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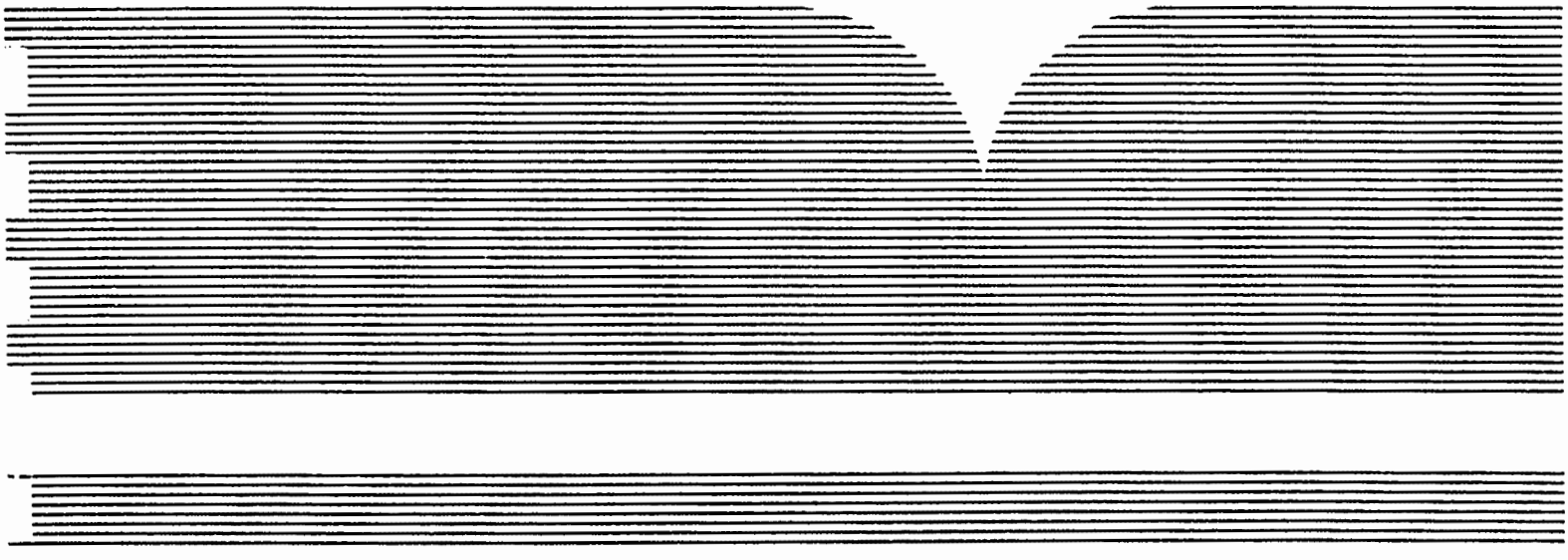

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16. Abstract (Limit: 200 words)

This document provides background information and support for regulations which have been designed to identify and list hazardous waste pursuant to Section 3001 of the Resource Conservation and Recovery Act of 1976. This document presents the Agency's rationale in determining the definition of infectious hazardous waste.

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BACKGROUND DOCUMENT

RESOURCE CONSERVATION AND RECOVERY ACT
SUBTITLE C - HAZARDOUS WASTE MANAGEMENT

SECTION 3001 - IDENTIFICATION AND LISTING OF HAZARDOUS WASTE

SECTION 250.14 - HAZARDOUS WASTE LISTS

INFECTIOUS WASTE

DECEMBER 15, 1978

U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF SOLID WASTE

Draft Background Document

Hazardous Waste Identification and Listing

Infectious Waste

Draft Background Document
Hazardous Waste Identification and Listing
Infectious Waste

3.1 Introduction

The purpose of this chapter of the background document is to present the Agency's rationale in determining the definition of infectious hazardous waste.

To date it has been the policy of the Agency under Section 3001 of the Act, to define chemical and physical hazardous waste characteristics such as toxicity, flammability, and corrosivity, in quantitative terms; i.e. criteria have been chosen that best quantify each hazardous characteristic, with certain hazard levels specified for each tested parameter (e.g., flashpoint for flammability, pH for corrosivity). For enforcement purposes, this method of quantitatively defining a hazardous waste is most desirable. It would follow then, that a similar type of definition for "infectious characteristics" would be the most useful one from a regulatory point of view.

Unfortunately, such quantification of infectious characteristics is not possible, as will be discussed in this document. Instead of specifying a certain number of infectious agents allowed to be present in a waste, the Agency has chosen to define infectious waste by specifying the sources where disease microorganisms may occur. After

clinical response in a host; yet for other disease agents it is known that hundreds or even thousands of organisms are necessary. Therefore setting a safe number of organisms for solid waste would involve specifying a safe level for each disease agent and providing a means to analyze for each one. Unfortunately, dose levels for all disease agents are not known at present and methods of environmental sampling and analysis for many disease agents have not been developed.

3.3 Indicator Organisms

Several EPA contacts have suggested the use of indicator organisms such as Salmonella spp., fecal coliforms, or S. aureus as an index of overall (i.e. viral, bacterial, fungal, parasitic) biological hazard of a waste. The problems associated with the use of indicator organisms have been recognized by EPA. For water standards, the Office of Water Program Operations originally suggested the use of fecal coliform as an indicator organism to determine the effectiveness of the chlorination process (40 CFR 133). This standard was later deleted (FR July 26, 1976) (1), with EPA recognizing that fecal coliform is "not an ideal indicator of pathogenic (sic) contamination" but is "a practical indicator of relative disease causing potential."

While microbial concentration standards may be applicable in the evaluation of the efficacy of wastewater treatment systems, their applicability as absolute quality standards remains to be demonstrated. A problem is that in some situations, the die-

degrees of severity from accidental inoculation or injection or other means of cutaneous penetration but which are contained by ordinary laboratory techniques.

Class 3

Agents involving special hazard or agents derived from outside the United States which require a federal permit for importation unless they are specified for higher classification. This class includes pathogens which require special conditions for containment.

Class 4

Agents that require the most stringent conditions for their containment because they are extremely hazardous to laboratory personnel or may cause serious epidemic disease. This class includes Class 3 agents from outside the United States when they are employed in entomological experiments or when other entomological experiments are conducted in the same laboratory area.

Class 5

Foreign animal pathogens that are excluded from the United States by law or whose entry is restricted by USDA administrative policy.

NOTE: It has been pointed out that the current CDC list does not include some agents of significance (e.g. Giardia, Ascaris, Legionnaires bacterium) as well as it does include one non-pathogen (Naegleria gruberi). The reader should keep in mind that the list is periodically revised. The most recently published list would be applicable.

It is interesting to note that not one of these definitions attempts to quantify numbers of disease organisms that would render a waste infectious and that it is these same States that have promulgated criteria for physical/chemical characteristics of hazardous waste on a quantitative basis similar to the ones EPA is considering. The approach that the Agency is taking to define infectious characteristics of waste, then, and the deviance of this approach from that of defining other characteristics of hazardous waste, is in line with the thinking proposed by the most progressive State hazardous waste management programs.

TABLE 1

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
California Department of Health		Assembly Bill No. 1593: An Act to Amend Section 25116. Ch. 6.5. Division 20, of the Health and Safety Code	"Infectious" means containing pathogenic organisms, or having been exposed, or reasonably being expected to have been exposed, to contagious or infectious disease. Articles which are "infectious" include, but are not limited to, the following: (1) Wastes that contain pathologic specimens, tissues, specimens or blood elements, excreta or secretions from humans or animals at a hospital, medical clinic, research center, veterinary institution, or pathology laboratory. (2) Surgical operating room pathologic specimens and articles attendant thereto which may harbor or transmit pathogenic organisms. (3) Pathologic specimens and articles attendant thereto from outpatient areas and emergency rooms. (4) Discarded equipment, instruments utensils and other articles which may harbor or transmit pathogenic organisms from the rooms of patients with suspected or diagnosed communicable disease.

TABLE

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
State of Maryland Department of Health and Mental Hygiene		Proposed Regulations for Medical Waste Disposal; "Subcommittee Report to the Task Force on Medical Waste Disposal - December 6, 1976"	The term medical wastes, encompassing materials hitherto called "infectious" "pathological", "contaminated", "special", and "hazardous" shall be replaced with the following new terms: (1) Hospital Medical Wastes - shall mean all solid waste generated within a hospital. Blood and blood products shall be included in this solid waste category. (2) Nursing Home Medical Wastes - shall be defined in two categories, as follows: (a) All disposable fomites from isolation areas, all dressings, pledgets, swabs, tongue depressors, plaster casts, body tissues, laboratory wastes, needles, syringes, I.V. apparatus, and medications (as permitted under Federal, State and local regulations). (b) Additional items which may be included in the above category include diapers and perineal pads.

TABLE -

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
Minnesota Department of Health, Health Facilities Division		Interpretive Policies for the Physical Plant: Handling and Disposal of Infectious Waste (Current DOH Guidelines)	Infectious Waste: (1) Hazardous Infectious Waste (same as above). (2) General Infectious Waste (contaminated): (a) Bandages, dressing, casts, catheters tubing, and the like, which have in contact with wounds, burns, or surgical incisions, but are not suspected or have been not medically identified as being of a hazardous infectious nature. (b) Discarded hypodermic needles and syringes, scalpel blades, and similar materials, when suspected or identified to be of a hazardous infectious nature. (c) Incinerator ashes from infectious waste.

TABLE -

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
Minnesota Pollution Control Agency, (CONT.)		Proposed, but to no longer be part of the hazardous waste regulations, HW-1	(2) Surgical and obstetrical wastes, pathological specimens, and disposal fomites from surgical operating rooms, outpatient areas, emergency rooms and similar areas where such wastes are generated. (3) Equipment, instruments, utensils, and fomites of a disposable nature from the rooms of patients with suspected or diagnosed communicable disease, or from the rooms of patients who by nature or their disease are required to be isolated by the State Board of Health. (4) Hypodermic needles and syringes, scalpel blades, suture needles and similar materials. (5) Mixtures of any of the wastes in (1) through (5) and other wastes that have been collected within the same container.

TABLE 4

State Definitions Of Infectious Waste

State Agency	Legislative Authority (if any)	Title of Regulation/ Guideline/ Document	Definition(s)
Pennsylvania Department of Environmental Resources	Pennsylvania Solid Management Act (35 (35 PS6-001), PL 241	Hazardous Waste Management Profile	General Classification of Hazardous Wastes (1) Pathogenic Materials (a) biological solids (b) laboratory wastes (c) infectious wastes (2) Other Hazardous Solid Waste (a) diseased animals
Texas Department of Health Resources		Comments to ANPR	Hazardous biological waste should include all pathological waste from chemical biological and contagious wards as well as animals dead of unknown disease and unstabilized domestic sewage.
State of Washington Department of Ecology		Washington Administrative Code (WAC) Hazardous Waste Regulation, Chapter 173-302 WAC	Waste containing etiologic agents are toxic dangerous wastes. Etiologic agent means a viable microorganism or its toxin, which causes or may cause human disease, and is limited to those agents listed in 42 CFR 72.25(c) of the regulations of the Department of HEW.

Table 2A.

Areas/Sources Identified as Sources of Infectious Wastes, By State

	California	Illinois	Maryland	Minnesota	New York	Oregon	Pennsylvania	Texas	Washington	Ontario
Abattoir										X
Animal Compounds										X
Veterinary Hospitals	X									
Health Services				X						
Hospital, "pathological waste"	X	X	X							X
Emergency Rooms	X			X						
Isolation Rooms	X		X	X			X			
Laboratory	X		X	X		X				X
Outpatient Areas	X									
Pathology Laboratory	X									
Surgical Operating Room	X			X						
Medical Clinics	X									
Nursing Homes			X							
Research Center	X									
Sewage Sludge							X			

Table 2 B (Cont.)

Items Identified, By State

	California	Illinois	Maryland	Minnesota	New York	Oregon	Pennsylvania	Texas	Washington	Ontario
Biological Solids						X				
Incinerator Ash From Infectious Waste			X							
Diseased Animals			X			X	X		X	

Haemophilus ducreyi, H. influenzae.
Herellea vaginicola.
Klebsiella--all species and all serotypes.
Leptospira interrogans--all serotypes.
Listeria--all species.
Mima polymorpha.
Moraxella--all species.
Mycobacterium--all species.
Mycoplasma--all species.
Neisseria gonorrhoeae, N. meningitidis.
Pasteurella--all species
Pseudomonas pseudomallei.
Salmonella--all species and all serotypes.
Shigella--all species and all serotypes.
Sphacrophorus necrophorus.
Staphylococcus aureus.
Streptobacillus moniliformis.
Streptococcus pyogenes.
Treponema careteum, T. pallidum, and T. pertenue.
Vibrio fetus, V. comma, including biotype El Tor, and V. parahemolyticus.
Yerscnia (Pasteurella) pestis.

FUNGAL AGENTS

Actinomycetes (including Nocardia species, Actinomyces species and Arachnia propionica).
Blastomyces dermatitidis.
Coccidioides immitis.
Cryptococcus neoformans.
Histoplasma capsulatum.
Paracoccidioides brasiliensis.

VIRAL, RICKETTSIAL, AND CHLAMYDIAL AGENTS

Adenoviruses--human--all types.
Arboviruses.
Coxiella burnetii.
Coxsackie A and B viruses--all types.
Cytomegaloviruses.
Dengue virus.
Echoviruses--all types.
Encephalomyocarditis virus.
Hemorrhagic fever agents, including Crimean hemmorrhagic fever (Congo), Junin, and Machupo viruses, and others as yet undefined.

tested. EPA would prefer to rely on such a list as a way to identify sources that may contain these etiologic agents. The CDC "Classification of Etiologic Agents on the Basis of Hazard," a more complete list which includes animal etiologic agents, will be used for source-identification purposes. (See Appendix VI of the regulation.)

EPA has previously defined infectious waste in "Guidelines for Thermal Processing and Land Disposal of Solid Waste," FR, August 14, 1974.(6) The definition, which is reprinted below, is felt to be unenforceable, as are most State definitions of infectious waste. Items specified in this definition would be included in the "sources," under the proposed approach. Also, this definition ignores the sewage sludge problem.

> "Infectious waste" means: (1) Equipment, instruments, utensils, and fomites of a disposable nature from the rooms of patients who are suspected to have or have been diagnosed as having a communicable disease and must, therefore, be isolated as required by public health agencies; (2) laboratory wastes such as pathological specimens (e.g., all tissues, specimens of blood elements, excreta, and secretions obtained from patients or laboratory animals) and disposable fomites (any substance that may harbor or transmit pathogenic organisms) attendant thereto; (3) surgical operating room pathologic specimens and disposable fomites attendant thereto and similar disposable materials from outpatient areas and emergency rooms.

3.9 Definitions (8, 9, 10)

For clarification the later discussions, the following definitions are provided:

ANIMAL WASTE - Waste generated from animal care or use; including bedding, egestion, excretions, secretions, tissue, remains, and any inedible by-products of animal processing for food and fiber-production.

AUTOCLAVE - An apparatus for effecting sterilization by steam under pressure. It is fitted with a gauge and a mechanical system which automatically regulates the pressure and the temperature to which the contents are subjected.

BACTERIA - Any of numerous unicellular microorganisms of the class Schizomycetes, occuring in a wide variety of forms, existing either as free-living organisms or as parasites, and having a wide range of biochemical, sometimes pathogenic, properties.

ENTERIC - of or within the intestine.

ETIOLOGIC AGENT - A viable microorganism or its toxin which causes, or may cause human disease. In the case of DOT Regulations, etiologic agents are (or are suspected to be) in relatively small concentrated samples which are shipped to special laboratories for identification.

FOMITE - An inanimate object such as an article of clothing, a dish, a toy, or a book, that is not itself corrupted but is able to harbor pathogenic organisms which may by that means be transmitted to others.

PROTOZOAN - Any of the single-celled, usually microscopic organisms of the phylum or subkingdom Protozoa, which includes the most primitive forms of animal life.

RICKETTSIA - Any of various microorganisms of the genus Rickettsia, carried as parasites by many ticks, fleas, and lice. Transmitted to man, they cause diseases such as typhus, scrub typhus, and Rocky Mountain spotted fever.

SOLID WASTE - Any garbage, refuse, sludge from a waste treatment plant, water supply treatment plant, or air pollution control facility and other discarded material, including solid, liquid, semisolid, or contained gaseous material resulting from industrial, commercial, mining, and agricultural operations, and from community activities, not including solid or dissolved material in domestic sewage, or solid or dissolved material in domestic sewage, or solid or dissolved materials in irrigation return flows or industrial discharges which are point sources subject to permits under section 402 of the Federal Water Pollution Control Act, as amended (86 Stat. 880), or source, special nuclear, or byproduct material as defined by the Atomic Energy Act of 1954, as amended (68 Stat. 923).

SEWAGE Sludge - The residue resulting from wastewater treatment.

3.10 Rationale for Regulation of Health Care Facilities Waste

The nature of waste generated by health care facilities is of concern to EPA due to a certain amount of potentially disease-contaminated materials found in the waste that are not normally found in other institutional solid wastes. Some studies have stated that the type and numbers of bacteria and viruses found in health-care solid waste are little different from that found in wastes generated from dwelling units, offices, factories and other institutions. Other researchers have given a completely opposite view and stated that health care facility wastes may be potentially dangerous to the environment due to their infectious content. (11)

Both hospitals and veterinary hospitals (for more specific breakdown by Standard Industrial Classification Code see §§250.14 (b) of the regulations) are health care facilities that are considered to be generators of infectious waste for purposes of the regulation. EPA realizes that there are different problems associated with the infectious wastes from the treatment of people vs. animals and by no means does the Agency intend to imply that these two types of health care facilities generate the same types and amounts of waste or should treat or dispose of their wastes by the same methods. A discussion of each type of health care facility and sources of waste associated with them are given below.

Hospitals

Theoretically, the difference between the biological hazard of waste generated in hospitsls, with their population of "sick" people, and the waste generated by dwelling units

incomplete at the time. It is these areas that infection potential of most waste is unknown. So, at some point, there is a reasonable possibility that infectious wastes can be intermixed with other wastes.

Three surveys have been made which cover quite extensively hospital practices with regard to waste collection and disposal (Iglar and Bond, 1971; (13) Burchinal and Wallace, 1971; (14) Esco/Greenleaf, 1972 (15)). The main interest, however, has been in evaluating the overall waste collection and disposal systems, with infectious wastes being considered as only one aspect of the overall situation. This section is concerned with discussing the infectious wastes which are identified in the literature.

The composition of infectious wastes is well known. They include items from surgery such as dressings, contaminated disposable items, drapes, and human tissue (amputated limbs, tissues, organs, placentas); items from pathology and the laboratory such as tissues, chemicals, bacteriological cultures, urine, blood, and feces; animal remains and biological specimens; and general infected material from the wards such as gauze dressings and bandages, swabs, plaster casts, sputum cups, paper tissues soaked with nose and throat secretions, and wound drainage.

Some authors distinguish between "pathological" wastes and "hazardous" or "infectious" wastes (Litsky, et al., 1972). (16) They call "pathological" materials those from surgery, laboratories, etc., and "hazardous" waste everything else--everything

those traditionally considered to be sources of infectious waste, but also ward areas, doctors' offices, outpatient clinics, and treatment rooms. Infectious waste averaged 43 percent of the total waste in the hospitals studied, and the general patient care areas generated almost three quarters of this infectious waste.

A survey in California (Anon, 1972b) (18) concluded that it was possible to safely separate and collect infectious waste within a hospital, but this does result in increased costs of waste handling. With an average total waste per patient day of 10.25 lbs., the average infectious waste measured was only 0.38 lbs.

Investigations by Bond and Michaelson (1964)(19) on the effects of waste handling upon air and surface contamination give some indication of what types of contamination to expect. They found that soiled laundry handling had by far the most significant influence on increased airborne bacteria.

Further investigations have been carried out on the solid waste itself. Armstrong (1969) (20) looked at refuse chutes with respect to airborne bacteria. He found that placing the refuse in bags reduces the number of airborne bacteria generated, and that the possibility exists for the transmission of viable organisms to other parts of the hospital by way of the refuse chute.

Research at the University of West Virginia Medical Center (Burchinal and Wallace, 1971; (14) Wallace, et al., 1972; (21) Smith, 1970; (22) Trigg, 1971 (23)) revealed that pathogenic organisms can be present in hospital solid waste in significantly high concentrations, and especially so if an organic substrate is present. Coliform counts ranged from less than one per gram of refuse at some stations to as high as 8.6 per gram. Fecal

taminated with viruses to established recovery times and rates. Vaccinia, Polio 1, Coxsackie A-9, and Influenza PR-8 were the viral strains used for inoculation. Paper and cotton fabric both held active viruses for long periods of time--from 5 to 8 days in most cases. Virus titer decreased in most cases at a steady rate with increasing time, implying that the agent loses its viability upon incubation.

An air samplying program was carried out at the Los Angeles County-USC Medical Center (Esco/Greenleaf, 1972).(15) Results are given in Table 9 and substantiate the earlier findings of Bond and Michaelson that laundry handling does generate considerably greater aerosols than does trash handling.

Estimates of the total waste generated by hospitals vary widely, ranging from about 10 lbs/patient/day to as much as 40-50 lbs/patient/day (Litsky, et al., 1972; (16) Oviatt, 1969;(24) Wallace, et al., 1972; (21) Anon, 1972b(18); Small, 1971(25)). Tables 10 and 11 give a breakdown of the types of wastes generated and the disposal costs for seven California hospitals. The great variation is caused by the quantity of disposable items used. The trend has been toward greater use of disposables because of decreased danger of cross-infection and supposedly greater economy. It has now become evident that "disposables" are really merely "throw-aways"; and their actual disposal presents a large problem. Even the cost advantage is open to question; Table 12 indicates that disposables cost more to handle and dispose of than reusables.

Table 10

Breakdown of Daily Waste Production (Lbs/Day) By Types of Wastes (Esco/Greenleaf, 1972)

Type of Waste	LAC-USC Medical Center	Long Beach General Hospital	Harbor General Hospital	Ranchos Los Amigos Hospital	John Wesley Hospital	Olive View Hospital	Mira Loma Hospital
# of Beds	3000	428	715	1188	259	725	232
Sharps, Needles, Etc.	75	3	22	40	8	20	5
Path. & Surgical	1000	trace	156	4	115	6	trace
Soiled Linen (Reusable)	45,000	3,740	13,600	16,320	2,900	5,630	1,120
Rubbish	16,200	540	6,569	2,760	717	1,722	362
Reusable Patient Items	trace	trace	trace	trace	trace	trace	trace
Non-combustibles	1,500	75	465	725	80	250	80
Non-grindable (a) Garbage	1,800	150	660	875	160	475	110
Food Service Items (Reusable)	9,000	1,400	2,400	4,200	800	2,500	600
Radiological	trace	—	trace	trace	—	trace	—
Ash & Residue	trace	—	20	20	50	20	25
Animal Carcasses	25	—	220	20	10	23	—
Food Waste (Grindable)	2,600	330	950	1,100	210	1,860	150
Total Production	77,700	6,238	25,062	26,064	5,050	12,506	2,452

Table 11

Annual, Daily, and Unit Operating Costs (Esco/Greenleaf, 1972)

	LAC-USC Medical Center	Long Beach General Hospital	Harbor General Hospital	Rancho Los Amigos Hospital	John Wesley Hospital	Olive View Hospital	Mira Loma Hospital
Quantity of Waste Produced							
Disposables (Tons/Day)	11.60	0.55	4.53	2.77	0.68	2.19	0.37
Reusables (Tons/Day)	27.25	2.57	8.00	10.26	1.85	4.06	0.86
Total Waste (Tons/Day)	38.85	3.12	12.53	13.03	2.53	6.25	1.23
Cost of System Operation							
Annual	$2,396,850	$223,600	$777,435	$656,340	$296,582	$750,585	$175,200
Daily	$ 6,566	$ 612	$ 2,130	$ 1,798	$ 813	$ 2,056	$ 480
Average Daily Cost per Ton							
Disposables	$ 305	$ 325	$ 327	$ 364	$ 664	$ 516	$ 551
Reusables	110	168	82	77	195	229	322
Total Wastes	170	197	170	168	321	329	390
Average Daily Cost/Bed Patient [Calculated based on total number of patients not total number of beds].							
Disposables	$ 1.76	$ 0.58	$ 2.73	$ 1.09	$ 2.65	$ 2.02	$ 1.42
Reusables	1.49	1.44	1.21	.85	2.13	1.65	1.91
Total Wastes	3.25	2.02	3.94	1.94	4.78	3.67	3.33

Disposable items are found in all the areas of the hospital, and have special application in burn therapy, aseptic techniques, and isolation cases. Typical items are found in Table 13. They are combinations of materials such as paper, plastic, rayon, acrylic, cellulose, nylon, glass and metal. The plastic content is much higher than the 2-3 percent found in municipal solid waste; one study of infectious waste found it to be 11.42 percent hard plastic and 7.09 percent soft plastic (Anon, 1972b).(18) Expenditures have risen from $30 million in 1966 to $126 million in 1970, and may rise to an estimated $900 million in 1978 (Fahlberg, 1973).(26) Further estimates say that a hospital can double its waste output by completely switching to disposable linen (Salkowski, 1970).(27) Disposables add two problems to the waste treatment process; first they increase the volume so that disposal systems are taxed and second the plastic components are hard to degrade. Also, it may be that some plasticizers are toxic. The John Hopkins School of Hygiene and Public Health in Baltimore has found that plasticizers in blood bags leach into the stored blood and go on to lodge in lungs, spleen, liver, and abdominal fat. Tests of embryonic heart cell cultures revealed that the cells died when plastic tubing was substituted for rubber (Anon, 1971b). (28)

When a simple a change as supplying paper towels to each patient's room was made at the Baylor University Medical

Center, it was found an additional wastebasket was then required. The maintenance cost from plugged toilets increased, and the labor charge for emptying and washing wastebaskets increased by 30 percent, but the number of cloth towels used did not decrease (Paul, 1964).(29) The pure bulk of the disposables presents the problem that most authors comment on, but other hazards are also present. Discarded needles and cutting edges remain a hazard to collection personnel. Scavenging of the dumping areas for useable items and play items for children show that spread of infectious disease is a real hazard in the disposal of disposables (Walter, 1964; (30) Mattson, 1974 (31)). Disease organisms can also be introducted to a landfill in great quantities via disposable linens and diapers (Ostertag and Junghaus, 1965; (32) Peterson, 1974 (33)).

Some indication of the numbers of disposable hypodermic needles used by individual hospitals can be obtained from the literature. Michaelson and Vesley (1966)(34) found from 14,000 to 833,000 used annually at various hospitals in 1966, and Baker (1971)(35) found over 550,000 used annually in 1968. There are proper ways to collect and destroy these items, such as collecting them at the individual nursing stations and returning them to central storage to be crushed and broken into fragments, then incinerated. They can also be collected in special boxes and sent directly to the incinerator, or collected at the nursing stations and sent to central service to be autoclaved and melted into one mass (Paul, 1964). (29) Some hospitals have even tried

Veterinary Hospitals

While veterinary hospitals have some of the waste disposal problems which hospitals caring for people have, these problems are mainly confined to disposing of dead animals, animal waste, and waste generated during treatment of animals. Animal waste includes waste generated from animal care or use, including excretions, secretions, tissue, remains, and any inedible by-products of animal processing for food and fiber production.

It has been pointed out to the Agency that the majority of diseases that could be transmitted through improper disposal of veterinary hospital waste are primarily ones that are transmitted only from animal to animal. It is true that several hundred diseases are transmitted from animal to animal, but more than 150 zoonotic diseases are transmitted between animals and man.

Decker and Steele (38a) report the human health problems that are created by pathogenic zoonoses. Some of the most significiant bacterial zoonoses are salmonellosis, staphlococcal and streptococcal infectious, tetanus, tuberculosis, brucellosis, leptospirosis, and colibacillosis. Animal wastes also play a significant role in the distribution of fungal diseases by providing nutrients for the survival and growth of fungi in man's environment.

soil associated with infected animals. Inhalation anthrax results from inhalation of anthrax spores. Gastrointestinal anthrax arises from ingestion of contaminated undercooked meat. Anthrax spreads among herbivorous animals through contaminated soil and feed and among omnivorous animals through contaminated meat, bone meal or other feeds. Biting flies and other insects are suspected of serving as vectors. Vultures have spread the organism from one area to another. The spores of Bacillus anthracis, the infectious agent, which resist environmental factors and disinfection, remain viable in contaminated areas for many years after the source-animal infection has terminated. (39)

Initial symptoms of inhalation anthrax are mild and non-specific, resembling common upper respiratory infection; acute symptoms of respiratory distress, fever and shock follow in from 3 to 5 days, with death shortly thereafter.

Gastrointestinal anthrax is more difficult to recognize, except that it tends to occur in explosive outbreaks; abdominal distress is followed by fever, signs of septicemia, and death in the typical case.

Untreated cutaneous anthrax has a fatality rate of from 5-20%, but with effective antibody therapy, few deaths occur. (39)

Salmonellosis

Although this disease is discussed in the section on sewage sludge, the important role that animals play in the transmission of the disease shall be stressed here.

Tuberculosis

Tuberculosis must still be considered as an important disease related to animal wastes. While bovine tuberculosis caused by Mycobacterium bovis has been effectively controlled in this country, it is occasionaly found in some wild animals, as well as in food animals and in pets.

Mycobacterium tuberculosis, the human type of tubercule bacillus, is capable of infecting cattle swine, and household pets.

Mycobacterium avium, the etiologic agent of tuberculosis in gallinaceous birds, is capable of producing tuberculosis in swine and of infecting cattle to such an extent that reactions are produced in routine tuberculin testing of cattle.

The bovine tubercle bacillus is transmitted to man through respiratory secretions, feces, and milk. In those few cases where infection of man with the bovine tubercle bacillus is known, there usually is an occupational contact with cattle. (38)

Brucellosis

Brucellosis is commonly an occupational disease of those with close contact with cattle and swine and their viscera and excreta. The disease in man and animals is caused by any one of three species of Brucella.

species host the leptospira, including the domestic food-producing species. Cattle and swine are the principal domestic animals involved--leptospirosis occurs in epizootic form in stables and feedlot herds. Dogs and rodents are frequently infected.

Leptospirae are transmitted from the animal host to man through a number of routes. Documented sources of human infection are rice fields, swimming "holes", sewers, and a number of occupations in which exposure to infected animals is by direct contact. (38)

The disease in man shows a wide range of symptoms and severity, depending on the species of leptospira involved, exposure, and the health of the individual. It presents symptoms similar to influenza, enteric viral infections, infectious gastroenteritis, and a number of other diseases. Fatality is low, but increases with advancing age and may reach 20% or more in patients with jaundice and kidney damage. (39)

Tularemia

The reservoir for Tularemia is normally wild animals, but is occasionally found in sheep. Mode of transmission is by inoculation of the skin, conjunctival sac or anal mucosa with blood or tissue while handling infected animals, as in skinning, dressing, or performing necropsies; or by fluids from infected flies, ticks, or other animals, or through the bite if arthropods including a species of deer fly. The

3.11 Rationale for Regulation of Laboratory Waste

Data are generally not available that can be used to show evidence of disease associated with laboratory waste. In a recently published study at the University of Texas (Pike, 1975) (42), some waste/disease data can be extracted from the 50-year data base of published and unpublished cases of laboratory-associated infections.

As shown in the reproduced table (Table 7), 46 cases of laboratory-acquired infections related to the (waste) source of discarded glassware are shown. Of these cases, 34 were related to bacteria, 10 related to viruses, and 2 to rickettsiae. Of the total number of reported laboratory-associated infections studied, the 46 associated with discarded glassware represent about 1% of the total.

The Center for Disease Control has determined that certain microorganisms are of potential hazard to human health and the environment, as published in the "Classification of Etiologic Agents on the Basis of Hazard." Since it has been determined by HEW that classes 2 through 5 are of potential hazard, then any laboratory dealing with these agents would be generating a potentially hazardous, infectious waste. Given that most hospitals and laboratories know which organisms are used in their work, the list is appended

TABLE 7 - Distribution of Cases According to Proved or Probable Source of Infection

Sources	Agents							
	Bacteria	Viruses	Rickettsiae	Fungi	Chlamydiae	Parasites	Unspecified	Total
Accident	378	174	45	33	14	38	21	703
Animal or ectoparasite	149	249	66	151	32	11	1	659
Clinical specimen	90	175	2	1	0	19	0	287
Discarded glassware	34	10	2	0	0	0	0	46
Human autopsy	56	9	4	0	0	1	5	75
Intentional Infection	14	1	0	0	0	4	0	19
Aerosol	101	92	217	88	22	2	0	522
Worked with the agent	381	213	100	62	43	28	0	827
Other	7	1	7	0	1	0	0	16
Unknown or not indicated	459	125	130	18	16	12	7	767
Total	1669	1049	573	353	128	115	34	3921

In this bulletin general requirements for land application of sludges are given. Reference is made to "Process Design Manual for Sludge Treatment and Disposal" (EPA 625/1-74-006; October 1974) which specifies in more detail the techniques for sludge stabilization.

The bulk of the information presented in this section of the background document is identical to that presented in the background document for §§257.4-5 (Land Criteria) to be used for Section 4004 of RCRA. (45) Section 4004 regulations will require sewage treatment plant sludge to be "stabilized" to "reduce public health hazards."

Pathogenic organisms occuring in sewage sludge cover a wide variety of bacteria, viruses and intestinal parasites. Their individual presence, as well as their numbers, will vary considerably from community to community depending upon rates of disease in the contributing population. (46) Routes of infection to humans and animals from sewage sludge may be through direct contact with contaminated environments or through the ingestion of contaminated food and water.

Bacteria

Among the bacteria that are commonly found in sewage sludge, is the group referred to as the "enteric bacilli" that naturally inhabit the gastronintestinal tract of humans. In their virulence for humans, the enteric baccilli fall into three general categories: pseudomonas species, salmonella species, and shigella species.

Shigella

The third category of enteric bacteria is the Shigella genus. The shigella cause in humans a disabling disease known as bacillary dysentery. This is an acute infection of the large intestines, resulting in diarrhea, which, if sufficiently severe, may be accompanied by bleeding from the colon. All known species of the genus Shigella are pathogenic for humans, with the following being the most common: S. dysenteriae, S. flexneri, and S. sonnei.

None of the enteric bacilli form spores. Spores are resistant bodies produced by large number of bacterial species that enable them to withstand unfavorable environmental conditions such as heat, cold, desiccation and chemicals. Since enteric bacilli are not spore formers, their survival span outside of their normal environment (human intestinal tract) is usually measured in days or months, compared to years for spore forming bacteria. Most sludge stabilization processes would create an unfavorable environment for enteric bacilli to survive.

A pathogenic bacterium frequently found in sewage sludge, although not an enteric organism, is the tubercle bacillus Mycobacterium tuberculosis. This organism is responsible for nearly all cases of pulmonary tuberculosis. Tubercle bacilli are very hardy organisms, and can withstand fairly extreme environmental conditions.

Infectious hepatitis is an acute infectious disease that causes fever, nausea, abdominal discomfort, followed by jaundice. It is caused by a resistant virus. The Hepatitis virus is shed from the body through the feces, and fecal-oral spread is probably the most common method of transmission.

Parasites

The third group of pathogenic organisms found in waste water treatment sludges are the intestinal parasites. Those parasites of concern to humans can be subdivided into two categories: (1) Protozoa, and (2) Helminths. Subgroups of the Protozoa group include amoebas, flagellates, and ciliates. Subgroups of the Helminths include trematodes and nematodes.

Protozoa

At least five species of amoebae live in the intestinal tract of humans, with Entamoeba histolytica being the only proven pathogen. Infection with E. histolytica may produce chronic diarrhea, amoebic hepatitis, abscess of the liver, brain, lung, and ulceration of the skin. Amoebae have two stages in their life cycles, a mobile form and a cyst form. The cysts are infective upon passage from the body, and are survive in a moist and cool environment. Giardia lamblia, another protozoan, is also found in sewage sludge. Like the amoeba, G. lamblia is a parasite of the human intestinal tract and is responsible for certain conditions such as diarrhea or symptoms referable to the gall bladder.

Balantidium coli is the only ciliate human parasite and is the largest of human protozoan parasites. It invades

solium, I. saginatta, and Hymenolepis nana. With the exception of the species of Hymenolepis, infection with the common human species results from eating raw or imperfectly cooked beef, pork, or fish in which the larvae have developed. Hymenolepis sp. on the other hand, need no intermediate host. It is able to complete its entire life cycle in a single host; thus, when eggs are ingested by man, the larvae migrate into the lumen of the intestine.

Numerous studies report that pathogenic organisms present in sludge are either killed or greatly reduced in number when exposed to various stabilization methods used.

The specific number of an organism necessary for the establishment of the potential for disease is related to various factors; etiologic agent, susceptibility of host etc. However, there is evidence that with many pathogens this dose may be rather high, in particular the enteric pathogens. DuPont et. al (49) reported that approximately 10^5 Salmonella cells (including S typhi) are required to cause a disease. This would tend to support the premise that by reducing the number of pathogenic organisms in sludge, the public health hazards associated with its use would be greatly minimized.

A review of the literature (7) has shown that there is a paucity of epidemiological data linking disease transmission of humans and animals directly to the landspreading of wastewater treatment sludges. The data that do exist, indicate

The stabilization process will reduce the pathogen population in sludge; the level of reduction will vary with the process used and numerous other variables, e.g., time, temperature, pH etc. Since available epidemiological evidence links disease transmission to the landspreading of unstabilized sludge and not stabilized sludge, it is evident that there is a correlation between the concentration of pathogens in the sludge and disease transmisssion.

Wastewater sludge stabilization is normally accomplished by anaerobic and aerobic digestion, and lime treatment. Lesser used methods include heat treatment, ponding and long time storage, chlorination, and composting. The stabilization of sludge by thermal irradiation is being addressed, but at this time the process is still in the experimental state.

As previously mentioned, the extent to which pathogenic organisms are reduced is related to the stabilization process used as well as other variables. Not all stabilization processes affect pathogenic organisms in the same manner, therefore, some processes are more effective in reducing the pathogen population than others. Also the levels of stabilization within a particular process will vary as to their effectiveness in reducing pathogenic organism numbers, e.g., anaerobic digestion of sludge for a two week period in the

the eggs of A. lumbricoides 0 to 45 percent.

Two groups (58,59) observed that there was 90 and 69 percent diminution of tubercle bacilli, while two others (60,61) noted "survival" of M. tuberculosis after anaerobic digestion.

McKinney et. al(62) found in their studies that approximately 93 percent of S. typhosa were removed after being exposed to anaerobic digestion process for 20 days. Kenner (63) reported that sludge treated by anaerobic digestion has been shown to contain Salmonella and Pseudomonas organisms.

Cram (54) reported from his studies, that activated sludge treatment does not affect the viability of E. histolytica cysts or ascarid eggs. Aeration in the activated sludge process for 5 months showed no effect on ascarid eggs except a slow reduction in numbers (64), Kabler (53) reported that studies indicate that activated sludge reduced S. typhosa and strains of bacilli 91 to 99 percent.

Enteric virus inactivation during the treatment of wastewater by the activated sludge process has been reported extensively in the literature. (65-70) Carlson (71) et al reported that after 6 months of aeration, polioviruses were removed or inactivated to a point at which infectiousness for mice was greatly reduced. Sproul (72) reported that virus removal of 90 percent or more has been obtained in a number of studies with activated sludge process. Kelly et al (73) reported that Coxsackie virus survived activated sludge treatment.

Table 4

Removal of viruses by bench scale activated sludge units

	Coxsackie virus A9		Poliovirus 1	
Test No.	Volatile solids (mg/l)	Virus Inactivated (Percent)	Volatile solids (mg/l)	Virus Inactivated (Percent)
1	600	98.8	200	79
2	650	96.1	400	88
3	1,000	99.2	600	90
4.	1,100	99.1	600	91
5	1,500	97.4	1,200	92
6	1,500	99.4	1,200	91
7			4,000	94

Bacterial inhibition from caustic conditions has long been known. (74) Studies have shown that Salmonella typhosa did survive in concentrations in the range of pH 11.01-11.50 longer than two hours, while Shigella dysenteriae was destroyed rapidly in all pH range studies; pH 11.01-11.50 produced 100% kill in 75 minutes. (75) However, the effectiveness of lime treatment on parasitic ova and viruses has not been demonstrated.

to sludge treatment are low pressure oxidation, heat drying and pasteurization. During the low pressure oxidation (LPO) process, the sludge temperature is elevated to between 350 and 400 F, pressure is raised to 180 to 210 psi, and the retention time is between 20 and 30 minutes. The process kills all pathogenic organisms due to the high temperature achieved and the retention time. Over 26 U.S. cities are currently using the LPO process.

Heat drying of sludge is presently being carried out in a number of U.S. cities. However, the numbers are declining because of cost of fuel necessary for the drying process, and also because the market for heat dried sludge did not develop as hoped. The temperature achieved during the heat drying process kills most bacteria.

Pasteurization is a process where the sludge is heated to a specific temperature for a period of time that will destroy pathogenic organisms. In most cases this is accomplished by the use of steam. Currently, pasteurization is used only in Europe.

While the technical literature presents some conflicting data as to the degree that pathogenic organisms are reduced by various sludge stabilization methods, it does generally indicate that the stabilization process will reduce most pathogenic organisms significantly. This reduction, in turn minimizes the public health risks associated with the landspreading of stabilized sludges.

in garden soil. Gudzhabidze (91) reported in the Soviet Union that Ascaris ova survived 2-5 years in soil of irrigated agriculture fields. The literature reviewed does not reveal any studies in the United States where Ascaris ova survived in sludge amended soils for more than one year.

Hess et al.(92) reported the survival of salmonellae on grass contaminated with sludge for 40 to 58 weeks in a dry atmosphere. McCarty and King (93) found that enteric pathogens could survive and remain virulent for up to two months. Rudolfs et. al. (94) concluded from field studies that the survival of representatives of the Salmonella and Shigella genera on tomato surfaces did not exceed seven days, even when the organisms were applied with fecal organic material. He attributed their short survival time to the lack of resistant stages; thus making them more vulnerable to adverse environmental conditions.

Martin (95), inoculating sterile virgin soils with E. typhosa, found they died out rapidly, but in sterilized contaminated soils growth occurred and the bacteria survived for numerous months. Rudolfs (94) in his literature review, found that the survival time of E. typhosa ranged from less than 24 hours to more than two years in freezing moist soils, but generally less than 100 days.

Approximately 90 different enteric viruses have been recovered from municipal sewage. However, there are few

Table 6

Survival times of Pathogenic Microorganisms in various media (89)*

Organisms	Medium	Type of Application*	Survival time
Ascaris Ova	Soil	Not stated	2-5 years
	Soil	Sewage	Up to 7 years
	Plants and Fruits	AC	1 month
Endamoeba Histolytics cysts	Soil	AC	8 days
	Tomatoes	AC	18-42 hours
	Lettuce	AC	18 hours
Enteroviruses	Roots of bean plants	AC	At least 4 days
	Soil	AC	12 days
	Tomato & pea roots	AC	4-6 days
Salmonella	Strawberries	AC	6 hours
	Soil	AC	74 days
	Soil	AC	70 days
	Soil	AC	At least 4 days
	Pea plant stems	AC	14 days
	Radish plant stems	AC	4 days
	Soil	AC	Up to 20 days
	Lettuce & endive	AC	1-3 days
	Soil	AC	2-110 days
	Soil	AC	Several months
	Lettuce	Infected feces	18 days
	Radishes	Infected feces	53 days
	Soil	Infected feces	74 days
Salmonella, other than typhi	Soil	AC	15-70 days
	Vegetables	AC	2-7 weeks
	Tomatoes	AC	Less than 7 days
	Soil	Sprinkled with domestic sewage	40 days
	Potatoes	Sprinkled with domestic sewage	40 days
	Carrots	Sprinkled with domestic sewage	10 days
	Cabbage and gooseberries	Sprinkled with domestic sewage	5 days
Shigella	Streams	Not stated	30 minutes to 4 day
	Harvested Fruits	AC	Minutes to 5 days
	Market tomatoes	AC	At least 2 days
	Market apples	AC	At least 6 days
	Tomatoes	AC	2-7 days
Tubercle Bacilli	Soil	AC	6 months
	Grass	AC	14-15 months

*Artifical Contamination

published reports on the survival of viruses in soil, and persistence on crops. Larkin et al. (96) described the persistence of polioviruses for 14 to 30 days on lettuce and radishes inoculated with sludge. According to Cliver (97) the soil is generally not a very adverse environment for viruses. Neither chemical nor biological inactivation occurs very rapidly, but enteroviruses do lose infectiousness as a function of time and temperature in the soil. Poliovirus 1, retained in sand from septic tank effluent, was inactivated at a rate of 13 to 18 percent per day at 20 to 25 C and at 1.1 percent per day at 6 C to 8 C. (97)

Rudolfs et al. (94) reported that unlike pathogenic bacteria, the parasitic amoeba, Endamoeba histolytica, forms resistant cysts which enable the organism to survive under adverse conditions. However, on the basis of laboratory and field studies on the survival of Endamoeba histolytica cysts, the cysts proved to be extremely sensitive to desiccation. Rudolfs concluded from his studies that field-grown crops contaminated with cysts of E. histolytica are considered safe in the temperate zone one week after contamination has stopped and after two weeks in wetter tropical regions.

It has been shown in the general survey of the literature (94) that certain parasite eggs, especially those of Ascaris, are markedly resistant to external conditions. Yoshida (98) found that mature eggs of A. lumbriocoides were still viable after five to six months under layers of soil in winter. He

3.13 Methods for Biological Examination of Solid Waste Bacteria

Mirdza L. Peterson of EPA has published "Methods for Bacteriological Examination of Solid Waste and Waste Effluents." (104) After examining methods currently available for measuring the bacteriological quality of solid waste, reliable methods were established which are best suited to routinely measure, under practical conditions, the bacteriological quality of solid waste in and around waste processing areas. These methods were not developed to be an all-inclusive battery of tests for microorganisms in solid waste; rather, these methods test for only a few of the possible microorganisms in the solid waste.

Three procedural lines of investigation were undertaken in this effort: (1) to develop methods suitable for indicating the sanitary quality of solid waste before and after processing or disposal; (2) to develop methods suitable for determining the efficacy of operational procedures in removing or destroying the microorganisms; and, (3) to develop methods suitable for indicating the health hazard of solid waste in which pathogenic species may be present in small numbers. Methods presented in this publication are ones for determining: total viable bacterial cell number, total coliforms, fecal coliforms, heat-resistant spores, and enteric pathogens, especially Salmonella sp.

The determination of approximate total viable bacteria multiplying at a temperature of 35 C may yield useful information concerning the sanitary quality of a waste entering a processing or a disposal site, and provide useful information in judging

a long exposure time (1-1/2 to 2 hr), even in an autoclave (121 C) to be heated throughly so that the center reaches a sporocidal temperature. Other reports (107) point out that although internal air temperatures of municipal incinerators usually range from 1200 to 1700 F (650 to 925 C) in continuous operation, intermittent use, overcharging of the incinerator, and high moisture content of the waste may slow the process and interfere with sterilization of the residue.

Fecal pollution of the environment by untreated and improperly disposed waste may add enteric pathogenic bacteria to a body of water or a water supply. The most common type of pathogen which may be found in untreated waste is Salmonella. The wide distribution of the many types of Salmonella in many species of animals with which man has contact or may use as food makes it difficult to prevent transmission to man. (108) Infections may occur through food, milk, or water contaminated with infected feces or urine, or by the actual ingestion of the infected animal tissues. (109) Salmonella has been found in many water supplies (110), polluted waters (111-113), raw municipal refuse and in incinerator residue (111-117)

General laboratory procedures, sample collection and preparation procedures, and bacteriological examination procedures for the organisms mentioned above can be found in Appendix A-3.1.

Parasites

The FDA has recently prepared a methodology for Ascaris determination in vegetable and sludge samples (118). The

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APPENDIX A-3

Methods for Biological Examination of Solid Wastes

Culture media.

The use of dehydrated media is recommended whenever possible, since these products offer the advantages of good consistency from lot to lot, require less labor in preparation, and are more economical. Each lot should be tested for performance before use.

Measurement of the final pH of a prepared culture medium should be accomplished colorimetrically after autoclaving and cooling. Acceptable pH range is 7.0 ± 0.1.

Media should be stored in a cool, dry, and dark place to avoid dehydration, deterioration, and adverse light effects. Storage in the refrigerator usually prolongs the shelf-life of most media. Media should not be subjected to long periods of storage, because certain chemical reactions may occur in a medium even at refrigerator temperatures.

Many of the media referred to below can be obtained from commercial sources in a dehydrated form with complete information on their preparation. These media will therefore be listed but not described in this section. Described in this section are those media that are formulated from ingredients or from dehydrated materials. Culture media (Difco or BBL products) are listed as follows:

Bacto-agar
Bismuth sulfite agar
Blood agar
Brain heart infusion broth
Brilliant green agar
Brilliant green lactose bile, 2 percent
Coagulase mannitol agar
Dextrose
E. C. broth
Eosin methylene blue agar, Levine
Fluid thioglycollate medium
Gelatin
H-broth
Indole nitrite medium
KCN medium

COLLECTION AND PREPARATION OF SAMPLES

Method for Collection of Solid Waste or Semi-Solid Waste Samples

Equipment and materials.

Necessary items are as follows:

1. Sample containers, specimen cups, sterile, 200-ml size (Falcon Plastics, Los Angeles)
2. Sampling tongs, sterile (stainless steel, angled tips, 18 in. long)
3. Shipping container, insulated, refrigerated, 6 by 12 in. I.D.
4. Disposable gloves

Procedure.

1. Using sterile tongs, collect 20 to 40 random 100- to 200-g samples and place in sterile sampling containers. When collecting samples from contaminated sources, wear disposable gloves and avoid contaminating the outside of the container.
2. Identify samples on tag and indicate time and date of sampling. If incinerator residue samples are taken, record operating temperatures of incinerator.
3. Deliver samples to laboratory. It is recommended that the examination be started preferably within 1 hr after collection;* the time elapsing between collection and examination should in no case exceed 8 hr.

Method for Collection of Liquid Samples-Quench and Industrial Waters or Leachate

Equipment and materials.

Necessary items include a screw-capped, 250-ml, sterile sample bottle or a 16-oz, sterile plastic bag.

Procedure.

Collect sample in bottle or plastic bag, leaving an air space in the container to facilitate mixing of the sample before examination. When collecting samples from contaminated sources, wear disposable gloves and avoid contaminating the outside of the container.

Identify and deliver samples to laboratory. When shipping samples to laboratory, protect containers from crushing and maintain temperature below 10C during a maximum transport time of 6 hr. Examine within 2 hr. If water sample contains residual chlorine, a dechlorination agent such as sodium thiosulfate is added to collection bottles to neutralize any residual chlorine and to prevent a continuation of the bactericidal action of chlorine during the time the sample is in transit to the laboratory. Enough sodium thiosulfate is added to the clean sample bottle before sterilization to provide an approximate concentration of 100 mg per liter in the sample.

*If sample is shipped to a laboratory for analysis and examination cannot begin within 1 hr of collection, the container must be insulated and sample maintained below 10 C during the maximum transport of 6 hr. Such samples should be refrigerated upon receipt in the laboratory and processed within 2 hr.

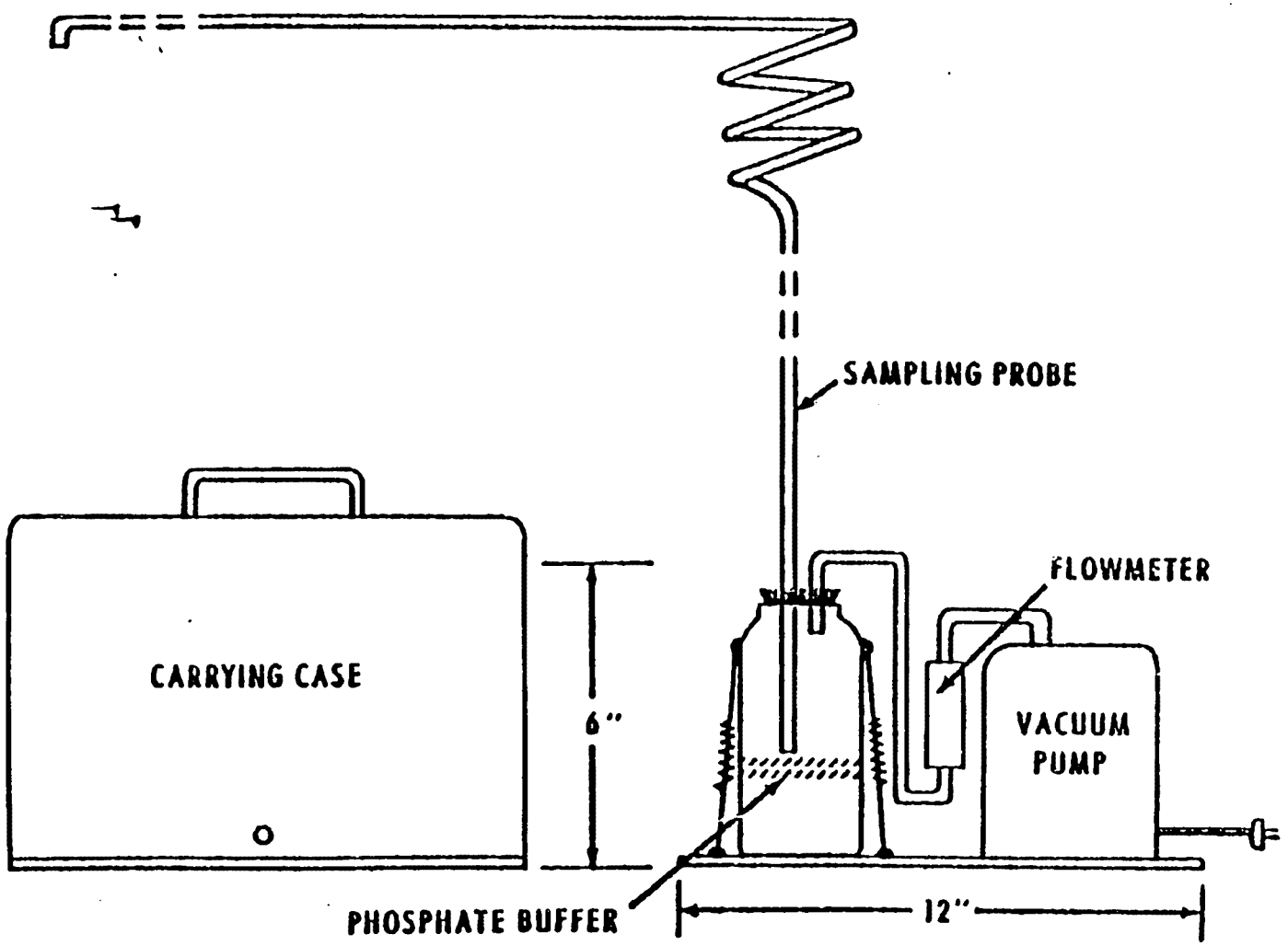

Figure 1. Portable sampler for microorganisms in incinerator stack emission.

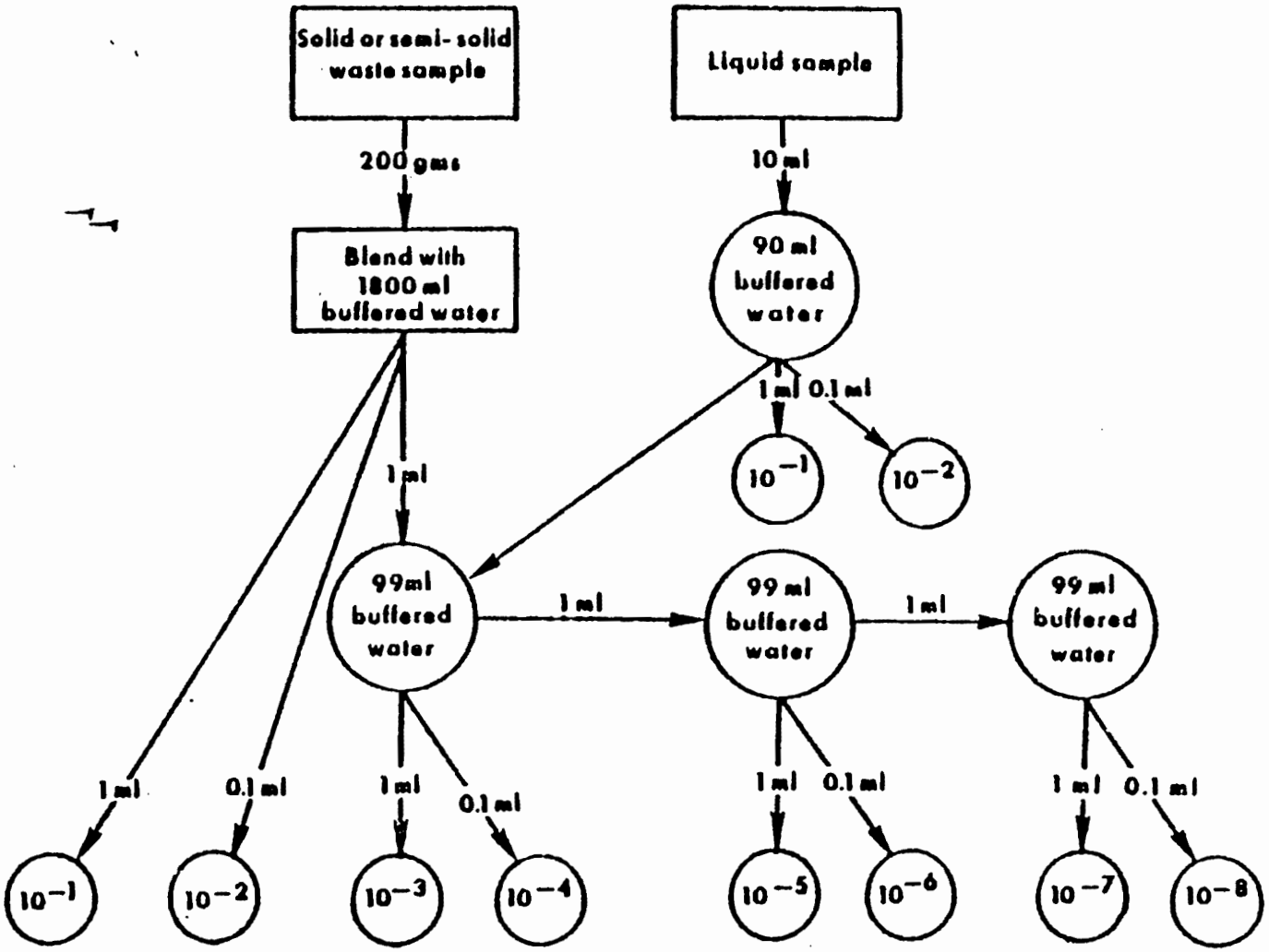

Figure 2. Preparation of decimal dilutions.

Methods for Presence of Members of Coliform Group

The presence of fecal matter in waste and related materials is determined by the standard tests for the coliform group described in Standard Methods for the Examination of Water and Waste Water (3). The completed Most Probable Number (MPN) procedure is employed. The testing method includes the elevated temperature test (44.5 C) that indicates the fecal or nonfecal origin of coliform bacteria. Comparative laboratory studies conducted showed that the MPN estimate is the most suitable method for achieving a representative enumeration of the coliform organisms in solid waste and waste effluents (9).

Equipment and materials.

1. Pipettes, sterile—deliveries to 10 ml, 1 ml (1.1 ml), and 0.1 ml
2. Media prepared in fermentation tubes:
   - Lauryl tryptose broth
   - Brilliant green lactose bile broth, 2 percent
   - Lactose tryptose broth
   - E.C. broth
3. Media for plating:
   - Eosin methylene blue agar plates
   - Nutrient agar slants
4. Dilution blanks, phosphate buffer solution, sterile, 99-ml or 90-ml amounts
5. Incubator, adjusted to 35 C ± 0.5 C
6. Water bath, adjusted to 44.5 C ± 0.2 C

Procedure for total coliform group.

Presumptive Test.

1. Inoculate a predetermined volume of sample into each of 5 lauryl tryptose broth tubes. The portions of the sample used for inoculation should be decimal multiples and submultiples of 1 ml.
2. Incubate the fermentation tubes at 35 ± 0.5 C for 24 ± 2 hr.
3. Examine for the presence of gas. If no gas is formed, incubate up to 48 ± 3 hr. Record the presence or absence of gas formation at each examination of the tubes, regardless of the amount.

Confirmed Test.

1. Submit all presumptive test tubes showing any amount of gas at the end of 24- and 48-hr incubation to the confirmed test. Using a sterile platinum loop 3 mm in diameter, transfer one loopful of medium from the presumptive test fermentation tube to a fermentation tube containing brilliant green lactose bile broth.
2. Incubate the inoculated brilliant green lactose bile broth tube for 48 ±3 hr at 35 ± 0.5 C. The presence of gas in any amount in the fermentation tube of the brilliant green lactose bile broth within 48 ± 3 hr indicates a positive confirmed test.

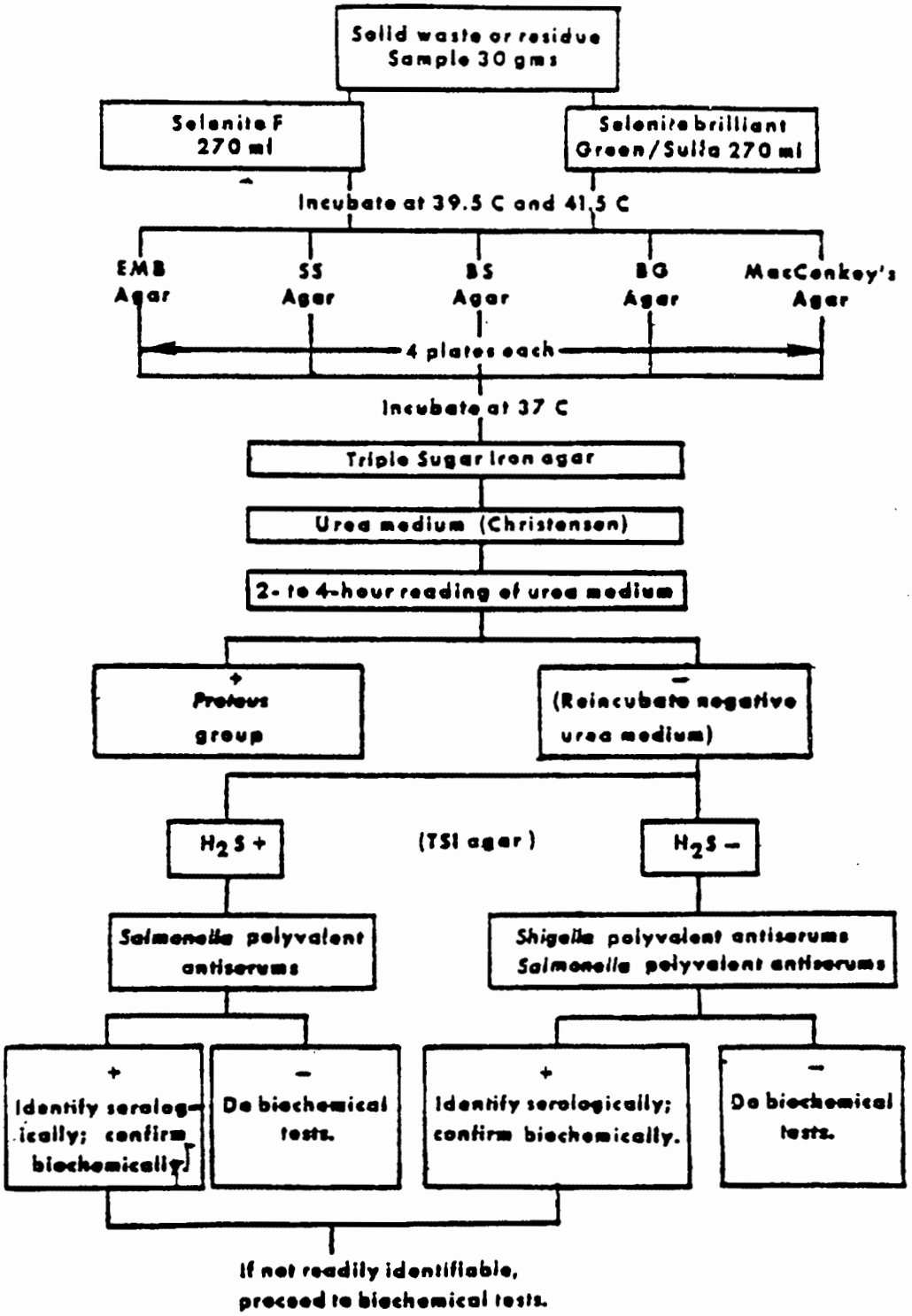

Figure 3. Isolation and preliminary identification.

3. After incubation, streak one loopful from each enrichment medium on each of four plates of Salmonella-Shigella and other selective enteric media.
4. Incubate the plates at 37 C for 24 to 48 hr and pick suspicious colonies to triple sugar iron agar slants.
5. Incubate the slants at 37 C for 24 hr and complete identification by appropriate methods as described by Edwards and Ewing (20). Isolation, preliminary identification, and biochemical testing are described in Figure 3 and in Table 2.

Procedure to detect pathogens in quench or industrial waters and in leachate.

1. Place enough sterile diatomaceous earth on the screen of a stainless steel membrane filter holder to form a 1-in. layer.
2. Filter 800-ml sample through the earth layer.
3. Remove one-half the diatomaceous earth layer with a sterile spatula and place into 90 ml of Selenite F enrichment broth; place other half of the earth layer into 90 ml of Selenite brilliant green/sulfa enrichment broth. Shake both flasks to mix.
4. Incubate both flasks in a water bath at 39.5 C for 16 to 18 hr.
5. Proceed as directed in steps 3 through 5 of Procedure to Select Pathogens in Solid Waste and Incinerator Residue.

Method for Examination of Stack Effluents

As described in Methods for Collection of Incinerator Stack Effluents (using the Armstrong sampler), the microorganisms are impinged into a 300-ml phosphate buffer solution.

Filter 100 ml of the "inoculated" phosphate buffer solution through a 0.45μ HA membrane filter (3).
2. Transfer membrane filter with sterile forceps to a culture plate containing trypticase soy agar.
3. Incubate culture plate under constant saturated humidity for 20 hr (± 2 hr) at 35 C.
4. After incubation, remove cover from culture plate and determine colony count with the aid of a low-power (10-15 magnifications) binocular, wide-field microscope. Characterize colonies using specific isolation media.
5. Remove a 10-ml portion of the "inoculated" phosphate buffer solution and examine for viable heat-resistant spores as directed in steps 1 through 6 of the procedure under Method to Determine the Presence of Viable Heat-Resistant Spore Numbers.

Microbial counts are reported as organisms per cubic foot of air. If the sample is not taken under isokinetic conditions, the results are qualitative. If the stack velocity is known and remains relatively constant, however, the flow rate of the sampler can be adjusted to isokinetic conditions to yield quantitative results.

Method for Examination of Dust

As described in Methods for Collection of Dust Samples, the Andersen sampler is used with two types of media—trypticase soy agar (TSA-BBL product) containing 5 percent sheep blood, and eosin methylene blue agar (EMB-Difco product). The TSA/blood agar is used to isolate a wider range of fastidious organisms such as *Staphylococci*, *Streptococci*, and *Diplococci*. The EMB agar is used to isolate gram-negative bacteria. The plates are incubated aerobically at 37 C for 24 hr. (Preliminary studies showed that few organisms in the dust would grow under anaerobic conditions.) Enumeration of colonies is made with a Quebec colony counter. Microbial count is reported as organisms per cubic foot of air. At times, when microbial counts are high, the sampling time is 0.25 min, thus yielding 0.25 cu ft air.

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A-3.3 DETERMINATION OF ASCARIS spp. EGGS in SOLID WASTE

1. Materials

1.1 Balance: 10 g - 1 kg capacity.

1.2 Beakers: 150 ml & 600 ml

1.3 Bottle: 125 ml, Wheaton.

1.4 Bottle shaker.

1.5 Brush: B-8695 Scientific Products.

1.6 Centrifuge: rotor radius 14.6 cm.

1.7 Centrifuge tubes: 15 ml and 50 ml.

1.8 Cheesecloth: FSN 8305-00-205-3496.

1.9 Counter: differential

1.10 Culture dish: with 2 mm grid.

1.11 Inverted microscope

1.12 Pipettes: Pasteur type and 5 ml serological.

1.13 Rubber bulb: ca. 2 ml

1.14 Tray: round, 10.5 inches diameter, 3 inches high e.g., Beckman Instrument Co. 82-018.

2. Reagents

2.1 Saline: 0.85% NaCl in H_2O.

2.2 Nacconol: 0.4% of concentrate in H_2O

2.3 Hydrochloric acid: 2% solution in H_2O.

2.4 Solvent: alcohol:acetone:xylene in 1:1:2 ratios.

3. Sample Preparation

3.1 Vegetable Samples

3.1.1 The sample size for vegetables is 1 kg. Leafy vegetables occuring in heads (cabbage, lettuce

3.2.4 Rinse the bottle 3 times with 5 ml saline and add each rinse to the beaker.

3.2.5 Transfer the contents of the beaker to seven 15 ml centrifuge tubes.

3.2.6 Rinse the beaker 3 times with 5 ml of saline and add each rinse to the centrifuge tubes.

4. Centrifugation Procedure

4.1 Centrifuge the tubes collected in 3.1 and/or 3.2 at 2,000 rpm (radius 14.6 cm) for 4 minutes.

4.2 Remove and discard the supernatant.

4.3 Add 2 ml of saline to each tube.

4.4 Combine the sediments into one tube using a Pasteur pipette to transfer the sediment and to rinse each tube 3 times with 2 ml of saline. Each rinse is also added to the collecting tube.

4.5 When the collecting tube is full, it is balanced with a blank, centrifuged at 2,000 rpm for 4 minutes; supernatant is discarded. Repeat if necessary.

4.6 Add saline to the 15 or 50 ml graduation mark on the collecting tube and resuspend the sediment; centrifuge at 2,000 rpm for 4 minutes.

4.7 Discard the supernatant; add 2 ml of saline and resuspend the sediment.

4.8 Transfer the suspension to the culture dish; rinse the tube 3 times with 2 ml of saline and add each rinse to the culture dish. Add 8 ml of the 2% hydrochloric acid to the dish (to prevent mold growth) and cover the dish.

incubation (step 6 above), fertilized eggs develop into embryonated eggs which contain a second-stage nematode larva in a cuticular sheath. Types of Ascaris spp. eggs are illustrated in the following references.

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2.6 Incubate cultures for 4 weeks, making weekly examinations (make smears of suspicious colonies; identify fungi by cultural characteristics.)

3. Actidione and chloromycetin inoculation

3.1 Prepare two tubes of Sabouraud's agar and two tubes of Sabouraud's agar containing 0.5 mg Actidione per ml and 0.05 g of chloromycetin per liter.

3.2 Inoculate with a small portion of concentrated sediment.

3.3 Incubate all tubes at 25 C and examine weekly.

3.4 At the end of 6 weeks make smears of suspicious colonies and identify by cultural characteristics.