

PIPETTING HAZARDS IN THE SPECIAL VIRUS CANCER PROGRAM

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I. HISTORY

Although this subject is new to the Cancer Program, it is an old one in microbiology. The use of mechanical pipettors to prevent laboratory-acquired infection appeared in the German scientific journals as early as 1907. (Reinhardt, 1918) There were three of these publications in 1908. (Reinhardt, 1918) In 1918 an Austrian physician (Reinhardt, 1918) described 21 different pipetting devices. He stated "with the aid of the devices described, it is possible to work more quickly than with the oral pipette."

This early attention to pipetting hazards arose because several cases of typhoid in laboratory personnel were ascribed to oral aspiration of culture, urine, or feces through a pipette.

From Germany in 1915 Kisskalt (Kisskalt, 1915) reported 50 cases of laboratory-acquired typhoid fever dating back to 1885. There were six deaths. The method of infection was known in 23 cases, and in 16 of these, the pipette was the cause.

In the early days of medical microbiology the pipette and the hypodermic syringe were often the first methods by which infection occurred in the laboratories, as gathered from reports dated:

- 1893 tetanus - syringe (Riesman, 1898)
- 1894 cholera - pipette (Riesman, 1898)
- 1897 brucellosis - syringe (Birt, 1899)
- 1898 glanders - syringe (Riesman, 1898)
- 1898 diphtheria - pipette (Riesman, 1898)

In more modern times there are reports of laboratory infections by means of the pipette with quite a variety of microorganisms (Sulkin, 1963). In the intestinal group: typhoid, shigella, salmonella, cholera; among others, anthrax, brucella, diphtheria, hemophilus influenzae, leptothrix, meningococcus, streptococcus, syphilis, tularemia; among viruses, mumps (Sulkin, 1963; Enders, 1945), Coxsackie virus (Shaw, 1950), viral hepatitis (Kuh, 1950), Venezuelan equine encephalitis (Fort Detrick, 1958), chikunguny (Shah, 1965), and scrubtyphus (Van den Ende, 1946).

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II. SURVEYS OF CAUSES OF INFECTION

In any consideration of the frequency or method of contracting laboratory infection, it is important to realize that laboratory infections often are not recognized as such, and are poorly reported in the literature or in response to questionnaires (Phillips, 1961). During the survey by Sulkin and Pike, published in 1951 (Sulkin, 1951), only half of about 5,000 laboratories in the U.S. replied to the questionnaire, and of the 1,342 cases reported only one-third had previously been acknowledged by inclusion in a publication.

The means of infection usually is unknown. A definite accident or action responsible for the infection has been found in 12% (Sulkin, 1969, b) or 16-25% (Pike, 1965) of collected cases, and under conditions of organized systematic investigation in no more than 35% (Phillips, 1965).

Non-hospitalized cases, those with mild symptoms, or infections recognizable only by serological change are just as important as overt severe infection to indicate accidents or inadequate techniques. Yet these usually go unrecognized and unreported. At Fort Detrick these mild infections accounted for 27% of the 423 cases.

The limitations to our knowledge described above may be the reason so few viral infections have been ascribed to pipetting accidents.

Among the reported causes of laboratory infection, the pipette has been implicated as follows:

TABLE 1
Laboratory Infections Due to Oral Pipetting

Years	Cases with Known Accident	% Due to Pipetting	References
1893-1950	921	17	Reitman, 1955
1930-1950	215	15	Sulkin, 1951; Pike, 1965
1950-1963	156	6	Pike, 1965
1930-1967	165*	13	Sulkin, 1969, b
1930-1968	460	18	Sulkin, 1969, a

* Viral and rickettsial

If one reads the accounts of accidents reported in 1915 and recalls recent experience, it is apparent that there has been little change in oral pipetting accidents: the cotton plug is loose and therefore does not stop an onrush of fluid, or a can of unplugged pipettes is absentmindedly selected, or the wrong size pipette is picked up and excessive suction used, or clogged material in or at the tip of the pipette suddenly is loosed, or the technician has a nose cold that encourages mouth breathing and loss of the usual degree of control of suction, or the pipette inadvertently emerges too soon from the liquid, etc. Such accidents probably are reported infrequently, but

from such data as is available, one infection has resulted from each 3, or 5, reported accidental aspirations (Phillips, 1966). Accidents in Germany (Kisskalt, 1915) with intestinal pathogens showed that infection often could not be prevented by rinsing the mouth with mercuric bichloride, 70% alcohol, or hydrogen peroxide solutions.

In addition to oral aspiration, pipetting can cause human infection by inhalation of aerosols. These are created when drops fall to a hard surface, during forceful mixing by alternate suction and expulsion, by blowing out the last drop, by forceful ejection of a 1 or 10 ml inoculum into a culture fluid, or by inhalation through an unplugged pipette of aerosol created in the pipette during mixing (Birt, 1899). Analysis of the particle size of aerosols produced by the first two of these procedures showed the particles to be in the respirable range of 1.0 to 7.5 microns diameter (Kenny, 1968). To determine the probable inhaled dose from these and other accidentally produced aerosols, a nomogram has been prepared for each of several accidents or operations (Dimmick, 1972). This nomogram reflects the relationship between the microbial titer of the source fluid, the operation, its duration, the yield of respirable particle-sized aerosol to which the technician would be exposed, the microbial decay rate, the breathing time, the probability that a particle will contain at least one microbial unit, and the variations due to good or poor technique. A possible, but unproved, advantage of a mechanical pipettor is that the source of the infectious aerosol is not so close to the nose as it is in oral pipetting.

A third category of oral contamination arises from placing a contaminated finger on the proximal end of the pipette. Although this is mentioned by several authors (Sulkin, 1963; Reitman, 1955; Phillips, 1966, Darlow, 1969), there seems to be no experimental microbiological data or reported infections from this source. However, it is probable that oral contamination through pipetting was demonstrated long ago by chemists.

III. APPLICATION TO THE SPECIAL VIRUS CANCER PROGRAM (SVCP)

A. Quantitation

A major difficulty in organizing a program of precautionary laboratory practices in the SVCP is that there have been no recognized human laboratory-acquired cases of cancer or leukemia. But a necessary assumption is that the SVCP will be successful in isolating an infectious or triggering substance. Then, past experience with known infectious microorganisms teaches us that it is only a question of time before a susceptible laboratory worker accidentally absorbs an adequate inoculum by some means or other.

The first step in assessing this danger has been to prepare lists of moderate-risk and low-risk oncogenic viruses (Wedum, 1972). To keep these lists up-to-date will require the cooperation of all who work with oncogenic viruses or their equivalent.

Another important step, in relation to the pipetting hazard and all other operational hazards, is to arrive at some estimate of the fluid volume of the minimum infective or triggering inoculum. As an introduction to this subject, Table 2 lists the infective human doses for several

PIPETTING HAZARDS IN THE SPECIAL VIRUS CANCER PROGRAM

viruses, in terms of the number of infective doses per milliliter of experimental material that the technician handles. Some of these viruses appear in various ways in the cancer/leukemia research program. This information supports the proposition that it is not uncommon for one infective dose of tissue culture to be sufficient to infect man if it is placed in contact with susceptible cells (Plotkin, 1967), or if it is placed in an immunologically depressed host. Consideration of this proposition, and the data in Table 2, suggests that realistic assessment of risks in cancer research would be assisted by a policy that resulted in early determination of the minimal infectious or triggering inoculum, for each promising oncogenic agent, chemical or biological, for tissue culture, mouse or monkey, and presumably, for man. What is needed is not the ID₅₀ but the minimal ID, which would apply to the most susceptible laboratory worker. It seems obvious that any material containing 1000 human infective doses per ml can cause problems in some experiments.

TABLE 2
Minimal Human Infective Dose in Volunteers (Plotkin, 1967)

Viral Agent*	Inoculation	Human Infective Dose <i>a</i> /	No. of Volunteers <i>g</i> /	Titer Per ml <i>a</i> /, <i>h</i> /
Venezuelan encephalitis (Smith, 1956; Sutton, 1954)	Subcutaneous	1 ^{<i>e</i>}	14	1
West Nile Fever (Southam, 1954)	Intramuscular	1 ^{<i>d</i>}	84	1
Adenovirus 4 (Couch, 1966a; Couch, 1966b; Knight, 1967)	Inhalation	1	15	1
Coxsackie A21 (Couch, 1965; Gerone, 1966)	Inhalation	<18	28	10 ⁹
Poliovirus 1 (Koprowski, 1956; Dulbecco, 1954)	Ingestion	2 ^{<i>b</i>} , ^{<i>e</i>}	75	1
Adenovirus 7 (Couch, 1963)	Conjunctival swab	320	4	10
Adenovirus 7 (Plotkin, 1967; Couch, 1963)	Ingestion	10,000	15	10
Measles (Markham, 1962)	Subcutaneous	10 ^{<i>b</i>}	34	1
Measles (Okuno, 1962)	Intranasal spray	0.2 ^{<i>b</i>}	183	1
Adenovirus 27 (Kasel, 1963; Knight, 1963)	Conjunctival swab	30	13	1
Influenza A2 (Alford, 1965)	Nasopharyngeal	<790	32	1
Influenza A2 (Knight, 1967)	Inhalation	3	11	
Parainfluenza 1 (Plotkin, 1967)	Nasal drops	<1.5	21	
Rubella (Green, 1965)	Pharyngeal spray	1 ^{<i>b</i>}	8	1
Rubella (Plotkin, 1965)	Subcutaneous	30 ^{<i>b</i>} , ^{<i>f</i>}	5	10 ²
Rubella (Plotkin, 1965)	Nasal drops	60 ^{<i>b</i>} , ^{<i>f</i>}	5	10 ²
Respiratory syncytial (Kravetz, 1961; Morris, 1961)	Intranasal spray	<160-640	41	3
SV40 (Morris, 1961)	Nasopharyngeal	10,000	35	50
Rhinovirus (Couch, 1966a; Cate, 1965)	Inhalation	<1	43,8	10

Postscript to Table 2

*Numbers in parentheses refer to references.

a/Infectious tissue culture dose 50 (TC₅₀) unless otherwise specified.

b/Children, c/Guinea Pig, or d/Mouse infective unit.

e/Plaque-forming units. f/Interference units.

g/May include volunteers with variously sized challenge doses, and sometimes control volunteers.

h/Titer of culture or materials, before dilution for inoculation.

i/ $10^{6.3}$ before centrifugation and resuspension of the pellet.

j/Per ml of culture or material before dilution.

There was illness after all inoculations except poliovirus, rubella (nasal drops), adenovirus (nasal drops), SV40 virus, adenovirus 7 (ingestion), and measles (subcutaneous); in these there was serological conversion.

B. Legalities

1. The Occupational Safety and Health Act

The Williams-Steiger Occupational Safety and Health Act of 1970 (OSHA) (OSHA, 1970) can be expected to have serious applications, about 10 to 20 years from now, to oncogenic research now under way. During that time some cancers or leukemias will appear, in the normal course of events among present laboratory personnel. Claims will be made that the disease resulted from laboratory exposures. Questions then will arise: What precautions were taken? Were they equal to standards of practice issued by OSHA during 1978 to 1993? Were they equal to good practices recognized as early as 1973? What records were kept of accidents and occupational exposure?

The Act specifically states that "each employer shall furnish...a place of employment...free from recognized hazards...likely to cause death or serious physical harm."

"The Secretary (of Labor)...shall issue regulations requiring employers to maintain accurate records of employee exposures to potentially toxic materials or harmful physical agents which are required to be monitored or measured under Section 6." (Section 6 concerns establishment of occupational safety and health standards).

"The term 'national consensus standard' means any occupational safety and health standard or modification thereof which (i) has been adopted and promulgated by a nationally recognized standards-producing organization under procedures whereby it can be determined by the Secretary that persons interested and affected by the scope or provisions of the standard have reached substantial agreement on its adoption, (ii) was formulated in a manner which afforded an opportunity for diverse views to be considered and (iii) has been designated as such a standard by the Secretary, after consultation with other appropriate Federal agencies."

A major purpose of this Conference on Biohazards in Cancer Research is to begin the development of a "National Consensus Standard." Pipetting is one small part of the consensus standards.

2. Existing Standards on Pipetting of Known Infectious Microorganisms

At the beginning of this exposition, mention was made of the situation in Germany in 1907-1918. In West Germany a government regulation was published in 1956 (Anon, 1956; Phillips, 1961) that prohibited all mouth pipetting in medical, dental, and veterinary laboratories even if infectious agents were not used. However, during visits to representative German laboratories in 1960 it was found that this regulation was "commonly disregarded" (Phillips, 1961). Phillips (Phillips, 1966) also states, "In several European countries Federal Regulations applying to all medical laboratory workers also prohibit mouth pipetting of dangerous substances. In one country infection due to mouth pipetting is ground for denial of work-loss compensation." During 1959-60, in 102 laboratories among 111 visited in 60 cities in 18 countries, he found 38% of the institutes had a rule prohibiting oral pipetting (Phillips, 1965; Phillips, 1961).

At the Naval Biomedical Research Laboratory the simple rubber medicine dropper bulb was made mandatory during the war (1943-45) (Wedum, 1950) and continues to this day "...to operate 1.0 ml pipettes to an accuracy of $\pm .05$ ml, and the ease of operation never ceases to surprise the neophyte or the experienced worker..." (Chatigny, 1969). The early regulations at Fort Detrick specified cotton-plugged pipettes for all infectious or toxic materials, but only recommended pipettors. Strong pressure for use of pipettors, and the variety of them available soon caused their general acceptance. A mandatory requirement was not issued until 1963. At the National Institutes of Health, a "Chemical and Biological Safety Guide" issued in 1965, states "Pipettes for handling infectious materials should be plugged with cotton. The use of safety pipetting devices is recommended when applicable." The many pipetting devices now commercially available indicates their widespread use. Nineteen are listed and ten have been evaluated for each of eight qualities, as part of the material presented to representatives of SVCP contractors who attend the course now being given by the University of Minnesota School of Public Health, under contract with the National Cancer Institute.

3. Standards on Pipetting Applicable to the SVCP

The Office of Biohazard and Environmental Control of the National Cancer Institute has prepared "Minimum Standards of Biological Safety and Environmental Control for Contractors of the SVCP (August 1972)." Item 6, Pipetting, reads "There shall be no mouth pipetting in any of the laboratories." This is the basis for the discussion on pipetting at this Conference.

However, an independent Federal agency, the USPHS Center for Disease Control, has issued "Operational Requirements for Safe Laboratory Handling of Hazardous Microorganisms" (ANON, 1972). These requirements, which include pipetting requirements, apply within CDC, but they also constitute an important precedent when considering biological materials in the SVCP, because the requirements for a number of SVCP agents are listed. "Bulb Pipetting (is) Required" for: all adenoviruses; all types of Coxsackie A & B; cytomegalo-; ECHO-; herpes-; murine viruses including ectromelia, LCM, murine hepatitis, etc.; reo-; respiratory syncytial-; rhino-; simian-; vesicular stomatitis and other rhabdoviruses, and all newly discovered agents.

C. RESPONSIBILITY

Responsibility is a necessary accompaniment of authority given to direct the activities of other persons in a laboratory research program. But there is a tendency for a scientist who supervises other scientific personnel to concentrate on responsibility for high quality and productivity in research, and ignore responsibility for the personal safety of persons under his research direction. This situation probably arises from a misapplication of respect for the individuality of his assistants.

However, it is a well-established principle of human relationships, beginning with the parent and child and extending to the government of a nation, that he who supervises or governs others has a responsibility to plan for prevention of their injury. Every good safety program depends upon a policy that each supervisor must consider the prevention of injury to employees under his direction to be as much a part of his job as quantity and quality of production. To that end, he should train, supervise, correct, enforce, and investigate. He also must set a good example. Rules and regulations are necessary, but what the supervisor does, establishes what the assistant will do, regulation or no regulation.

D. POLICY ON PIPETTING

The present SVCP policy is "no oral pipetting," because oral pipetting is a route of infection that is easily interrupted by a convenient and easily learned procedure. Oral pipetting is not approved in laboratories where there are no infectious or toxic materials because experience shows it is difficult to maintain usage of pipettors in one laboratory room or in any one operation in a room, when oral pipetting is permitted elsewhere in that room or elsewhere in the laboratory area. Furthermore, in media preparation areas oral pipetting may be the source of non-specific contamination of media, such as by mycoplasma.

IV. CONCLUSIONS AND RECOMMENDATIONS

1. Unrestricted oral pipetting of infectious, toxic, or otherwise hazardous materials inevitably causes infection or other injury in someone.
2. Eventually, OSHA will issue a Federal regulation that includes standards for pipetting of hazardous materials.
3. The SVCP should seek to establish a preliminary "national consensus standard," assisted by attendees of the present Conference, in regard to categorization of hazardous materials in the SVCP program and the use of pipetting devices in SVCP laboratories.

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PIPETTING HAZARDS IN THE SPECIAL VIRUS CANCER PROGRAM

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