated to the improvement of health, was contacted by NIH to provide liaison services among industry, regulatory agencies of government, professional and technical organizations, and consumer representatives in developing a standard that would be agreeable to users and manufacturers of class II cabinets. The first meeting composed of NSF personnel, manufacturers, government representatives, and scientific consultants convened on 23 January 1973 in Ann Arbor, Mich. After the general meeting, in which the scope and other particulars were discussed, the participants divided into three working task groups to formulate a rough draft. After seven or eight meetings in Ann Arbor, depending on committee participation, and five rough drafts, standard number 49 was completed and adopted on 11 June 1976. The participating manufacturers submitted class II cabinets to NSF for testing, evaluation of performance, and compliance with all facets of the standard, and in February 1978, NSF published a list of manufacturers with cabinet models that met standard number 49.

On 20 November 1978, the committee reconvened in Ann Arbor to revise standard number 49. The changes included the following. (i) The nomenclature of cabinets classified as venting or nonventing was changed; nonventing cabinets were redesignated class II, type A, and class II, type B cabinets were divided into class II, type B1, B2, and B3 cabinets, depending on the volume of air recirculated and exhausted (B1), total exhaust with no recirculation of air (B2), and venting added to class II, type A cabinets with increased face velocity (B3). (ii) UV lighting was omitted. (iii) Noise levels were raised from 65 to 67 dB. (iv) Light intensity readings ranging from 90 to 120 ft-c (1 ft-c = 10.76 lx) were changed to 80 to 150 ft-c. (v) For microbiological tests, the universal standard all-glass impinger (AGI-30) (17) was adopted; slit-type air samplers (224), located outside the class II cabinet to simulate the breathing zone of a person, were replaced by two AGI-30 samplers in the personnel protection test; and an additional cross contamination test was added so that tests could be performed on both sides of the cabinet. (iv) Times at which certification and recertification should be performed were stated. (viii) The face velocity in type B1 cabinets may be measured at the work access opening. (ix) A Smoke patterns test was added. The revision was adopted in May 1983.

NSF policy is to update all standards to the present-day state of the art. On 18 January 1989, the committee convened in Ann Arbor to once again revise standard number 49. The proposed changes included the following. (i) Definitions for low-, moderate-, and high-risk biological agents were replaced with current terminology: biosafety level 1, 2, 3, and 4 agents (207). (ii) The stability of the cabinet must comply with the specifications of Underwriter Laboratories, Inc., Northbrook, Ill. (iii) Cabinet sizes of 24 and 30 in. (ca. 61 and 76 cm, respectively) were included. (iv) Airflow grids for 24- and 30-in. cabinets were set, respectively, at 3 and 4 in. (ca. 7.6 and 10 cm, respectively). (v) Slit-type air sampler flow was changed from 1 to 2 cfm to 1 ± 0.5 cfm. (vi) Face plates must list model and serial numbers, voltage requirements, and air velocity nominal set points.

Periodically, NSF updates the list of class II cabinets that meet the requirements of NSF standard number 49. This list, available from NSF, should be examined before the purchase of a class II cabinet. Thus, to protect oneself as well as fellow employees, the environment, and the experiment, only class II safety cabinets that are listed by NSF as meeting standard number 49 should be purchased.

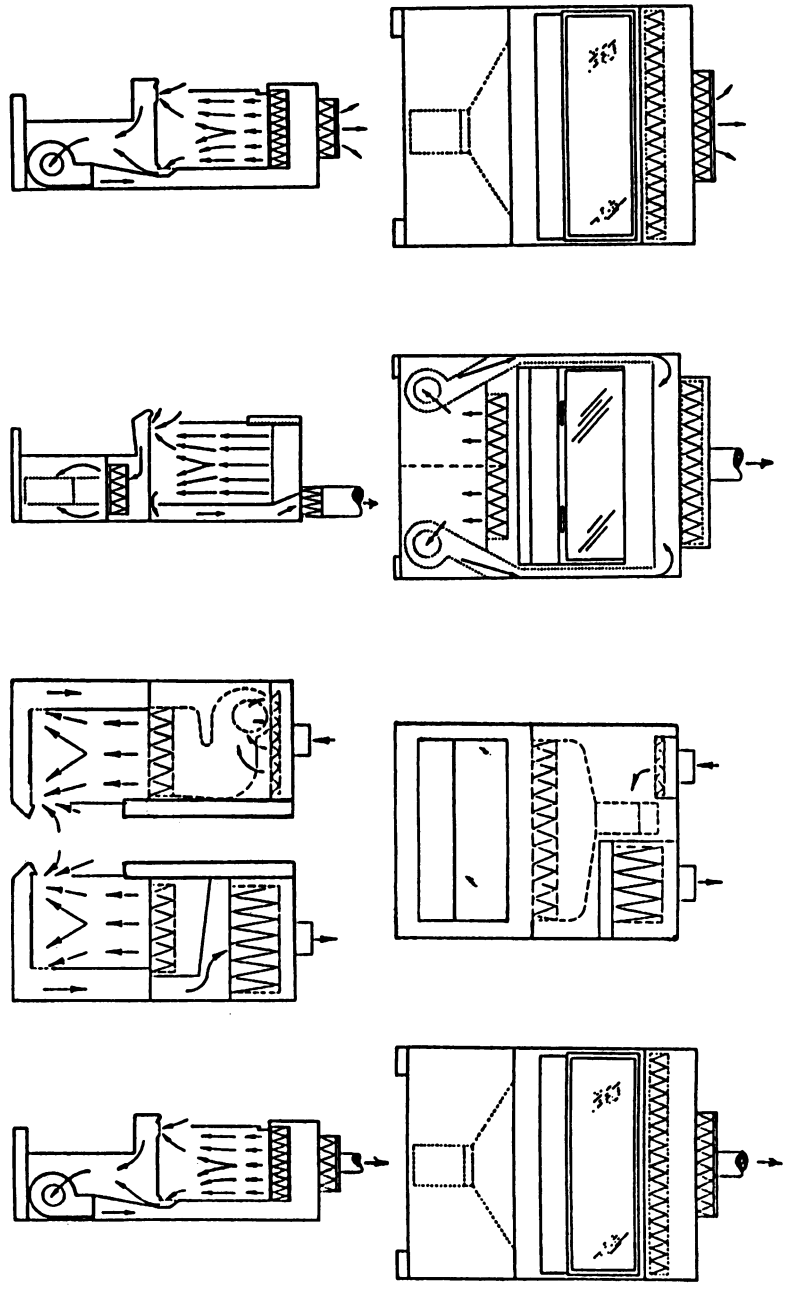
TYPES OF CLASS II SAFETY CABINETS

Figure 7 is a modification of a line drawing of Kent and Kubica (100) illustrating the differences among class II biological safety cabinets. Type B1 replaces the old classification type B. In each cabinet, air entering from the laboratory or pharmacy passes through the front air intake grill, but in type B1 cabinets, the HEPA filters are located below the work area. Air passes through the HEPA filters before traversing to the blower, airflow plenum, and upper plenum for 30% of the air to recirculate and 70% to egress through the exhaust filter. The blower, motor, fan, and plenums past the fans do not contain viable particulates. Face velocity in type A cabinets is 75 fpm, whereas in type B cabinets, face velocity is 100 fpm. This type of construction ensures that all ducts and plenums are under negative pressure. This cabinet may be used for biological agents treated with "minute quantities" of toxic chemicals and "trace amounts" of radionuclides (145).

Many changes have occurred in the manufacture of class II cabinets to provide greater protection. In older models of type B1, a polyurethane diffuser was used to maintain the distribution of the HEPA-filtered air into the work area. Federal standard 209b requires that an area with 100 or fewer 0.5-μm particles per ft^3, as determined with a particle counter, be termed "class 100" (63), but this level was difficult to obtain with the release of oxidized particles from the diffuser. Manufacturers are now fabricating a new type B1 cabinet in which the polyurethane diffuser is replaced with a metal diffuser and three HEPA filters are used.

Type B2 is a total exhaust cabinet. A bench-top type B2 cabinet is shown in Fig. 7. Air enters at the top of the cabinet and passes through a prefilter before it passes through the HEPA filter into the work area. Filtered air makes only one traverse through the cabinet and is exhausted through the exhaust HEPA filter. There is no recirculation, and this cabinet may be used for biological agents treated with toxic chemicals and radionuclides.

Type B3 is essentially a type A cabinet. Downward velocity is approximately 75 fpm of the air recirculated, but air is exhausted through the HEPA filter into a duct. The face velocity has been raised from 75 to 100 fpm. Type B3 cabinets require a separate external blower to remove the exhaust air.



Class	II	II	II	II
Type	A	B1	B2	B3
Inflow (fpm)	75	100	100	100
Downflow (fpm)	75	50	50	75
Exhaust (cfm)	45	65	65	65
% Recirculation	70	30	0	70
Use: Biohazards	+	+	+	+
Toxics, Volatiles	-	±	+	±
Protection User, Product	U/P	U/P	U/P	U/P

FIG. 7. Variations in class II cabinets. U, User; P, product.

INSTALLATION OF SAFETY CABINETS

The ideal location for a safety cabinet is a remote area such as the back wall of the laboratory well away from personnel traffic, doors, and air currents produced by heating and air conditioning vents, with due consideration for safe evacuation in the event of a fire or explosion. Rapid movements of the body as well as rapid opening and closing of laboratory doors create air currents that disrupt critical airflows at the access opening of the safety cabinet and allow test microorganisms to escape from the safety cabinet into the laboratory (38, 112, 128). Rake (168) demonstrated that cross drafts cause an outflow of organisms from the cabinet. She created controllable cross drafts while aerosolizing test microorganisms in the cabinet and determined that the loss of microorganisms was proportional to the velocity of airflow across the front of the safety cabinet.

Class II, Type A Biological Safety Cabinets

This safety cabinet does not require venting to the outside environment, as it was designed to discharge air through a HEPA filter directly into the laboratory. It is important that a minimum of 8 in. (ca. 20 cm) of clearance is maintained between the top of the cabinet and the ceiling to avoid interference with the exhaust flow (197). In addition, a filter guard should be installed at a slant over the exhaust HEPA filter to prevent storage of material on top of the cabinet that may block the exhaust HEPA filter and affect airflow adversely. The cabinet must be certified to confirm that the exhaust HEPA filter and its mounting frame seals are free of leaks that would permit the passage of microorganisms and contamination of the laboratory environment.

Class II, type A cabinets may also be vented into a common exhaust plenum through solid or thimble connections. A solid connection joins the exhaust duct directly to the cabinet and exhausts only the volume of air passing through the exhaust HEPA filter. A thimble connection leaves an air space between the cabinet and exhaust duct that draws in supplemental air from the laboratory to prevent a remote exhaust fan from influencing exhaust flow volume adversely. Building codes in the United States no longer permit cabinets located against an outer wall to be ducted directly to the outside through the side of the building. Now the exhaust duct must run to the roof to exhaust effluent air.

First (59) and NSF (145) state that when a cabinet is vented through a common exhaust plenum, erratic exhaust flow rates may result when other hoods on the same system go on and off, affecting the face velocity of the cabinet. Therefore, a canopy connection (every manufacturer has such a transition piece) is recommended when a class II, type A cabinet is connected to a multicabinet exhaust system. Examples of the canopy and thimble connections are shown in Fig. 8. The air gap should be tested with smoke to verify that internal air turbulence does not create outward air leakage at points around the perimeter. All duct systems receiving exhaust air from class II, type A cabinets should use an auxiliary fan located on the roof to maintain the entire duct system under negative pressure and not introduce additional resistance to the exhaust side of the cabinet, which may affect inflow velocity adversely. The auxiliary fan compensates for the added resistance of the exhaust duct. The auxiliary fan should be interlocked with the cabinet's fan, and an alarm should be located on or by the cabinet to signal whenever both motors fail to operate in unison. If the cabinet is connected to the combined building heating, ventilation, and air conditioning exhaust systems (not recommended), air must not be recirculated. In addition, a modulating damper should be installed in the duct to control room air loss and maintain constant pressure in the duct.

The main advantage of using hard ducting (i.e., the absence of an air gap between the cabinet and exhaust duct) is that it conserves energy by reducing the loss of conditioned air from the laboratory. When hard ducting is used, a damper should be installed in the exhaust duct close to the cabinet to control exhaust static pressure applied to the cabinet and to serve as a seal when the cabinet is decontaminated with formaldehyde. Static pressure should be measured directly above the exhaust filter of the cabinet, and negative static pressure should be adjusted with the damper to equal the airflow resistance of the duct system from the cabinet to the roof fan. For all ducting arrangements, it must be remembered that at some point a clogged exhaust HEPA filter must be removed and replaced with a new filter. Therefore, a section of the duct system must be removable. When a HEPA filter must be removed from the top of the cabinet, it is not advisable to use hard-ducted connections. Figure 9 shows methods that may be used to exhaust effluent air from a safety cabinet connected to an exhaust system by hard ducting.

Class II, Type B1, B2, and B3 Biological Safety Cabinets

Table 4 was compiled from data furnished by manufacturers of NSF-listed safety cabinets and shows the airflow rates and static pressures required to exhaust each model in the certified operating mode. If the listed values cannot be achieved in a field-installed cabinet, one should contact the manufacturer for instructions.

First (59) and NSF (145) state that type B cabinets should be hard ducted to a dedicated external exhaust that discharges outside the building at a height and location that permit no recirculation. Air should not be discharged near the roof, where turbulence and downdrafts may cause the effluent air to enter the building air inlets. A damper should be installed in the duct close to the cabinets to permit airflow adjustment and for use during formaldehyde decontamination. Backflow dampers should never be used in the duct because, all too frequently, they fail to function because of corrosion, erosion, and lack of maintenance.

Type B cabinets with an internal exhaust fan still require the installation of an external exhaust fan on the roof of the building, and the two should be interlocked with an alarm located on or by the cabinet to indicate a loss of exhaust airflow. The external roof blower should be sized to handle the required airflow, taking into consideration the pressure losses in the duct system from the cabinet to the roof fan.

Discharging the effluent air from the safety cabinet to the atmosphere above the roof and above adjacent buildings and terrain features is very important, because pathogenic microorganisms that may escape through a leaking HEPA filter will be diluted, dried, and subjected to lethal UV irradiation. Laboratory-associated infections due to improper discharge of exhaust air from laboratories have been reported (87). One method for determining satisfactory air discharge height and location is to place a smoke bomb in the exhaust duct downstream of all air filters and observe the dispersion of smoke in the atmosphere under many different wind directions and turbulence patterns. Figure 10 shows a recommended method for discharging effluent air safely. No rain cap or other obstruction should be placed over the top of the discharge stack, as such an obstruction forces effluent air

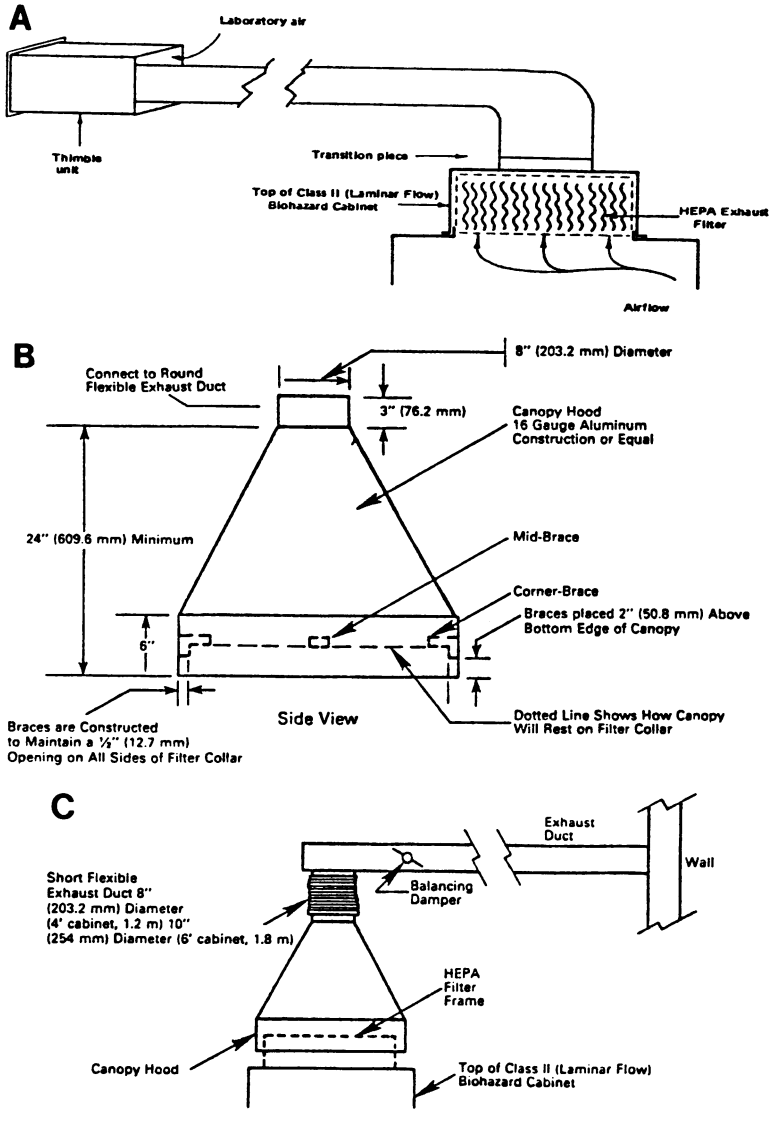


FIG. 8. Venting safety cabinets by thimble (A) (144) or venting canopy (B) (145) connection. (C) Suggested way of connecting to existing exhaust duct (145). Reprinted with permission of the publisher.

downward toward air intakes and the ground instead of dispersing and diluting it rapidly in the atmosphere.

CERTIFICATION OF CLASS II (LAMINAR FLOW) BIOLOGICAL SAFETY CABINETS

Some of the most important questions often asked are, "How do I know my cabinet is working properly?" and "How long will the HEPA filters last?" Before a class II cabinet is sold, the manufacturer performs numerous tests to verify that design specifications and standards have been met. One problem still remains: the safety cabinet must be shipped to the user. The user who believes that he or she is working with a safety cabinet that meets design specifications soon may have contaminated experiments or become exposed to hazardous substances. The manufacturer's warranty does not always ensure that a class II cabinet is operating properly.

Why, when, and how often should a class II cabinet be certified? Baldwin and Errico (11) performed in-place testing of HEPA filters in class II cabinets. They found that the failure rate in initial certification was due to faulty filter installation and that subsequent failures were caused by carelessness, such as the use of punctured or burned HEPA filters. However, these data were obtained from 1972 to 1974, when many of the 19 manufacturing companies were fabricating safety cabinets for the first time. Their inexperience often resulted in poor workmanship and shipping practices.

As the number of manufacturers fabricating class II cabinets decreased, improved shipping procedures, stricter quality control (especially in welding and removing of jagged metal edges, silicone grease, and sealant), implementation of government specifications, and new manufacturing techniques reduced the number of cabinets failing initial certification from 61 to 27% in 2 years. In 1980, only 2% of NSF-listed cabinets failed initial certification, illustrating the influence that NSF standard number 49 had on manufacturing workmanship and stringent quality control.

Every NSF-listed class II cabinet is subjected to a battery of tests by the manufacturers: cabinet leak, HEPA filter

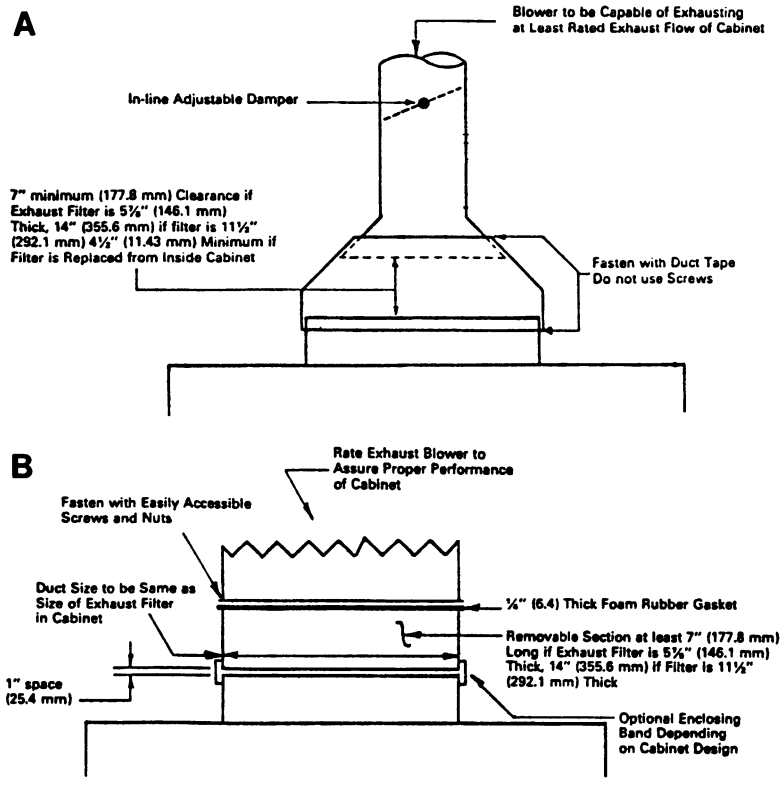


FIG. 9. Suggested methods of hard ducting a venting system with methods to accommodate filter testing and removal (145). (A) Double canopy. (B) Alternate method without transition piece. Reprinted with permission of the publisher.

leak, airflow smoke patterns, velocity profile (downflow), work access opening (face velocity), and lighting intensity. Most of the manufacturers test for electrical leakage and ground circuit resistance. Every 10th cabinet of a particular model is subjected to additional tests: noise level, vibration, drain trough spillage, and temperature increase. In their operation and instruction manuals, manufacturers should include the results of the tests performed with each NSF-listed cabinet. Every manufacturer states that these tests were performed before shipment and advises that the cabinet be certified on site after installation.

Revised NSF standard number 49 states that class II biological safety cabinets should be certified prior to use, at least annually, when HEPA filters are changed, when maintenance repairs are necessary, and when the cabinets are relocated. In addition, "More frequent recertification may be considered for particularly hazardous or critical applications, or workloads" (145). All federal standards and guidelines state that there should be an initial certification, an annual certification thereafter, and a certification whenever cabinets are relocated or filters are replaced (55, 137, 142) and that class II cabinets are for work with biosafety level 2 and 3 agents (207). Furthermore, many times it is the certifier who determines more frequent certification without knowing what work is being performed and without consulting with the principle investigator.

At this time, the reader must be informed that there is no license or examination for certifiers of safety cabinets. One-week courses are offered (80, 94) and include history, theory, airflow and filtration principles, and practical on-site testing of class II biological safety cabinets. These courses serve as a good beginning, but there are limitations, because over 80 cabinets are listed by NSF. Workshops (48) have been held, manufacturers (e.g., The Baker Co., Inc., and NuAire Inc.) have classes, and slide-cassette recordings (138) are available, all for instructing personnel in class II certification methods.

Certifying the Certifiers

On 31 January 1984, in Atlanta, Ga., personnel from government, manufacturers, academia, and certifiers met and discussed the ways and means of "certifying the certifiers." A committee was formed, and numerous meetings were convened in an attempt to come to an agreeable and amicable treatise. The personnel involved became a standing committee of American Biological Safety Association in October 1985, but after numerous meetings in 2 years, the final outcome was a failure to produce a method to certify the certifiers and was so reported to the membership of American Biological Safety Association in October 1987.

On 16 October 1989, in New Orleans, La., an ad hoc committee was formed to explore various organizations and methods for certifying the certifiers. Because of the impact that NSF standard number 49 had on biological safety cabinets, NSF was the logical choice.

In April 1990, the Committee for NSF Accreditation of Biological Safety Cabinet Certifiers met. NSF has many years of experience in certification programs, as field inspections and certifications, ranging from those of food service equipment, e.g., dishwashers and refrigerators, to wastewater treatment devices, have been an integral part of NSF since its inception. Decisions on the type and level of certifiers were made. There will be one level of certifier, and

individuals, not companies, will be accredited. Proposed personnel qualifications are as follows. (i) The certifier must have a high school diploma or certificate equivalent to a high school diploma. (ii) The certifier must complete a training course in biosafety cabinets (Harvard University or Johns Hopkins University) and have 1 year of experience in actively certifying cabinets. (iii) If a training course has not been completed, a minimum of 3 years of work experience in certifying cabinets is required. (iv) The certifier must pass a written examination based on material in NSF standard number 49 (145) and U.S. Department of Health and Human Services publication number (CDC) 84-8395 (207) and a practical evaluation, involving performing certification of a class II cabinet, given by NSF personnel. (v) After accreditation, the certifier must accrue CEUs or obtain equivalent training credits relevant to biological safety, engineering, or related subjects. A meeting of the accreditation committee in November 1990 resulted in a commitment of NSF to announce by June 1991 an accreditation program for safety cabinet certifiers.

Field Testing

Certification of a class II biological safety cabinet must be done at the permanent location of the cabinet. Using generalized statements, we deem the following to be the most important tests and believe that the user should have basic knowledge of these tests. (For technical details [equipment, procedure, measurement, concentration, and volume, etc.], refer to NSF standard number 49 [145].) (i) The velocity profile measures the airflow that descends through the cabinet's work space from the supply HEPA filter to ensure that the correct velocity is maintained and is uniform, as turbulence is conducive to cross contamination. (ii) The work access opening airflow (face velocity) is calculated to determine the velocity of the supply air through the front access opening. This calculation is done by measuring with a thermoanemometer the air velocity above the exhaust HEPA filter or inside the duct if the cabinet is vented. (iii) The integrity of HEPA filters is ascertained by generating a fine-particulate aerosol of dioctylphthalate or an equivalent fluid and determining the degree of penetration with an aerosol photometer. Filter leaks found in HEPA filters may be repaired by sealing the hole with silicone sealant, but such repairs cannot cover over 5% of the surface of a HEPA filter. (iv) Airflow smoke patterns are determined to ensure that the airflow along the entire perimeter of the work access opening is inward, that the airflow in the work area is downward, with no refluxing or dead spots, and that ambient air does not enter the work area. (v) Lighting intensity, temperature increase, vibration, and noise level do not reflect containment performance, but it is essential to have sufficient lighting within the cabinet to prevent an accident, such as a needlestick.

Microbiological Testing

Microbiological testing in situ became one of the most important safety tests at Fort Detrick. Class I and complex modular class III cabinets, rooms, and aerosol exposure chambers, from the smallest unit to the million-liter test sphere, were microbiologically tested with tracer organisms (*Serratia marcescens*, *Bacillus subtilis* subsp. *niger* spores, and *Escherichia coli* bacteriophages). Microbiological testing of class II safety cabinets (127, 128, 169) is a continuation of the microbiological testing of class I safety cabinets formulated by personnel in the Industrial Health and Safety Division, Fort Detrick, and later refined by Barbeito and Taylor (13) to meet the high level of competence required for personnel and environmental protection. After modifications, the microbiological testing that is used for class II cabinets was formulated by Barkley (14). He used a solid metal cylinder extending from the back of the cabinet to 6 in. (ca. 15 cm) outside the cabinet to simulate an arm in the class II cabinet. In the first revision of standard number 49, the test was modified by placing two AGI-30 samplers outside the cabinet at the level approximating the user's face, and time sequences were modified by reducing the control sampling time and extending the test sampling time (145).

When the advisory committee was formulating NSF standard number 49, it was suggested that dioctylphthalate and/or chemical tests be used. Harper (74) concluded that chemical tests were not as efficient or sensitive in detecting small particles as were microbiological tests. It was the consensus of the advisory committee that the only performance test that ensures that a class II safety cabinet is working properly in protecting personnel, product, and environment is the microbiological test.

A class II safety cabinet listed by NSF indicates that a representative cabinet from a cabinet model series has successfully passed the microbiological challenge test. The airflow velocity and flow parameters for this representative cabinet are recorded by the manufacturer and NSF. Field airflow tests on cabinets from the same model series indicating that these parameters are met ensure the cabinets are working properly.

NSF listing indicates only that a cabinet will function properly when operated as a single unit after installation in accordance with the manufacturer's instructions. This assurance is not applicable for a cabinet that has been modified or installed in a location that does not conform to the manufacturer's installation instructions. When such circumstances exist, it would be prudent to verify performance through microbiological testing. It is recommended that such testing be conducted at the manufacturer's facility so that modifications can be made, if necessary, before the cabinet is shipped to the user. Such an arrangement would not be suitable, however, if the test environment could not be made to simulate the selected use location because of extenuating circumstances.

The Baker Co., Inc., was the first manufacturer to construct a microbiological aerosol test facility for cabinet testing. At least one cabinet from each production run is moved to the test laboratory, where it is subjected to and must pass microbiological tests before it is shipped to the user. The data obtained in such tests (95, 196) have led to the improved performance of class II cabinets. Other manufacturers followed this lead and constructed test facilities in which they now perform routine and special microbiological tests.

The following are three special situations in which microbiological testing at the permanent location of a class II cabinet was considered to be appropriate. (i) A pharmaceutical company required a special class II safety cabinet. Two 6-ft (ca. 183-cm) class II cabinets were connected, the outer frames were removed on the abutting sides, and a connecting metal frame was installed (Fig. 11). The cabinet met or exceeded all field tests of NSF standard number 49 but failed the personnel and product protection microbiological tests. Numerous test spores were recovered outside the cabinet, especially by the samplers positioned around the steel cylinder and simulated facial area. Data from the product

TABLE 4. Volume of air and static pressure required to vent class II cabinets[a]

Manufacturer	Model number	Window opening (in.)	Volume (cfm)	Static pressure (in. water gauge)
The Baker Co., Inc., Sanford, Maine	SG-250	8	170	0.02–0.04
	SG-400	8	268	0.02–0.04
	SG-400	10	335	0.02–0.04
	SG-600	8	408	0.02–0.04
	SG-600	10	510	0.02–0.04
	B40-112	8	260	0.02–0.04
	B60-112	8	390	0.02–0.04
	NCB-B4	8	256	0.9
	NCB-B6	8	374	0.9
	4-TX	8	702	1.4
	6-TX	8	1,148	2.0
	BC-4	8	492	2.1[b]
	BC-6	8	840	2.1[b]
Becton Dickinson Microbiology Systems, Cockeysville, Md.	60631	8	240	0.2–0.75
	60633	8	384	0.2–0.75
	10278	8	288	0.2–0.75
	10279	8	440	0.2–0.75
Bellco Glass Inc., Vineland, N.J.	8001-74000	8	183–207	0.5
	8001-74000	10	233–260	0.5
	8001-76000	8	306–343	0.45
	8001-76000	10	406–454	0.45
	8011-74000	8	183–207	0.5
	8011-74000	10	233–260	0.5
	8011-76000	8	306–343	0.45
	8011-76000	10	406–454	0.45
Canadian Cabinets Co. Ltd., Nepean, Ontario, Canada	BM4-2A-49	8	183–297	0.5
	BM4-2A-49	10	233–260	0.5
	BM4-2B-49	8	244–262	0.5
	BM6-2A-49	8	306–343	0.45
	BM6-2A-49	10	406–454	0.45
	BM6-2B-49	8	407–444	0.45
ENVIRCO, Inc., Albuquerque, N.M.	10274	8	240	0.2–0.75
	10276	8	384	0.2–0.75
	10448	8	288	0.2–0.75
	10449	8	440	0.2–0.75
Forma Scientific, Inc., Marietta, Ohio	1000	10	295–340	0.25–0.5
	1102	10	405–430	0.25–0.5
	1104	10	420–470	0.25–0.5
	1106	10	295–340	0.25–0.5
	1108	10	335–375	0.25–0.5
	1110	10	420–470	0.25–0.5
	1112	10	260–285	0.25–0.5
	1118	8	155–170	0.25–0.5
	1122	10	260–280	0.25–0.5
	1124	10	220–245	0.25–0.5
	1126	10	345–380	0.25–0.5
	1128	10	270–300	0.25–0.5
	1132	10	380–425	0.25–0.5
	1136	10	350–400	0.25–0.5
	1148	8	800–950	1.50–2.0
	1162	8	240–270	1.50–2.0
	1166	8	360–390	1.00–2.0
	1168[c]	8	1,075–1,250	1.50–2.0
The Germfree Laboratories Inc., Miami Fla.[d]	BF-4	8	211	
	BF-6	8	344	
	BKF-4	10	306	
	BKF-6	10	473	
	TE-4	8	700	
	TE-6	8	1,050	
Hitachi Ltd., Tokyo, Japan	SCV-1300 EC	8	320–345	0.5–1.2

Continued on following page

TABLE 4—*Continued*

Manufacturer	Model number	Window opening (in.)	Volume (cfm)	Static pressure (in. water gauge)
ICN Biomedicals, Inc., Huntsville, Ala.	BSA 4A/B3	8	260	0.2–0.9
LABCONCO Corp., Kansas City, Mo.	36204	10	202	NA
	36205	8	211	NA
	36208	10	265	NA
	36209	8	281	NA
	36212	10	402	NA
	36213	8	421	NA
	36210	8	770 ± 30	1.2–3.2
	36214	8	1,165 ± 45	1.2–3.2
NuAire Inc., Plymouth, Minn.	407-400	10	319	0.05–0.1
	407-600	10	489	0.50–0.1
	408-300	8	191	0.05–0.1
	408-400	10	319	0.05–0.1
	408-600	10	489	0.05–0.1
	410-400	8	240	0.50–1.0
	410-600	8	350	0.50–1.0
	415-400	8	648	1.50–2.2
	415-600	8	946	1.50–2.2
	420-400	8	240	0.50–1.0
	420-600	8	350	0.50-1.0
	425-200	8	136	0.05–0.1
	425-300	8	201	0.05–0.1
	425-400	10	336	0.05–0.1
	425-600	10	502	0.05–0.1
	427-400	8	244	0.50–1.0
	427-600	8	355	0.50–1.0
	430-400	8	609	1.50–2.2
	430-400	10	674	1.50–2.2
	430-600	8	896	1.50–2.2
	430-600	10	990	1.50–2.2

[a] One inch equals 2.54 cm. NA, Not applicable.
[b] Requires exhaust HEPA filter.
[c] Formerly model number 1156.
[d] Manufacturer will furnish data on static pressure.

protection tests demonstrated there was a small zone of increased airflow near the inlet grill that literally bounced the test spores out of the safety cabinet. The metal bar that joined the two class II cabinets was removed, and a new, modified junction was installed, after which the cabinet passed all of the succeeding microbiological tests. (ii) At a multistory laboratory, the safety cabinets specified by the laboratory director were not purchased by the contractor, even though all air requirements had been calculated for the particular model. The cabinets were installed and inspected. Face velocities were elevated, with average ranges from 102 to 118 fpm. Airflow smoke patterns appeared to be satisfactory. Because no microbiological tests had been performed, as the cabinets were not NSF listed, and because, at the time, the manufacturer did not have the facilities to microbiologically test cabinets, the laboratory director requested that the cabinets be tested microbiologically because of the hazardous work that would be performed in the laboratory. Four of 11 cabinets failed the product protection test, because numerous colonies of test bacteria were recovered on the agar plates covering the work trays. The face velocities of these four cabinets were decreased, and airflow smoke patterns appeared to be satisfactory, but again two cabinets failed the product protection test. Closer examination revealed that the work trays were slightly warped, with bent edges, and new trays were ordered and received from the manufacturer. The remaining two cabinets successfully

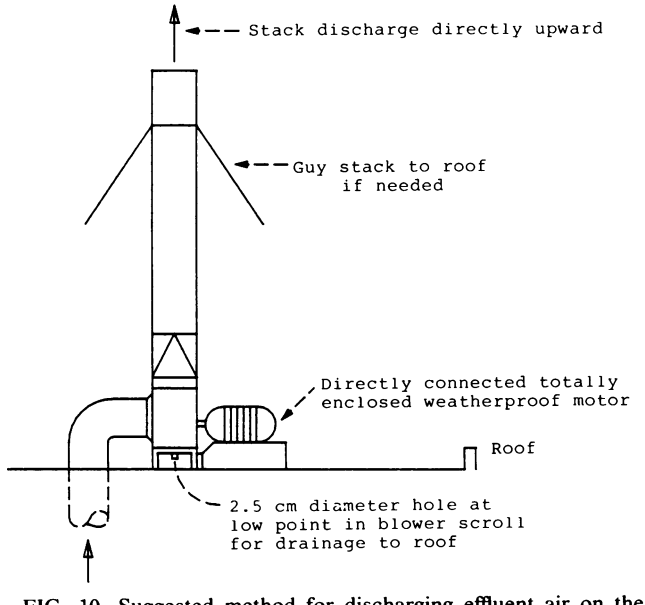


FIG. 10. Suggested method for discharging effluent air on the roof from a biological safety cabinet (59). Reprinted from A. C. Stern, *Air Pollution*, 3rd ed., vol. 4, Academic Press, Inc., Orlando, Fla., with permission of the publisher.

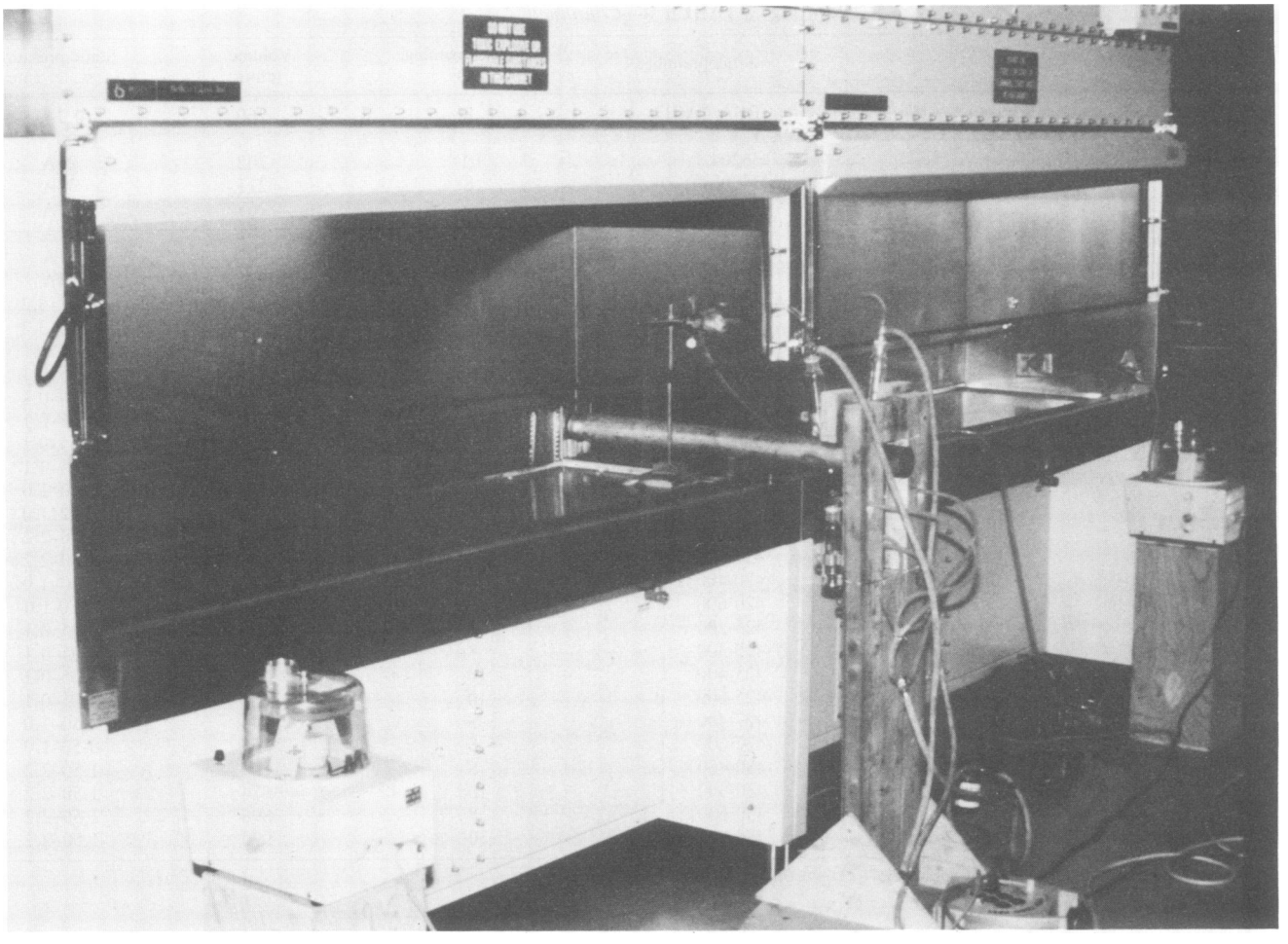


FIG. 11. Personnel protection microbiological test performed on a specialized class II cabinet.

passed all microbiological tests. The airflow smoke patterns may have been misleading because smoke is difficult to observe, especially in a rapid airflow. The microbiological tests prevented the use of cabinets with defective work trays that created an air disturbance and could have contaminated all work performed in the cabinets. (iii) In 1978, a laboratory in which specimens were prepared and cultured daily for the presence of *M. tuberculosis* was remodeled. Old wooden cabinets were replaced with two class II safety cabinets installed perpendicular to each other because of space limitations (Fig. 12). A stainless steel pass-through box connected the 6-ft and 4-ft (ca. 122-cm) cabinets so that prepared concentrated material could be passed to a person who in turn would inoculate the selective media. This pass-through box eliminated the need to remove infectious specimens from the 6-ft cabinet for transfer to the 4-ft cabinet and the possibility of dropping an infectious culture on the floor. Face velocity in the 6-ft cabinet was set higher than that in the 4-ft cabinet to ensure that aerosolized microorganisms would migrate toward the 6-ft cabinet and the 4-ft cabinet would remain relatively free of contamination. Many personnel and product protection tests were performed, because the number of test spores exceeded the allowable number. Eventually the range of high and low face velocities was established for each cabinet. Each year, the face velocities were set to fall within the established range. When the HEPA filters were changed or when face velocities fell to the low end of the established range, the cabinets were microbiologically tested. No tuberculin conversions have occurred since these procedures were instituted.

These three examples illustrate the importance of microbiological testing. Representative models of all NSF-listed cabinets must pass microbiological tests to ensure personnel, product, and environmental protection. Although microbiological testing is expensive and time-consuming when performed by manufacturers, NSF, or in the field, it is necessary to ascertain microbial containment and the fulfillment of the cabinet's purpose—protection.

Improper Certifications

Certification of a biological safety cabinet is essential, for without a proper working equipment, safety cannot be assured. Many large companies purchase cabinets from only one manufacturer and send personnel for training to learn proper certification methods. In 1985, Kruse and Kruse (108) presented 20 examples of improper certifications that they observed in their 13 years of experience. Five are presented to illustrate how improper certification or lack of knowledge of various cabinets can result in serious problems.

(i) An inspection label was affixed to a pharmacy clean bench stating that the cabinet was certified to meet federal

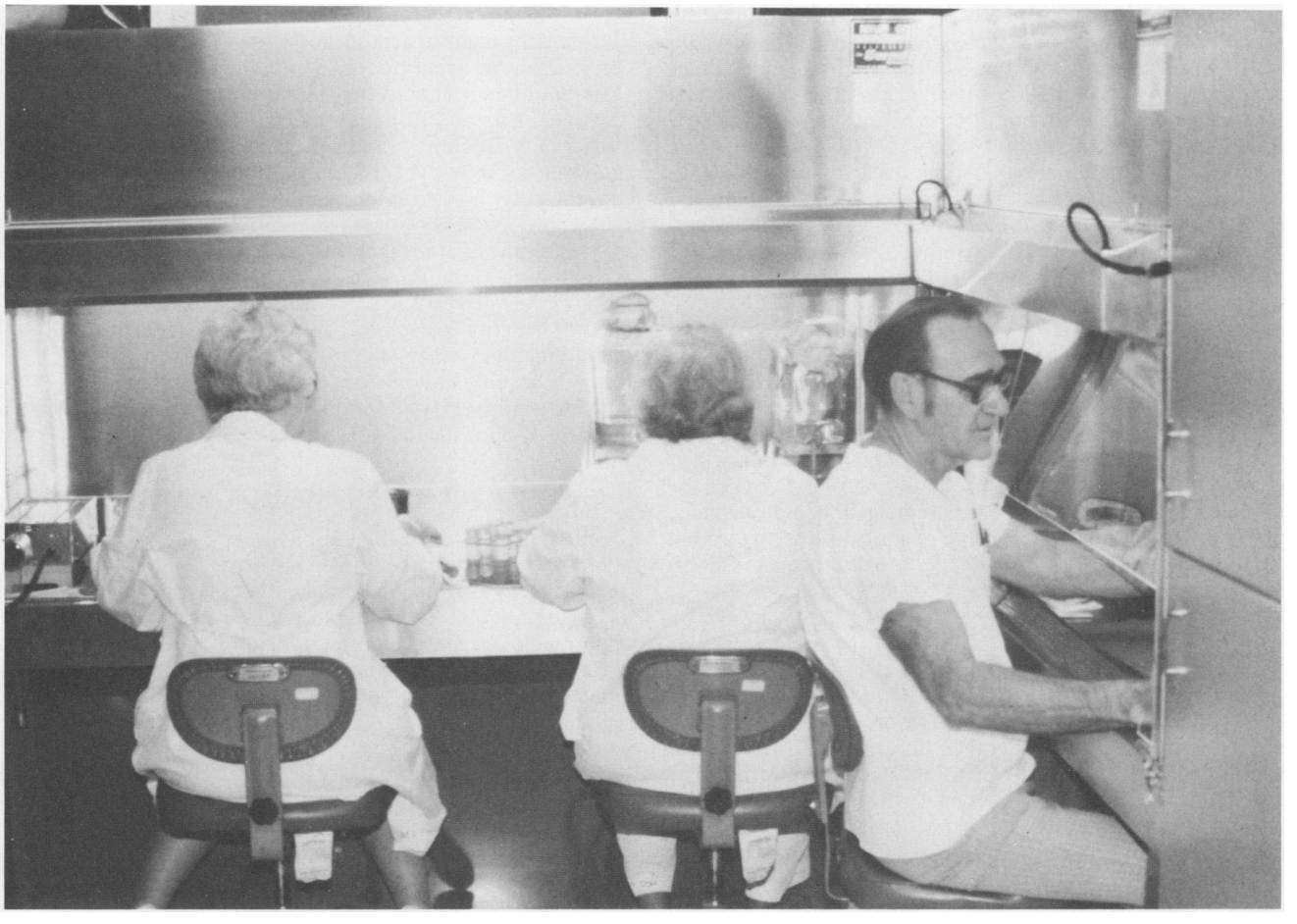


FIG. 12. Specialized class II cabinetry. Two cabinets are connected by a transfer cabinet to contain infectious microorganisms.

standard 209b (63), NIH-03-112c (141), National Cancer Institute general-purpose clean-air biological safety cabinet (135), and NSF standard number 49 (144, 145) specifications. The pharmacists did not know that a horizontal-airflow clean bench cannot meet NIH-03-112c, for this specification is for a class II, type A safety cabinet; the National Cancer Institute specification is for a class II, type B1 safety cabinet; and NSF standard number 49 is for class II safety cabinets. All of these are vertical-airflow cabinets.

(ii) Personnel complained of a heat buildup inside their class II, type A safety cabinet when the motor was running. Smoke placed at the work access opening did not enter the cabinet but was blown toward the operator. Examination revealed that the metal plate placed by the manufacturer over the exhaust HEPA filter to protect the filter during transit had never been removed. However, the cabinet had been inspected four times in 2 years and had been certified each time, with an inspection label affixed to the cabinet.

(iii) When a class II, type A cabinet in which mycology and mycobacteriology procedures were performed was inspected, a sprinkler head was discovered in the work area below the metal diffuser. From the sprinkler head, a galvanized iron pipe extended toward the side and penetrated the side wall of the cabinet. A second iron pipe penetrated the side wall of the cabinet in the area in which the motor was located. It was impossible to test the supply HEPA filter because dioctylphthalate filled the entire room and the photometer could not be calibrated. Closer examination revealed that the two pipes passed through two large holes in the side of the cabinet that were closed with metal covers that were aesthetic, not functional, because they did not seal the holes. After the cabinet was sealed and decontaminated, the motor plenum area was opened, and a sprinkler head was found 4 in. (ca. 10 cm) above the motor. Had the motor overheated, it would have set off the sprinkler system. The two pipes and sprinkler heads were removed, and the holes were sealed with silicone and metal plates, but the cabinet could not be pressurized. Because of the age of the cabinet, a new NSF-listed cabinet was purchased.

(iv) In a remodeled laboratory, five new NSF-listed class II, type B3 cabinets were found to have sprinkler heads below the supply HEPA filters with pipes penetrating the side walls. On the contractor's blueprints, every class II cabinet was marked "biological hood." Because state law required every hood to have a sprinkler system, be it in a laboratory or over a kitchen stove, a sprinkler system was installed. The sprinkler heads were removed, steel plates were welded over the holes, and the cabinets were sealed with metal plates and tested with halide gas to ensure tightness before they were certified.

(v) A hospital laboratory had two class II, type B3 cabinets in two rooms located back-to-back but separated by a wall. Ducting from each exhaust HEPA filter traversed upward, past a guillotine-type damper and into the intersti-

tial space, where it connected to an identical set-up from the cabinet in the adjoining room. The scientist who worked with cell cultures in the two cabinets complained about contamination. Examination revealed that the certifier had certified both cabinets but did so while operating each cabinet separately. With both cabinets operating, the face velocities were well below 75 fpm, well below the established specification of 100 fpm. Closer examination revealed that holes had been drilled in each duct below the guillotine-type damper, not above the damper, as specified in NSF standard number 49. Holes were drilled in the ducts in the interstitial space and, by careful manipulation of the dampers with both cabinets operating, face velocities over 100 fpm were obtained for each cabinet. This example illustrates a lack of knowledge of where holes should be drilled in a duct for correct airflow measurements.

In light of such examples of improper test methods and workmanship, the NSF accreditation committee is justified in demanding technical courses plus 1 year of experience for a certifier, although even 1 year may be insufficient, because a certifier should know the principles of airflow, microbial aerosols, and filtration to protect the users of safety cabinets.

Selection of Certifiers

The selection of a person or firm to certify biological safety cabinets is largely a subjective procedure. Currently, there is no mechanism or organization to objectively assess the training and experience of cabinet certifiers or certifier credentials on the basis of demonstrated knowledge or ability. In addition, as Bryan and Marback (19) state, "The proper performance of the laminar-airflow hood is often certified by an outside certifying agency or contractor whose testing procedures and findings must be relied upon almost completely and without question by the pharmacy (laboratory) practitioner." Consequently, it is essential that owners and users of class II safety cabinets have a working knowledge of such equipment and of NSF standard number 49, which details performance standards and certification procedures. Pharmacists also must have knowledge of federal standard 209b if they are using horizontal-airflow clean benches.

Fees for decontamination, filter replacement, and certification of biological safety cabinets may vary widely between certifiers and geographic areas. The lowest fee is not always a bargain, and the highest fee may not ensure that certification will be satisfactorily performed.

The owner or user should be involved from the beginning. Bid requests should state the type of cabinet, model number, and serial number of each cabinet to be certified to conform with NSF standard number 49.

The owner or user of safety cabinets should be involved in the selection of the certifier. Determination of the competence of individual certifiers may include a review of their attendance at technical courses (Harvard University or Johns Hopkins University), government-sponsored courses, and classes given by cabinet manufacturers. Additionally, it is pertinent to ask for references, including the names of biological safety cabinet owners for whom certifications have been performed previously. Prospective certifiers should be asked to describe the step-by-step procedures and equipment used to certify cabinets and to explain the meaning of the information recorded on the certification test report, which should be presented immediately after certification of the cabinet.

Certification is only as good as the test equipment used. Equipment used to test airflows and HEPA filter integrity, etc., must be accurate and in good working condition. All test equipment should be calibrated at least annually to a National Bureau of Standards Traceable Standard to ensure uniformity, accuracy, and serviceability, and a certificate of calibration should be issued stating to what standard the equipment was calibrated.

Cabinet owners or users should read and become familiar with the information included in the operator's manual that the manufacturer provides with each cabinet. Manufacturer's technical service representatives should be contacted for information on cabinet performance, certification, malfunctions, and other operational issues.

Only when a national organization accredits certifiers will owners or users know which certifiers are competent to inspect and certify class II safety cabinets.

SAFETY CABINETS IN PHARMACY AND MEDICINE

Biological safety cabinets have been used extensively in pharmacy and medicine over the past decade for preparing potentially hazardous pharmaceuticals to dispense or administer to patients. Beginning in the early 1960s (170), hospital pharmacies began promoting the admixing of drugs in intravenous solutions as a pharmacy activity (2). Although pharmacies had been involved in this function to a limited degree, nursing personnel traditionally had this responsibility in most patient care settings. Arguments in favor of shifting this responsibility to pharmacists included (i) more expertise; (ii) a better foundation in physical and chemical drug incompatibilities and stabilities; (iii) less waste; and (iv) because of centralization of the activity, the ability to use modern horizontal-airflow clean-air benches to reduce the risk of product microbiological contamination. Before this shift, only the type I fume hood was used in pharmacies for manufacturing and compounding pharmaceuticals with noxious odors or fumes.

The horizontal-airflow cabinet or clean bench was rapidly adapted for use in pharmacies. Intravenous rod tubing on which parenteral agents and diluents could be hung was installed. The airflow provided protection for the product, and the fear of contaminants in the admixtures was greatly decreased. The clean bench appeared to address the pharmacist's concerns, for the product was protected from airborne contamination by the clean air flowing from the HEPA filter. However, improper training and/or techniques created many problems. Often, excess fluid in syringes was expelled into the HEPA filter and material hardened on the filter. Many filters became soiled because of the amount of expelled fluid. Changing prefilters was not part of routine maintenance, and because many of the prefilters were located near the floor, they became loaded with dust and debris, resulting in reduced horizontal airflow.

Even before the advent of hazardous anticancer agents, many pharmacists noticed an increase in allergic reactions and dermatitis in personnel who manipulated drugs at clean-air benches. Personnel known to be allergic to antibiotics such as penicillin developed the same or similar allergic responses when they prepared penicillin solutions. Thus, the protection for the product afforded by clean-air benches did not provide protection for the worker, who was exposed to aerosols and particles of proteinaceous materials.

The earliest documented concern for handling potentially hazardous drugs, in particular, anticancer agents, appeared in 1970 (150). In 1979, increased mutagenic activity was

noted (51) in concentrates of urine taken from nurses working in oncology units, as compared with those of a nonexposed control group. Other reports and studies that further documented the dangers of exposure of nurses to cytotoxic and mutagenic agents subsequently appeared (35, 83, 92, 117, 153, 154, 183, 193, 213). The potentially more concentrated exposure of pharmacy personnel to these agents was addressed by a study, published in 1982 (8), which demonstrated various levels of mutagenic substances (using Ames methodology [5, 151]) in the urine of pharmacy personnel who admixed cytotoxic and genotoxic anticancer drugs at horizontal-airflow clean benches. The levels of these agents rose and fell during the work week. The tests were repeated under the same circumstances for admixing anticancer drugs, but pharmacy personnel wore masks and gloves. Again, levels of mutagens rose and fell during the work week. Control groups of nonexposed individuals had no measurable levels of mutagens. When several members of the test group were retested while working at a class II biological safety cabinet, levels of mutagens were undetectable throughout the work week, clearly illustrating the protection afforded to pharmacy personnel by the class II cabinet.

In March 1981, questionnaires were mailed to the entire U.S. membership of the Oncology Nursing Society; 547 were completed and returned (158). Approximately half (50.5%) of the respondents reported using a hood while preparing drugs in inpatient service. The horizontal-airflow clean bench was noted as the type used most of the time. In outpatient service, only 26% of the personnel reported using a hood, again with no differentiation as to type. In this survey, >30% of persons preparing antineoplastic agents in the outpatient setting reported symptoms such as skin and eye irritations, headaches, dizziness, nausea, nasal sores, and vomiting.

Anderson et al. (6) reported that the pharmacists at the London Hospital took over antineoplastic agent reconstitution services to eliminate potential hazards facing an inexperienced medical staff. D'Arcy (41) concurred with this type of action by pharmacists, because many inexperienced personnel had minor to severe local toxic reactions, allergic reactions, or both.

Specific details of why a horizontal-airflow clean bench should not be used for preparing hazardous agents were described previously (178). A pharmacy technician developed an urticarial rash on his arms and body almost immediately after mixing amsacrine at a horizontal-airflow clean bench. The same reaction occurred even when he wore gloves, mask, and gown. When the pharmacist prepared the drug 2 days later, a vial broke and a portion of the drug spilled on her hands. She immediately washed her hands, but 4 h later she developed nausea and vomiting. In this hospital, there were nine reactions to amsacrine when a horizontal-airflow clean bench was used, but when a class II vertical-airflow biological safety cabinet was used for mixing amsacrine, not one toxic reaction occurred.

The ability of drugs used in the treatment of cancer to be carcinogens themselves is well documented (31, 47, 76, 120, 124, 180, 186, 203). Harrison (78) listed 30 antineoplastic drugs that induced mutagenicity in vitro or carcinogenicity in vivo in animal models. The World Health Organization (89, 90) classified several of the more common anticancer drugs as human carcinogens. Pharmaceutical agents classified as causally associated with cancer in humans included azathioprine, busulfan, chlorambucil, cyclophosphamide, melphalan, and MOPP treatment regimen (combination of procarbazine, nitrogen mustard, vincristine, and prednisone). Agents classified as probably carcinogenic for humans included actinomycin D, cisplatin, dacarbazine, doxorubicin, nitrogen mustard, procarbazine, uracil mustard, and all alkylating agents. Several investigators have shown that drugs can be released into the environment as aerosols through normal manipulations, such as preparing dosages for dispensing or administration to patients, in the workplace (85, 103, 149). The potential for exposure of health care workers to these agents has led to the establishment of government and professional standards and procedures for handling antineoplastic agents (4, 21, 34, 39, 45, 158, 192).

Guidelines published by the U.S. Occupational Safety and Health Administration (155) note "Two essential elements to ensure proper workplace practices: education and training of all staff involved in handling any aspect of cytotoxic drugs; and a biological safety cabinet." The class II biological safety cabinet offers effective personnel protection from airborne particulates and, at the same time, provides product protection. Several reports in the literature discuss the use of class II safety cabinets in the "medication preparation environment" (10, 37, 46). The term medication preparation environment is used because, although many anticancer drugs are prepared by pharmacy personnel in hospitals, most doses are prepared by nursing and technical personnel in physicians' offices, clinics, and home care settings. However, the requirement for personnel safety is the same. The U.S. Occupational Safety and Health Administration recommends (155) the use of class II vertical-flow containment hoods. In 1984, the National Study Commission on Cytotoxic Exposure (146, 147) further recommended a class II, type A biological safety cabinet as a minimum, with outside venting if feasible, or a class II, type B3 biological safety cabinet.

Outside venting of biological safety cabinets in pharmacies has not been a strong recommendation to date because of the belief that drugs are released into the environment only as aerosols or dry particles, which are effectively entrapped in exhaust HEPA filters. Vapors, fumes, gases, and other nonparticulate releases are not of concern now but may be in the future with new agents. For similar reasons, charcoal filters in the HEPA exhaust system do not appear to be of any value with these agents. For new installations, however, it seems prudent to install a class II, type B1, B2, or B3 safety cabinet to cover future eventualities. Some pharmacists now advocate that all horizontal-airflow clean benches used for preparing pharmaceuticals be retired (166). With the still unknown consequences from chronic low-level exposures of personnel to such drugs as antibiotics, steroids, and hormones, it is not prudent to continue working with equipment that "blows the drug into your face."

In a pharmacy, a biological safety cabinet is effective in preventing exposure to potentially hazardous drugs only when used in conjunction with related safety procedures. In addition to learning how to work in a vertical- rather than a horizontal-airflow environment, personnel must (i) wear gloves and barrier garments (114); (ii) practice aseptic techniques that prevent or minimize the release of drugs into the air (40, 223); (iii) work over an absorptive barrier surface drape; and (iv) dispose of hazardous wastes cautiously (62, 77, 209).

All of these recommendations prevent exposure by the use of various barriers. The concepts of protective intervention (167) apply in (i) situations in which direct exposure to hazardous agents is possible or potential and (ii) situations in which direct exposure is known or probable. The former

situations include the normal preparation and administration of drugs. Here, protective intervention includes meticulous aseptic techniques and maintenance of closed drug transfer systems, use of protective gloves and barrier gowns, and use of a biological safety cabinet as a work environment for drug manipulations. Work should be performed over a disposable absorptive barrier drape to trap any released particles or droplets. The use of commercially available disposable chambered devices that snap on to the top of a drug vial has been advocated. These act to equilibrate air pressure differences and/or entrap released aerosol particles caused by these pressure differences in syringe or closed-vial solution transfers. Their need or utility has not been proven, but their use may be prudent when a biological safety cabinet is not available.

The latter situations include instances of drug spillage, broken containers, handling of patient excreta, and disposal and elimination of hazardous wastes. Pharmacists must also consider recent concerns about HBV and HIV transmission. These exposures occur outside the biological safety cabinet environment and require stricter techniques and barriers, such as the use of heavier barrier gowns and gloves or double layers of gloves. Cytotoxic or hazardous drug spill kits that provide items such as absorbents, towels, scoops, disposal bags, heavy-duty gloves and gowns, respiratory masks, and warning signs and labels are available commercially. Spilled drugs need to be contained, removed, and disposed of properly. Remnant quantities should be diluted with copious water and removed by mopping or wet vacuuming. Excreta from patients receiving cytotoxic agents should be handled with the same barrier approach, with heavy latex or polyvinyl chloride gloves to prevent exposure. Durable textile items, such as clothing, which come into contact with these agents should be well laundered by machine if disposables are not used. Patient excreta should be dispatched into the sanitary sewer system. Until better methods are developed, the traditional methods of waste disposal by water dilution and sewer treatment continue to be the most acceptable practices for liquid wastes.

The overall issues of solid waste disposal are still being debated, but segregation, containment, and high-temperature incineration methods are currently preferred (209). Chemical inactivation of cytotoxic drugs is under study, but no simple or safe procedure appears feasible as yet for use in the workplace (146).

A compilation of 19 journal articles with 170 references which reviews and discusses several of the most important subjects related to the safe handling, preparation, and utilization of cytotoxic agents is available (18). The articles in the compilation have practical applications for office, clinic, or hospital pharmacies.

The laminar flow cabinets in pharmacies must be certified. Bryan and Marback (19) described laminar airflow certification in detail, emphasizing precautions for pharmacists. Most certifications of the horizontal-airflow clean bench use federal standard 209b. Airflow should be measured with a thermoanemometer on a 4- to 6-in. (ca. 10- to 15-cm) grid across the entire surface of the HEPA filter, and no measurement should be made closer than 12 in. (ca. 30 cm) from the HEPA filter. Average air velocity should be 90 fpm, with a uniformity of ±20% and no refluxing or turbulence. The prefilter should be checked for cleanliness, because the usual practice of changing prefilters monthly increases the life expectancy of HEPA filters. A particle count should be ascertained by counting the number of particles in the airstream from the HEPA filter from which air cleanliness is derived, e.g., class 100 = <100 0.5-μm particulates per ft^3 and class 1000 = <1,000 0.5-μm particulates per ft^3, etc. Bryan and Marback emphasized that the particle counter monitors only the environment of the containment area and does not replace the photometer when testing for leaks in the HEPA filter. They further described certification of the class II biological safety cabinet with NSF standard number 49.

Recent guidelines (4) recommended recertifying pharmacy vertical-airflow safety cabinets every 6 months, but we believe that annual recertification is sufficient. The historical performance over time of the HEPA filter, air velocity profiles, measurement of filter back pressures, and particulate load in the ambient air should be the deciding factors.

The recently revised American Society of Hospital Pharmacists guidelines (4) (Goal II, Sections 5.f. and 5.g.) describe a weekly washing decontamination procedure for the interior of the safety cabinet with simple cleaning agents. The procedure involves cleaning and raising the work tray in the cabinet and cleaning out the spillage tray. The documented rationale for performing the decontamination procedure is a concern that contaminated dust and other particles that are not entrapped in the exhaust may escape from the cabinet either by airflow disruptions or if the cabinet blower is turned off. We believe that such a decontamination procedure poses an unnecessary exposure risk to untrained personnel and that the biological safety cabinet interior should be cleaned only of obvious spills. More thorough cleaning should be done at the time of certification by personnel following all protective intervention procedures for spill removal. More research on the use of biological safety cabinets for containment of and protection from hazardous drugs is needed.

The degree and significance of these hazards are not resolved, nor is the ultimate effectiveness of protective measures. The effects of exposure of health care workers to hazardous drugs may not become evident for many years. The maintenance of records of personnel potentially (or knowingly) exposed to hazardous agents has been advocated (201), so that a data base can be established for future epidemiological studies.

To date, no studies that have adequately identified acute or chronic effects from handling hazardous agents, with or without protective measures, have been carried out. However, prudence dictates that protective intervention is necessary to prevent needless exposure of personnel handling any known carcinogen. Guidelines and regulations must be clean, simple, and rigid. An editorial (9) entitled "How Real is the Hazard?" was answered by an article entitled, "Can We Risk Finding Out?" (88) Regardless of the controversy, the class II biological safety cabinet is now a cornerstone for personnel and product safety in the preparation of pharmaceutical agents.

REPLACEMENT OF HEPA FILTERS

"How long will a HEPA filter last?" This question has been asked more than any other because replacement of HEPA filters is expensive. In most cases, the cabinet must be decontaminated before the filters are changed, and the cabinet must then be recertified. It is extremely difficult to state the "life" remaining in a filter, for each cabinet operates under different conditions. The number of particles in the immediate environment, how many hours the cabinet is operated, various techniques used by the worker, and outside influences, such as construction, all determine the life of a HEPA filter. Tests performed by one of us have

TABLE 5. Recommended biosafety levels for bacterial and mycoplasmal agents

Agent	Biosafety level recommended for the following laboratory function[a]:			Agent	Biosafety level recommended for the following laboratory function:		
	A	B	C		A	B	C
Acinetobacter calcoaceticus	2	2	2	*Mycobacterium africanum*	2	3	3
Acinetobacter lwoffii	2	2	2	*Mycobacterium avium-intracellulare*	3	3	3
Actinomyces spp.	2	2	2	*Mycobacterium bovis BCG*	2	3	2
Actinobacillus spp.	2	2	2	*Mycobacterium chelonei*	2	3	2
Aeromonas hydrophilia	2	2	2	*Mycobacterium fortuitum*	3	3	3
Arachnia propionica	2	2	2	*Mycobacterium kansasii*	3	3	3
Bacillus anthracis[b]	2	3	2	*Mycobacterium leprae*[c]	2	3	3
Bacillus cereus	1	1	1	*Mycobacterium marinum*	2	3	3
Bacillus subtilis	2	2	2	*Mycobacterium scrofulaceum*	2	3	3
Bacteroides spp.	2	2	2	*Mycobacterium simiae*	2	3	3
Bartonella bacilliformis	2	2	2	*Mycobacterium szulgai*	3	3	3
Bordetella bronchiseptica	2	2	2	*Mycobacterium tuberculosis*	3	3	3
Bordetella pertussis	2	3	2	*Mycobacterium ulcerans*[c]	2	3	3
Borrelia spp.	2	3	2	*Mycobacterium xenopi*	2	3	3
Brucella spp.	3	3	3	*Mycoplasma pneumoniae*	2	3	2
Campylobacter fetus (all subspecies)	2	3	2	*Neisseria* spp.	2	3	2
Chlamydia spp.	2	3	2	*Nocardia* spp.	2	3	2
Clostridium botulinum[b]	2	3	2	*Pasteurella* spp.	2	3	2
Clostridium chauvoei	2	3	2	*Plesiomonas shigelloides*	2	2	2
Clostridium difficile	2	2	2	*Proteus* spp.	2	2	2
Clostridium sordellii	2	2	2	*Pseudomonas aeruginosa*	2	2	2
Clostridium spp.	2	3	2	*Pseudomonas mallei*[d]	3	3	3
Clostridium tetani[b]	2	3	2	*Pseudomonas pseudomallei*	3	3	3
Corynebacterium spp.	2	3	2	*Salmonella arizonae*	2	2	2
Edwardsiella tarda	2	2	2	*Salmonella choleraesuis*	2	3	2
Enterobacter aerogenes	2	2	2	*Salmonella enteritidis* (all serotypes)	2	3	2
Erysipelothrix rhusiopathiae	2	3	2	*Salmonella typhi*	2	3	2
Escherichia coli	2	2	2	*Serratia marcescens*	2	2	2
Escherichia coli K-12	1	1	1	*Shigella* spp.	2	3	2
Francisella novicida	2	2	2	*Staphylococcus aereus*	2	3	2
Francisella tularensis	3	3	3	*Staphylococcus epidermidis*	2	2	2
Fusobacterium necrophorum	2	3	2	*Streptobacillus moniliformis*	2	3	2
Haemophilus spp.	2	2	2	*Streptobacillus* spp.	2	3	2
Klebsiella pneumoniae	2	3	2	*Treponema* spp.	2	3	2
Lactobacillus spp.	1	1	1	*Vibrio cholerae*[b]	2	3	2
Legionella pneumophila	2	3	2	*Vibrio parahaemolyticus*	2	2	2
Legionella-like organisms	2	3	2	*Vibrio vulnificus*	2	3	2
Leptospira interrogans (all serovars)	2	3	2	*Yersinia enterocolitica*	2	3	2
Listeria monocytogenes	2	3	2	*Yersinia pestis*[b]	3	3	3
Moraxella spp.	2	2	2	*Yersinia pseudotuberculosis*	2	3	2

[a] A, Activities involving the use or manipulation of small quantities or low concentrations of cultures or other materials known to contain or suspected of containing the agent; B, activities involving the use or manipulation of large quantities or high concentrations of cultures or other materials known to contain or suspected of containing the agent; C, activities involving the use or manipulation of vertebrate animals with a natural or induced infection with the agent.

[b] Vaccination recommended for at-risk personnel.

[c] Personnel should wear gloves.

[d] Possession or use restricted by the U.S. Department of Agriculture.

shown that a cabinet turned on 15 to 20 min before work commences will be free of contaminating particles (104). Unless it is absolutely required for a specific procedure, operating a cabinet 24 h a day is unnecessary and may drastically shorten the life of a HEPA filter. In many laboratories, the work day is 8 h, but the cabinet is left running for the remaining 16 h. During those 16 h, the HEPA filter removes particulates from the room but the life of the filter is shortened. A class II, type A cabinet turned on 20 min before work commenced and turned off at the end of the work day was still certified with the original HEPA filters installed by the manufacturer 10 years previously when the cabinet was fabricated.

DO'S AND DO NOT'S OF CLASS II CABINET TECHNIQUES

Biological safety cabinets have limitations. No cabinet can replace good aseptic and procedural control techniques. Class II biological safety cabinets are currently the most widely used primary containment devices in clinical, research, microbiological, and pharmaceutical laboratories. These devices are suitable for manipulations of infectious agents, recombinant DNA molecules, cytotoxic drugs, and low- to moderate-risk oncogenic viruses, for which biosafety level 1 to 3 containment equipment and practices are recommended. Class II biological safety cabinets are partial con-

TABLE 6. Recommended biosafety levels for viral and rickettsial agents

Agent	Biosafety level recommended for the following laboratory function[a]:		
	A	B	C
Adenoviruses (human)	2	2	2
Arenaviruses: lymphocytic choriomeningitis			
Viscerotrophic strains	2	3	2
Neutrophic strains	3	3	3
Coronaviruses	2	2	2
Herpesvirus group			
Herpesvirus hominis[b]	2	3	2
Cytomegalovirus	2	3	2
Epstein-Barr virus	2	3	2
Herpesvirus simiae	4	4	4
Pseudorabies virus	2	3	3
Varicella virus	2	3	2
Myxoviruses and paramyxoviruses			
Canine distemper virus (Snyder-Hill strain)	1	1	1
Influenza viruses	2	3	2
Measles virus[d]	2	2	2
Mumps virus[d]	2	2	2
Newcastle disease virus	2	3	2
Parainfluenza viruses			
Human	2	3	2
Respiratory syncytial virus	2	3	2
Subsclerosing panencephalitis virus	2	3	2
Picornaviruses			
Coxsackieviruses	2	2	2
Echoviruses	2	2	2
Poliomyelitis viruses			
Wild type[d]	2	3	2
Attenuated[d]	2	2	2
Rhinoviruses (human)	2	2	2
Poxviruses			
Cowpox	2	3	2
Molluscum contagiosum	2	3	2
Monkeypox	3	4	3
Orf	2	3	2
Paravaccinia	2	3	2
Tanapox	2	3	2
Vaccinia	2	3	2
Variola major and minor[c]	R	R	R
Whitepox[c]	R	R	R
Yabapox	2	3	2
Papovaviruses			
Simian virus 40	2	3	2
B-K virus	2	3	2
Spongiform encephalopathy viruses			
Creutzfeld-Jacob agent[b]	2	3	2
Kuru agent[b]	2	3	2
Retroviruses (human)			
HIV[e]	3	3	
Human T-cell lymphotrophic virus types I and II	3	3	
Rotaviruses			
Togaviruses: rubella virus	2	2	2
Rickettsial agents			
Coxiella burnetii	3	3	3
Rickettsia spp.	3	3	3
Rickettsia prowazekii[d]	3	3	3
Rickettsia rickettsii	3	3	3
Rochalimaea quintana	3	3	3
Vole rickettsia	3	3	3
Other agents			
Parvovirus B19 (fifth disease agent)	2	3	2
Hepatitis viruses[b,d]	2	3	2
Norwalk agent	2	3	2

[a] A, Activities involving the use or manipulation of small quantities or low concentrations of cultures or other materials known to contain or suspected of containing the agent; B, activities involving the use or manipulation of large quantities or high concentrations of cultures or other materials known to contain or suspected of containing the agent; C, activities involving the use or manipulation of vertebrate animals with a natural or induced infection with the agent.

[b] Personnel should wear gloves.

[c] The importation, possession, and use of variola major, variola minor, and whitepox viruses are restricted to the designated World Health Organization Collaborating Center for Poxviruses, CDC, Atlanta, Ga.

[d] Vaccination recommended for at-risk personnel.

[e] Normal clinical laboratory specimens should be handled as biosafety level 2; biosafety level 3 should be used for experimental purposes, with consideration for risk assessment.

tainment devices, and their capabilities and limitations must be clearly understood. The expectation of class II biological safety cabinets is that they will provide protection to the user and the immediate laboratory environment from aerosols (respirable particles 1 to 5 μm in diameter) and larger particles generated by manipulations within the work chamber. The level of protection, however, is relative and is largely dependent upon two variables: mechanical performance of the biological safety cabinet according to the manufacturer's specifications and good laboratory practices that minimize aerosol generation and interference with the protective inward airflow of the cabinet.

The rate of inward airflow through the work access opening of the biological safety cabinet—the protective air barrier—varies from 75 to 125 fpm in the various models of class II cabinets. The ability of the protective air barrier to entrap aerosols within the work area may be compromised by user ignorance and poor technique. For example, the rapid insertion into or withdrawal from the class II safety cabinet of the user's arms may sufficiently disrupt the inward airflow to allow the escape of aerosolized particles. Escaped particles commonly migrate parallel to the protective view screen on the front of the cabinet to the user's breathing zone.

The following recommendations constitute the basic do's and do not's in the use of a class II biological safety cabinet.

Do's

(i) Become knowledgeable about the equipment to be used. Before selecting or using a biological safety cabinet, read the owner's manual and become thoroughly familiar with the performance characteristics of the particular model you will be using. (ii) Be aware that the various class II safety cabinets provide both user protection and high air quality within the work chamber and that class II, type A

TABLE 7. Recommended biosafety levels for fungal agents

Agent	Biosafety level recommended for the following laboratory function[a]:			Agent	Biosafety level recommended for the following laboratory function:		
	A	B	C		A	B	C
Absidia spp.	2	2	2	*Loboa loboi*	2	2	2
Aspergillus spp.	2	2	2	*Madurella mycetomi*	2	2	2
Blastomyces dermatitidis	2	3	2	*Microsporum* spp.	2	2	2
Candida spp.	2	2	2	*Mucor* spp.	2	2	2
Coccidioides immitis	3	3	3	*Paracoccidioides bra siliensis*	3	3	3
Cryptococcus neoformans	2	3	2	*Rhizopus* spp.	2	2	2
Dermatophilus congolensis	2	2	2	*Sporothrix schenckii*	2	2	2
Epidermophyton spp.	2	2	2	*Trichophyton* spp.	2	2	2
Geotrichum spp.	2	2	2	*Trichosporon* spp.	2	2	2
Histoplasma capsulatum	3	3	3	*Xylohypha bantania*	2	2	2
Histoplasma farcinimosum[b]	3	3	3				

[a] A, Activities involving the use or manipulation of small quantities or low concentrations of cultures or other materials known to contain or suspected of containing the agent; B, activities involving the use or manipulation of large quantities or high concentrations of cultures or other materials known to contain or suspected of containing the agent; C, activities involving the use or manipulation of vertebrate animals with a natural or induced infection with the agent.

[b] Possession or use restricted by the U.S. Department of Agriculture.

and B3 cabinets recirculate through HEPA filters approximately 70% of the intake air within the cabinet. This recirculation feature reasonably precludes the use of toxic, flammable, or explosive chemicals in these models. (iii) Be aware that the exhaust air from the class II, type A safety cabinet discharges into the laboratory and that volatile nonparticulates may pass through the HEPA filters and enter the work environment. (iv) Be aware that the air requirements (±1,200 cfm) of class II, type B2 cabinets are high and that installation and use of this model may require major modifications of the ventilation systems of laboratory and pharmacy facilities to provide sufficient air. (v) Plan and organize the work to be conducted within the safety cabinet. A checklist is a useful adjunct in properly setting up a safety cabinet for use. Checklist items include media, equipment, pipettes, discard pans, and other items that will be used in the activity. Anticipate the order of events and place items in the work chamber accordingly to ensure that the procedure can be completed without passing materials in or out through the air barrier. (vi) Use the following start-up procedures: ensure that the front intake grill and exhaust grill are unobstructed during cabinet start-up and use; turn on the safety cabinet's lights and blower fan; decontaminate the interior work surfaces of the safety cabinet by wiping them thoroughly with 70% ethyl or isopropyl alcohol; wash hands and arms and put on long-sleeved coats or cuffed gowns and gloves; place equipment and media, etc., for the planned activity in the work area of the safety cabinet—the materials should be arranged to segregate clean and contaminated items and to minimize movement within the cabinet; place discard receptacles in the rear of the work area; use a microburner or incinerator, because a large open flame may damage the HEPA filter and disrupt airflow; select and use an automatic pipettor appropriate for the planned activity; conduct manipulations in the center of the work area, not over the intake grill; if small equipment (clinical centrifuge and vortex mixer, etc.) is to be used within the safety cabinet, do not perform other activities within the cabinet when this equipment is operating; use special care with syringes and needles or operations necessitating scapels and cannulas, etc.; when work is complete, let the cabinet run for 15 min more to remove infectious or hazardous particles; wipe the cabinet with 70% ethyl or isopropyl alcohol; and keep laboratory doors closed and minimize personnel movement within the room during safety cabinet use.

Do Not's

(i) Do not place receptacles for discarding used pipettes or other glassware on the floor or on a laboratory cart or table outside the biological safety cabinet. (ii) Do not block intake or rear grills with paper or equipment. (iii) Do not rapidly insert or withdraw arms. (iv) If the safety cabinet has a UV lamp, do not turn this lamp on while the cabinet is being used. (v) Do not place items that will be used in the planned activity on carts and tables, etc., outside the cabinet. All supplies and equipment for the activity should be placed in the work area before the activity is begun. (vi) Do not place or tape paper notes and directions on the window. This action will limit the user's field of vision and may block or decrease lighting intensity. (vii) Do not leave an open flame in the cabinet when activity is completed.

Shut Down

(i) When planned activity is completed, allow the cabinet blower to continue operating for 15 to 20 min to purge the work area of airborne contaminants. (ii) Remove inoculated media, pipette cans, and other equipment from the cabinet. Cover and remove discard pans. (iii) Decontaminate the work surface and inner surfaces by thoroughly wiping them with 70% ethyl or isopropyl alcohol. (iv) Turn off the blower and lights.

WHICH ORGANISM IN WHICH SAFETY CABINET?

The question most frequently asked after the length of time that HEPA filters will last is, "How do I know with which microorganisms I can work in my safety cabinet?" We refer to publication number (CDC) 88-8404 (208), but not all microorganisms are listed. Thus, we have compiled Tables 5 through 10 from the draft guidelines (30), which were more extensive than the available document. To use these tables, locate the organism in the alphabetical listing in the appropriate category (bacteria, viruses, fungi, parasites,

TABLE 8. Recommended biosafety levels for parasitic agents

Agent	Biosafety level recommended for the following laboratory function[a]:			Agent	Biosafety level recommended for the following laboratory function:		
	A	B	C		A	B	C
Acanthocheilonema spp.	2	2	2	*Leishmania* spp.	2	2	2
Acanthamoeba spp.	2	2	2	*Linguatula* spp.	2	2	2
Ancylostoma spp.	2	2	2	*Loa* spp.	2	2	2
Angiostrongylus spp.	2	2	2	*Macracanthorhynchus* spp.	2	2	2
Ascaris spp.	2	2	2	*Necator* spp.	2	2	2
Babesia spp.	2	2	2	*Naegleria* fowleri	2	3	2
Balantidium spp.	2	2	2	*Naegleria gruberi*	1	1	1
Brugia spp.	2	2	2	*Onchocerca* spp.	2	2	2
Capillaria spp.	2	2	2	*Opisthorchis* spp.	2	2	2
Clonorchis spp.	2	2	2	*Paragonimus* spp.	2	2	2
Cysticercus spp.	2	2	2	*Plasmodium* spp.	2	2	2
Dicrocoelium spp.	2	2	2	*Pneumocystis carinii*	2	2	2
Dipetalonema spp.	2	2	2	*Schistosoma* spp.[b]	2	2	2
Diphyllobothrium spp.	2	2	2	*Strongyloides* spp.	2	2	2
Dipylidium spp.	2	2	2	*Taenia* spp.	2	2	2
Dracunculus spp.	2	2	2	*Toxascaris* spp.	2	2	2
Echinococcus spp.	2	2	2	*Toxocara* spp.	2	2	2
Entamoeba histolytica	2	2	2	*Toxoplasma* spp.	2	2	2
Enterobius spp.	2	2	2	*Trichinella* spp.	2	2	2
Fasciola spp.	2	2	2	*Trichomonas vaginalis*	2	2	2
Fasciolopsis spp.	2	2	2	*Trichostrongylus* spp.	2	2	2
Giardia spp.	2	2	2	*Trichuris trichiura*	2	2	2
Hymenolepis spp.	2	2	2	*Trypanosoma* spp.	2	2	2
Heterophyes spp.	2	2	2	*Wuchereria* spp.	2	2	2
Isospora spp.	2	2	2				

[a] A, Activities involving the use or manipulation of small quantities or low concentrations of cultures or other materials known to contain or suspected of containing the agent; B, activities involving the use or manipulation of large quantities or high concentrations of cultures or other materials known to contain or suspected of containing the agent; C, activities involving the use or manipulation of vertebrate animals with a natural or induced infection with the agent.

[b] Personnel should wear gloves when handling infected tissues or animals.

arboviruses, or oncogenic viruses) and identify the recommended biosafety level in the laboratory function columns.

DECONTAMINATION OF BIOLOGICAL SAFETY CABINETS

When maintenance must be performed on safety cabinets in which infectious materials have been handled, the cabinets must be decontaminated to ensure safe working conditions and prevent infection of maintenance or certifying personnel. Safety cabinets must be decontaminated when (i) HEPA filters are changed; (ii) the cabinet is relocated; or (iii) there is a large spill of infectious material. The most widely used method for decontaminating biological safety cabinets of infectious material is the depolymerization of paraformaldehyde by heat (204) to produce formaldehyde gas. The proper procedure is as follows. (i) Calculate the safety cabinet's volume by multiplying the height, width, and depth of the cabinet. (ii) Multiply the cabinet's volume (cubic feet) by either 0.25 or 0.3 to determine the gram weight of paraformaldehyde required. (iii) Place an electric frying pan in the cabinet. (iv) Raise the relative humidity (must be at least 60%) either by placing a beaker of water on a hot plate or by placing water in the electric frying pan. (v) Place the measured amount of paraformaldehyde in the electric frying pan. (vi) Secure the cabinet by taping the cabinet with plastic sheeting and ensure that all openings are sealed; if the cabinet is ducted, remove the duct connection and seal the opening with plastic sheeting and tape. (vii) Turn on the frying pan. (viii) After 25% of the paraformaldehyde depolymerizes, turn on the cabinet's blower for 5 to 10 s to disseminate the formaldehyde gas throughout the cabinet and through the HEPA filters. (ix) Repeat step viii after 50, 75, and 100% of the paraformaldehyde depolymerizes (this is a change from an earlier procedure [139], but experiments [104] with *Histoplasma capsulatum* and *M. tuberculosis* revealed that turning on the blower for 3 to 5 s when only 50 and 100% of the paraformaldehyde had depolymerized did not kill these two infectious microorganisms). (x) Let the safety cabinet stand for at least 1 h or, when resistant organisms such as *M. tuberculosis* or systemic fungi have been used in the cabinet, let the formaldehyde gas remain in the cabinet for at least 2 h. (xi) To neutralize the formaldehyde gas, add the same amount of NH_4HCO_3 as of paraformaldehyde and turn on the frying pan and the cabinet's blower until the NH_4HCO_3 has dissipated; let the safety cabinet stand for at least 1 h before opening seals (formaldehyde is explosive when mixed with air in concentrations between 7.0 and 73% by volume, but these concentrations are never approached when standard decontamination procedures are used [156]).

A commercial machine (Certek, Inc., Raleigh, N.C.) that contains paraformaldehyde, neutralizer, and water is available. One seals the cabinet and inserts tubing from the machine through the plastic sheeting into the work area of the safety cabinet. The machine raises the relative humidity, releases formaldehyde gas, sets the contact time up to 10 h, and neutralizes the formaldehyde gas.

This method of decontamination is only appropriate for infectious microorganisms. There is no known method of

TABLE 9. Recommended biosafety levels for arboviruses[a]

Agent	Biosafety level recommended for the following laboratory function[b]:			Agent	Biosafety level recommended for the following laboratory function:		
	A	B	C		A	B	C
Absettarov	4	4	4	Issyk-kul	3	3	3
Aino	3	3	3	Itaituba	3	3	3
Akabana	3	3	3	Japanese encephalitis	3	3	3
Araguari	3	3	3	Junin	4	4	4
Batama	3	3	3	Kairi[c]	3	3	3
Batken	3	3	3	Khasas	3	3	3
Bhania	3	3	3	Korean hemorrhagic fever (Hantaan)	3	3	3
Bimbo	3	3	3	Koutango	3	3	3
Bluetongue				Kumlinge	4	4	4
Indigenous	2	3	2	Kyasanur Forest disease	4	4	4
Exotic	3	3	3	Kyzylagach	3	3	3
Bobaya	3	3	3	Lassa	4	4	4
Bobia	3	3	3	Louping ill[c]	3	3	3
Buenaventura	3	3	3	Lymphocytic choriomeningitis	3	3	3
Cabassou	3	3	3	Machupo	4	4	4
Chikungunya	3	3	3	Marburg	4	4	4
Chim	3	3	3	Mayaro	3	3	3
Cocal	3	3	3	Middelburg	3	3	3
Congo-Crimean hemorrhagic fever	4	4	4	Mosqueiro	3	3	3
Dhori	3	3	3	Mucambo[d]	3	3	3
Dugbe	3	3	3	Murray Valley encephalitis	3	3	3
Eastern equine encephalitis[d]	2	3	3	Nariva	3	3	3
Ebola	4	4	4	Ndumu	3	3	3
Everglades[d]	3	3	3	Negishi	3	3	3
Ganjam[c]	3	3	3	New Minto	3	3	3
Garba	3	3	3	Nodamura	3	3	3
Germiston	3	3	3	Northway	3	3	3
Getah	3	3	3	Omsk hemorrhagic fever	4	4	4
Gordil	3	3	3	Oropouche	3	3	3
Guaratuba	3	3	3	Orungo	3	3	3
Hanzalova	4	4	4	Ouango	3	3	3
Hypr	4	4	4	Oubangue	3	3	3
Ibaraki	3	3	3	Paramushir	3	3	3
Inhangapi	3	3	3	Piry	3	3	3
Inini	3	3	3	Ponteves	3	3	3
Israel turkey meningo	3	3	3	Spondweni	3	3	3
Powassan	3	3	3	St. Louis encephalitis	3	3	3
Razkan	3	3	3	Tamdy	3	3	3
Rift Valley fever[c,d,e]	3	4	4	Telok Forest disease	3	3	3
Rochambeua	3	3	3	Thogoto	3	3	3
Rocio	3	3	3	Tiacoyalpan	3	3	3
Russian spring-summer encephalitis	4	4	4	Tonate	3	3	3
Sagiyama	3	3	3	Vesicular stomatitis virus (Alagoas)	3	3	3
Sakpa	3	3	3	Venezuelan equine encephalitis			
Salanga	3	3	3	TC83[d,f]	2	3	3
Santa Rosa	3	3	3	Others[d]	3	3	3
Saumarez Reff	3	3	3	Wesselsbron[c]	3	3	3
Semliki Forest	3	3	3	Western equine encephalitis[d]	2	3	3
Sepik	3	3	3	West Nile	3	3	3
Serra do Navio	3	3	3	Yellow fever (17D)[d,f]	2	3	3
Slovakia	3	3	3	Zinga[c,d,e]	3	4	4

[a] Arboviruses not listed are assigned to biosafety level 2.
[b] A, Activities involving the use or manipulation of small quantities or low concentrations of cultures or other materials known to contain or suspected of containing the agent; B, activities involving the use or manipulation of large quantities or high concentrations of cultures or other materials known to contain or suspected of containing the agent; C, activities involving the use or manipulation of vertebrate animals with a natural or induced infection with the agent.
[c] Possession or use restricted by the U.S. Department of Agriculture.
[d] Vaccination recommended for at-risk personnel.
[e] Zinga virus is now recognized as being identical to Rift Valley fever virus.
[f] Provided not more than one passage from the vaccine strain.

decontamination appropriate for hazardous pharmaceutical agents. When HEPA filters must be changed in a cabinet in which hazardous pharmaceutical agents have been handled, one should wear appropriate clothing, gloves, and mask, etc. The filters should be placed in proper containers, labeled as hazardous waste, and discarded according to hospital policy.

In 1986, the American Sterilizer Company introduced vapor-phase hydrogen peroxide as a sterilant (157). Formal-

TABLE 10. Recommended biosafety levels for oncogenic viruses

Agent	Biosafety level recommended for the following laboratory function[a]:			Agent	Biosafety level recommended for the following laboratory function:		
	A	B	C		A	B	C
Rous sarcoma	1	2	2	Rat leukemia	1	2	2
Simian virus 40	1	2	2	Hamster leukemia	1	2	2
CELO	1	2	2	Bovine leukemia	1	2	2
Adenovirus type 7-simian virus 40	1	2	2	Dog sarcoma	1	2	2
Polyomavirus	1	2	2	Mason-Pfizer monkey virus	1	2	2
Bovine papilloma	1	2	2	Marek's disease virus	1	2	2
Rat mammary tumor	1	2	2	Guinea pig herpes	1	2	2
Avian leukosis	1	2	2	Lucke (frog)	1	2	2
Murine leukemia	1	2	2	Adenovirus	1	2	2
Murine sarcoma	1	2	2	Shope fibroma	1	2	2
Mouse mammary tumor	1	2	2	Shope papilloma	1	2	2
Epstein-Barr	2	3	2	Herpesvirus saimiri	2	3	2
Feline leukemia	2	3	2	Wooley monkey fibrosarcoma (SSV-1)	2	3	2
Feline sarcoma	2	3	2	Yaba poxvirus	2	3	2
Gibbon ape lymphosarcoma	2	3	2	Nondefective adenovirus type 2-simian virus 40 hybrids	2	3	2
Herpesvirus ateles	2	3	2				
Human T-cell lymphotrophic virus types I and II	3	3		RNA and/or DNA virus isolates from humans with possible oncogenic potential	2	3	2

[a] A, Activities involving the use or manipulation of small quantities or low concentrations of cultures or other materials known to contain or suspected of containing the agent; B, activities involving the use or manipulation of large quantities or high concentrations of cultures or other materials known to contain or suspected of containing the agent; C, activities involving the use or manipulation of vertebrate animals with a natural or induced infection with the agent.

dehyde and hydrogen peroxide were compared as decontaminating agents. A number of applications and future uses of the peroxide-air mixture were discussed. Research continues in space decontamination and practical applications of decontamination of equipment, including HEPA filters and biological safety cabinets.

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