Disinfection 101

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Introduction

Pathogenic organisms can be introduced into a veterinary clinic or animal housing facility through a variety of ways. For this reason, biological risk management (BRM) protocols are necessary to prevent, contain and eliminate the spread of disease. Disinfection protocols, when implemented correctly, can be a cost-effective means of reducing pathogenic organisms and are an important step in any biological risk management program. Prevention of disease is typically easier and more cost-effective than addressing an outbreak situation. Therefore, development and implementation of a step-by-step disinfection protocol for the control and prevention of infectious disease has become essential for farms and clinics.

Disinfection protocols may vary depending on the need of the farm or clinic. No single disinfectant is adequate for all situations. Disinfection protocols used on a daily basis will differ from those needed to control an infectious disease outbreak. However, both have one component in common; **thorough cleaning and washing prior to the application of any disinfectant is essential**.

The purpose of this handout is to provide 1) an overview of factors to consider when developing and implementing an effective disinfection protocol, 2) an overview of chemicals used for disinfection, their advantages and limitations, and 3) essential steps of an effective disinfection protocol. Following development of a disinfection plan, it is equally important to train personnel of the proper procedures to use and safety issues involved as well as to have the steps posted in prominent locations throughout the facility to serve as a reminder of proper disinfecting techniques.

Disinfectants Defined

Disinfecting agents are registered by the Environmental Protection Agency (EPA) as “antimicrobial pesticides” and are substances used to control, prevent, or destroy harmful microorganisms (i.e., bacteria, viruses, or fungi) on inanimate objects and surfaces. These antimicrobial products have traditionally included sanitizers, disinfectants, and sterilants. Data on a product’s chemistry, efficacy, toxicity to humans, animals and plants, and other parameters must be tested and submitted to the EPA prior to the marketing of the chemical.[1]

Chemical disinfectants can have various effects against microorganisms. Therefore, a basic understanding of the different chemical agents is important.

Biocide or germicide refers to chemical agents that kill microorganisms. These general terms includes disinfectants, antiseptics and antibiotics. Germicides and biocides generally react with proteins, specifically essential enzymes of microorganisms. Actions may include oxidation, hydrolysis, denaturation or substitution.[2] When a killing action is implied, the suffix *–cide* (e.g. biocide, bactericide, virucide, sporicide) is used, while *–static* (e.g. bacteriostatic, virostatic, sporostatic) is added when an organism’s growth is merely inhibited or it is prevented from multiplying.[3]

Sanitizers do not destroy or eliminate **all** bacteria or microorganisms, but reduce the number of microbial contamination on inanimate surfaces to levels that are considered safe from a public health standpoint. Many sanitizers are a formulation of a detergent and disinfectant.

Disinfectant describes a product applied directly to an inanimate object. It destroys or irreversibly inactivates most pathogenic microorganisms, some viruses, but not usually spores.[2,4,5,6] In comparison, **antiseptics** are applied to the surface of living organisms or tissues to prevent or stop the growth of microorganisms by inhibiting the organism or by destroying them.[2]

Sterilization refers to the process, either physical (i.e., extreme heat) or chemical (i.e. ethylene oxide), that destroys or eliminates all forms of life, especially microorganisms.[2,4,6]

Detergents serve to disperse and remove soil and organic material from surfaces allowing a disinfectant to reach and destroy microbes within or beneath the dirt.[2] These products also reduce surface tension and increase the penetrating ability of water, thereby allowing more organic matter to be removed from surfaces. [2] Some disinfectants have detergent properties (i.e., chlorine compounds, iodophors, QACs).

Detergents are classified in three categories: cationic, anionic and non-ionic. Cationic detergents are positively charged solutions, and with the exception or quaternary ammonium compounds (QACs), are seldom used a cleaning ingredients. Anionic detergents, or soaps, are negatively charged alkaline salts of fatty acids. They are less ideal for cleaning because they can be excessively foamy, creating a residue that may allow soil and microorganisms to accumulate. Nonionic (uncharged) detergents are very good emulsifiers, have good penetration and dispersion, are effective at lowering surface tension, and have reduced foaming properties. These products do not typically complex with metallic ions, such as those found in hard water. Most commercial detergents are a combination of anionic and non-ionic. [2]

Disinfectant Labels[7]

Product labels contain important information on the proper use and hazards of a chemical. This information may often be overlooked however **it is a violation of federal law to use a product in a manner inconsistent with its labeling**. Therefore, strict attention must be given to the proper use of a product with regard to its application, effectiveness, and associated hazards (human, animal, and environment). This information will assist in decisions for infection control efforts.

Label Claims

Disinfectants may have a range of uses and label claims, such as cleaner, deodorizer, sanitizer, disinfectant, fungicide, virucide or 'for hospital, institutional and industrial use'. Label claims are primarily determined by three test microorganisms, *Staphylococcus aureus*, *Salmonella cholerasuis*, and *Pseudomonas aeruginosa*.

- ***Limited efficacy*** is a claim of disinfection or germicidal activity against one specific microorganism group (eg. Gram-negative or Gram-positive). Gram-positive designation comes from effectiveness against *Staphylococcus aureus*, while Gram-negative bacteria claims must be effective against *Salmonella cholerasuis*. The label must specify the group against which the product is effective.
- ***General-purpose or broad-spectrum*** is a claim of effectiveness against Gram-positive **and** Gram-negative bacteria. This claim must be supported by efficacy testing against *Staphylococcus aureus* and *Salmonella cholerasuis*.

- ***Hospital or medical environment claim*** must be supported by efficacy testing against *S. aureus* **and** *S. cholerasuis* but **also** efficacy against the nosocomial bacterial pathogen, *Pseudomonas aeruginosa*.
- Claims against pathogenic fungi or other microorganisms are permitted, but not required, on the label following standardized testing procedures.

Other Important Information on a Product Label

- ***Effectiveness of Product Under Certain Conditions.*** Product testing for the EPA requires testing under "hard" water conditions up to 400 ppm hardness ($CaCo_3$) in the presence of 5% serum contamination to simulate the product's effectiveness under field conditions If the product is tested under additional conditions, it may be listed on the label.
- ***Active Ingredients.*** The active ingredients of the product are listed as percentages and are the chemicals responsible for the control of the microorganisms.
- ***Inert Ingredients.*** Inactive ingredients are often lumped into one statement and include items such as soaps or detergents, dyes or coloring agents, perfumes, and water.
- The ***Precautionary Statement*** describes the potential hazards of the product (to people or animals) and actions to take to reduce those hazards (i.e., wearing gloves or goggles). Specific "signal words" are used to indicate the degree of hazard. Descriptors used (from least harmful to most harmful) are: "Caution", "Warning", "Danger" and "Danger-Poison".
- The ***First Aid*** section lists the actions to take in the event of accidental swallowing, inhalation or contact with the product. A ***Notes to physicians*** area may be listed with specific medical information needed by medical professionals.
- ***Additional Precautionary Statements*** contained on the label includes additional safety and precautionary information such as ***environmental hazards***, ***physical or chemical hazards***, (i.e., corrosiveness or flammability), and ***storage and disposal*** information.
- The ***Directions for Use*** section tells what the product controls, as well as where, how and when to use it. Some products may have multiple uses (i.e., cleaning versus disinfection), require different dilutions and/or contact times for such specific actions (i.e., *-cidal* versus *–static*). The best application method to use with the product (i.e., spray directly or wipe on surfaces) will also be listed.

Considerations and assessment for a disinfection action plan

Before selecting a disinfectant to use, there are several factors that must be considered. Some disinfectants are effective for routine disinfection protocols at the farm or veterinary clinic level while others are necessary for outbreak situations. For an effective disinfection protocol, consideration should be given to the microorganism being targeted, the characteristics of a specific disinfectant, and environmental issues. Additionally, the health and safety of personnel and animals are always an important consideration.[2,5,8,9,10]

Microorganism considerations

Microorganisms vary in their degree of susceptibility to disinfectants. In general, Gram-positive bacteria are more susceptible to chemical disinfectants while mycobacteria or bacterial endospores are more resistant. The hydrophilic, non-enveloped viruses (adenoviruses, picornaviruses, reoviruses, rotaviruses) are more resistant to disinfection than lipophilic, enveloped viruses (coronaviruses, herpesviruses, orthomyxoviruses, paramyxoviruses, retroviruses).

For a comparison of microorganisms and their susceptibility to a various disinfectant chemical classes, see 'The Antimicrobial Spectrum of Disinfectants' table (Appendix 1).

Pathogenic microorganisms also vary in their ability to survive or persist in the environment (i.e., bedding, debris, feed), and in their potential routes of transmission. Additionally, some microorganisms are also effective at creating a biofilm that enhances their ability to persist in the environment and avoid the action of disinfectants.[2] These also are important considerations when selecting a disinfectant and protocol to use. Whenever possible, identification of the target microorganism should be done, however if the organism has not been identified, a broad-spectrum approach should be utilized until identification can be made.

Disinfectant considerations

An ideal disinfectant is one that is broad spectrum, works in any environment and is non-toxic, non-irritating, non-corrosive and relatively inexpensive. Unfortunately, no disinfectant is ideal.[11] Therefore careful consideration of the characteristics of a disinfectant are essential to select the most useful, effective and cost-efficient product.

Disinfectant concentration. Use of the proper concentration of a disinfectant is important to achieve the best results for each situation. Some products will have different dilutions depending on the desired use of the product (i.e., *-static* versus *–cidal* action). Although some disinfectants may be more efficacious at higher concentrations, these levels may be limited by the degree of risk to personnel, surfaces or equipment, as well as the cost of the chemical. However, over-dilution of a product may render the disinfectant ineffective to the target microorganism. The product label will list the best concentration to use for each situation. Be sure to consider any standing water or other water sources (i.e., rainfall) in the area as a potential dilution source for a disinfectant.

Application method. There are a variety of ways to apply disinfectants. Object surfaces or walls of a building may be treated with a disinfectant solution by wiping, brushing, spraying or misting. Portable items should be soaked in a container of disinfectant. Fumigation may be used in some situations but is inefficient in buildings with ill-fitting doors and windows, or damaged roofs.

Contact time. Appropriate contact times are essential. Disinfectants may vary in the contact time needed to kill versus inactivate microorganisms. For example, 70% isopropyl alcohol can destroy *Mycobacterium tuberculosis* in 5 minutes, whereas 3% phenol requires 2-3 hours.[2] The minimum contact time needed is normally stated on the product label. Areas being disinfected should be well soaked with the disinfectant selected to avoid drying before the end of the optimum contact time. Some chemicals may have residual activity (i.e., QAC) while others may evaporate quickly (i.e., alcohols).

Stability and storage. Some disinfectants (i.e., sodium hypochlorite) lose stability quickly after being prepared for use or when stored over long periods, especially in the presence of heat or light. Disinfectant product labels will list the shelf life of the concentrated product. To maximize stability and shelf life, products should be stored in a dark, cool location and preferably in stock concentrations. Use of an outdated or inactivated product may result in the use of a non-efficacious product and will lead to a false sense of security.[9]

Instructions for use. Misuse of a product is in violation of EPA regulation. The label of a disinfectant may include limitations of the product and must be followed carefully. This will ensure maximum effectiveness, as well as protect personnel, the treated items and the environment.

Safety precautions. Most disinfectants can cause irritation to eyes, skin and/or the respiratory tract, therefore, the safety of all personnel should be considered. Training on proper storage, mixing and application procedures is essential.[2] Personal protective equipment (PPE), such as gloves, masks and eye protection, should be worn during the mixing or application of disinfectants. All chemical disinfectant have a Material Safety Data Sheets (MSDS) listing the stability, hazards and personal protection needed, as well as first aid information. This information should be available to all personnel. A 3-ring binder containing this information in one easily accessible location may be useful.

Expense. Economic considerations are always important when selecting a disinfectant. Since disinfectants vary in cost, contact time and dilution, **costs should always be calculated on a per gallon of use/dilution rather than the cost of concentrate**. However, disinfection protocols are generally a cost-effective means of reducing pathogenic organisms. For example, a QAC that costs $68.00 per gallon of concentrate will cost $0.27 per diluted gallon (0.5 ounce concentrate per gallon of water). Considering a gallon of diluted disinfectant approximately covers 100-150 square feet (10-15 m^2), the cost for disinfecting a 500 square foot room is $1.35.

Environmental considerations

To disinfect a contaminated item or area, environmental factors can greatly impact the effectiveness of a disinfection plan. Organic load, surface topography, temperature, relative humidity, pH, water hardness or the presence of other chemicals are all important environmental factors to consider. Additionally the value of an item and the health and safety of humans, animals and the environment are also important considerations.

Purpose of disinfection protocol. Initial assessment of the farm or clinic and the needs for disinfection will be important in the development of an effective protocol as well as disinfectant selection. What are the goals for the disinfection protocol? Are they to prevent an infectious disease, minimize disease spread, or control an outbreak? Answers should be addressed to develop an effective disinfection plan. Ineffective disinfection methods can lead to a false sense of security, which can inevitably lead to further spread of a disease.

Organic load. Removal of all organic material prior to application of a disinfectant is essential. The level of organic material (i.e., soil, bedding, litter, feed, manure) on an item or in areas to be disinfected can greatly impact the efficacy of a product or protocol. Organic matter provides a physical barrier that protects microorganisms from contact with the disinfectant. Debris and organic material can also neutralize many disinfectants especially chlorine and iodine containing compounds.[2,9] Items or equipment removed from the area, including those used for cleaning (i.e., brooms, shovels, buckets, hoses), must be also be decontaminated before reuse or disposal.

Some disinfectants may have some efficacy or residual activity in the presence of organic material (i.e., phenols) and should be considered in circumstances where complete removal of organic debris is difficult. However, application of these products to a heavy organic load (i.e., non-cleaned surfaces) is not implied.

Surface topography. The type of surface to be disinfected can have a great impact on effectiveness of a disinfection plan. Porous, uneven, cracked, or pitted surfaces, especially wooden surfaces and earthen floors, can hide microorganisms and are difficult to disinfect. An ideal surface to be disinfected is smooth.[2]

Temperature. In general, most disinfectants work best at temperatures above 68^{o}F. However, elevated temperatures may accelerate evaporation of a disinfectant, which can reduce contact time and decrease efficacy. Colder temperatures may also reduce the efficacy of some products. Consideration of the exposure temperature will be important if you will be disinfecting outdoors. Additionally, some items or areas being treated will be heat sensitive.[2]

Relative humidity. Relative humidity can also influence the activity of some disinfectants. For example, formaldehyde fumigation requires a relative humidity in excess of 70% for effectiveness.

Water hardness. The water source used when cleaning and diluting disinfectants is also important. Water “hardness” can inactivate or reduce the effectiveness of certain disinfectants (i.e., QAC, phenols). “Hard” water contains calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions (leached from limestone and other minerals as groundwater passes over it). These ions can complex with cleaning compounds, leading to residue buildup, which may reduce their cleaning action. However, many detergents have chelating agents, such as EDTA, to help bind these ions.[2]

pH. The activity of some disinfectants is also affected by pH. For example, the efficacy of glutaraldehyde is dependant on pH, working best at a pH greater than 7. In contrast, QACs have the greatest efficacy at pH of 9-10. The pH can also affect the activity of phenolics, hypochlorite and iodine compounds.[2]

Presence of other chemicals. Other chemicals can affect the efficacy of some disinfectants. For example, iodine agents are inactivated by QACs, while phenols are commonly formulated with soaps to increase their penetrative ability.

Value of the item to be decontaminated. Equipment will have varying degrees of value and disposability. Items that are reuseable (i.e., otoscopes) will need to be handled differently than those that will be discarded.

Health, safety and the environment. The health and safety of humans and/or animals should always be a primary consideration when selecting a disinfectant. Most disinfectants have some level of hazard associated with their use. Some pose a serious threat to human and animal health (i.e., aldehydes, phenols, sodium hydroxide). Some cannot be used when animals are present or must be thoroughly rinsed away with potable water prior to restocking. Personnel training, personal protective measures and safety precautions should always be taken.

Environmental factors, such as runoff into creeks or ponds, must also be considered when selecting a disinfectant. Many agents are known ecological hazards for plants and aquatic life (i.e., sodium carbonate, hypochlorites, phenolics), therefore drainage, runoff, and biodegradability of disinfectants should be considered. Health and safety information of a disinfectant is required to be printed on the product's label.

Physical Disinfection

In addition to chemical disinfectants, heat, light and radiation may also be appropriately used to reduce or eliminate microorganisms in the environment. The use of **heat** is a one of the oldest physical controls against microorganisms and is fairly reliable method of sterilization.[3,12] Although both moist heat (autoclave, steam) and dry heat (flame, baking) can be used for inactivating microorganisms, moist heat is more effective and requires less time than dry heat.[5] **Sunlight and ultraviolet (UV) light** can have a detrimental effect on a number of microorganisms and may be a practical method for inactivating viruses, mycoplasma, bacteria and fungi, particularly those that are airborne. UV light sterilizing capabilities are limited on surfaces because of its lack of penetrating power.[2,5] Other forms of **radiation** are less frequently used but may include the use of microwaves or gamma radiation. Freezing is not a reliable method of sterilization[3] but may help to reduce heavy numbers of bacteria; some microorganisms are resistant to freezing.

Classification of Chemical Disinfectants (listed alphabetically)

Disinfectants are classified by their chemical nature and each class has its unique characteristics, hazards, toxicities and efficacy against various microorganisms. Environmental conditions, such as the presence of organic matter, pH or water hardness can also impact the action of a disinfectant. Therefore, before using any chemical disinfectant, **thoroughly read and follow the label instructions**.

The major classes of chemical disinfectants and their characteristics follow. A summarization of these characteristics can be found in the 'Characteristics of Selected Disinfectants' table in Appendix 2.

Disclaimer: The use of trade names in this material does not in any way signify endorsement of a particular product. They are only provided as examples. For additional product names, please consult the most recent 'Compendium of Veterinary Products'.

Acids

Examples: acetic acid, citric acid
Acidic disinfectants function by destroying the bonds of nucleic acids and precipitating proteins. Acids also change the pH of the environment making it detrimental to many microorganisms.[13] Concentrated solutions of acids can be caustic, cause chemical burns, and can be toxic at high concentrations in the air. These characteristics limit their use. The antimicrobial activity of acids is highly pH dependant. Acids have a defined but limited use as disinfectants.

Acetic acid is usually sold as glacial acetic acid (95% acetic acid) which is then diluted with water to make a working solution concentration of 5%. The concentrated form is corrosive to the skin and lungs, but the typical dilution (5%) is considered non-toxic and non-irritating. Acetic acid is typically applied by spraying, misting or immersing an item in a diluted solution. Household vinegar is a 4-5% solution of acetic acid (by volume). Acetic acid has poor activity in organic material[14].

Alcohols

Examples: ethanol, isopropanol

Alcohols are broad spectrum antimicrobial agents that damage microorganisms by denaturing proteins, causing membrane damage and cell lysis.[2,3] Alcohols are used for surface disinfection, topical antiseptic and hand sanitizing lotions. Alcohols are considered fast-acting capable of killing most bacteria within five minutes of exposure but are limited in virucidal activity and are ineffective against spores. [Ethanol is considered virucidal; isopropanol is not effective against non-enveloped viruses.] An important consideration with alcohols is the concentration used, with 70-90% being optimum. Higher concentrations (95%) are actually less effective because some degree of water is required for efficacy (to denature proteins).[2,5] Alcohols evaporate quickly but leave behind no residue. The activity of alcohols is limited in the presence of organic matter. Alcohols are highly flammable, can cause damage to rubber and plastic, and can be very irritating to injured skin.[2,14]

Aldehydes

Examples: formaldehyde, gluteraldehyde

Aldehydes are highly effective, broad spectrum disinfectants, which typically achieve sterilization by denaturing proteins and disrupting nucleic acids.[2,13] The most commonly used agents are formaldehyde and gluteraldehyde.[5] Aldehydes are effective against bacteria, fungi, viruses, mycobacteria and spores.[15] Aldehydes are non-corrosive to metals, rubber, plastic and cement.[14] These chemicals are highly irritating, toxic to humans or animals with contact or inhalation, and are potentially carcinogenic; therefore their use is limited.[5,12,14] Personal protective equipment (i.e., nitrile gloves, fluid resistant gowns, eye protection) should be worn if using these chemicals.

Formaldehyde is used as a surface disinfectant and a fumigant and has been used to decontaminate wooden surfaces, bricks and crevices of electronic and mechanical equipment.[2] Its use must occur in an air tight building, which must remain closed for at least 24 hours after treatment.[2] The efficacy of formaldehyde is dependant on relative humidity and temperature; optimum being humidity close to 70% and a temperature close to 57°F. [Formalin is 37% solution of formaldehyde in water].

Glutaraldehyde is primarily used as a disinfectant for medical equipment (e.g. endoscopes), but can provide sterilization at prolonged contact times.[2] A 2% concentration is used for high-level disinfection.[5] Its efficacy is highly dependant on pH and temperature, working best at a pH greater than 7 and high temperatures. It is considered more efficacious in the presence of organic matter, soaps and hard water than formaldehyde.[5,12] An example product is Cidex™.

Alkalis

Examples: sodium or ammonium hydroxide, sodium carbonate, calcium oxide
Alkaline agents work by saponifying lipids within the envelopes of microorganisms.[13] The activity of alkali compounds is slow but can be increased by raising the temperature. Alkalis have good microbicidal properties, but are very corrosive agents and personal protection precautions should be observed.[15]

Sodium hydroxide (lye, caustic soda, soda ash) is a strong alkali used to disinfect buildings but is highly caustic. Protective clothing, rubber gloves, and safety glasses should be worn when mixing and applying the chemical. Lye should always be carefully added to water. **Never pour water into lye**; a very violent reaction will occur as well as the production of high heat that can melt plastic containers. Sodium hydroxide is corrosive for metals. It is considered an effective FMD disinfectant.

Ammonium hydroxide is an effective disinfectant against coccidial oocysts however strong solutions emit intense and pungent fumes.[5] This substance is not considered effective against most bacteria. General disinfection should follow the use of this compound.

Sodium carbonate (soda ash, washing soda) has been used in a hot solution (180^{o}F) for disinfecting buildings, which have housed animals with FMD. It is more effective as a cleanser than a disinfectant since it lacks efficacy against some bacteria and most viruses. A 4% solution has been listed as an approved chemical for the FMD virus.[5] It has poor activity in the presence of organic material and can be deactivated by hard water. It can be irritating and requires protective clothing and is harmful to aquatic life.

Calcium oxide (quicklime) becomes lime wash when mixed with water. This has biocidal effects on some bacteria and virus and is sometimes spread on the ground following depopulation of infected premises and has also been used to retard putrefaction of buried carcasses after depopulation. It is not very effective against the FMD virus.

Biguanides

Example: chlorhexidine
Biguanides are detrimental to microorganisms by reacting with the negatively charged groups on cell membranes which alters the permeability.[5,13] Biguanides have a broad antibacterial spectrum, however they are limited in their effectiveness against viruses and are not sporicidal, mycobacteriocidal, or fungicidal.[5,15] Biguanides can only function in a limited pH range (5-7) and are easily inactivated by soaps and detergents. These products are toxic to fish and should not be discharged into the environment.

Halogens

Examples: chlorine or iodine compounds
Halogen compounds are broad spectrum compounds that are considered low toxicity, low cost and easy to use. They do lose potency over time and are not active at temperatures above 110^{o}F or at high pHs(>9).[15] Since these compounds lose activity quickly in the presence of organic debris, sunlight and some metals, they must be applied to thoroughly cleaned surfaces for disinfection.[6,9,11,12]

<u>Chlorine compounds</u> function through their electronegative nature to denature proteins[13] and are considered broad spectrum, being effective against bacteria, enveloped and non-enveloped viruses, mycobateria and fungi. At elevated concentrations, chlorine compounds can be sporicidal.[6,11] ***<u>Sodium hypochlorite (NaOCl)</u>*** is one of the most widely used chlorine containing disinfectants. [Commercial chlorine bleach contains 5.25% sodium hypochlorite in aqueous solution and 50,000 ppm available chlorine].[11,13] Biocidal activity is determined by the amount of the available chlorine of the solution. Low concentrations (2 to 500 ppm) are active against vegetative bacteria, fungi and most viruses. Rapid sporicidal action can be obtained around 2500 ppm, however this concentration is very corrosive so should be limited in its use. High concentrations are also irritating to the mucous membranes, eyes and skin.[6,11] Chlorine compounds are rapidly inactivated by light and some metals so fresh solutions should always be used. Hypochlorites should **<u>never</u>** be **<u>mixed</u>** with acids or ammonia as this will result in the release of toxic chlorine gas.[9] See Table 1 below for details regarding different bleach dilutions.

Table 1. Bleach dilutions.

Sodium hypochlorite %	**Bleach Solution Ratio**	**Bleach Dilution**	**ppm (available chlorine)**	**Comments** **Always use on cleaned surfaces.
0.025%	1:200	1.5 Tbsp (0.6 oz) bleach to 1 gallon water	250 ppm	Common household use
0.1%	1:50	1/8 C (1 oz.) bleach to 1 gallon water	1000 ppm	Commonly used
0.16%	1:32	1/2 cup (4 oz.) bleach to 1 gallon water	1562.5 ppm	Commonly used
0.5%	1:10	1.5 cups (12 oz.) bleach to 1 gallon water	5000 ppm	This is a very strong solution and should be used on a limited basis.
3.33%	2:3	2 parts bleach to 3 parts water	33,333 ppm	Effective for FMD virus – but use with caution

<u>Iodine Compounds</u> are broad spectrum and considered effective for a variety of bacteria, mycobacteria, fungi and viruses.[15] Iodines function by denaturing proteins to interfere with the enzymatic systems of microorganisms.[13] Iodines are often formulated with soaps and considered relatively safe.[11] Concentrated iodine compounds can be irritating to the skin, can stain clothes or damage rubber and some metals.[9] Iodine agents are inactivated by QACs and organic debris.[9]

<u>Iodophors</u> are iodine complexes that have increased solubility and sustained release of iodine. One of the more commonly used iodophors if povidone-iodine. They are good for general use and are less readily inactivated by organic matter than elemental iodine compounds. The dilution of iodophors actually increases the free iodine concentration and antimicrobial activity.

Oxidizing Agents

Examples: hydrogen peroxide, peracetic acid, Virkon-S®

Oxidizing agents are broad spectrum, peroxide based compounds that function by denaturing the proteins and lipids of microorganisms.[13] Peroxygen compounds vary in their microbiocidal range, but are considered effective on hard surfaces and equipment. In their diluted form, these agents are relatively safe but may be irritating and damage clothing when concentrated.[11]

Hydrogen peroxide in the home is in diluted form (3-10%) whereas industrial use involves concentrated solutions (30% or greater). Hydrogen peroxide (at a 5-20% concentration) is considered bactericidal, virucidal (non-enveloped viruses may be resistant), fungicidal and at high concentrations sporicidal. Its activity against mycobacteria is limited.[15]

Peracetic acid (e.g. OxySept 333®) is a strong oxidizing agent and is a formulation of hydrogen peroxide and acetic acid. It is considered bactericidal, fungicidal, sporicidal and virucidal.[5,15] It is also effective against mycobacteria and algae and has some activity in the presence of organic material.

Virkon-S® (potassium peroxymonosulfate and sodium chloride) is a peroxygen molecule, organic acid and surfactant combination, with a wide microbial spectrum of activity and some efficacy in the presence of organic material.[9]

Phenols

Examples: One Stroke Environ®, TekTrol®, Pheno-Tek II®

Phenols are broad spectrum disinfectants that function by denaturing proteins and inactivating membrane-bound enzymes to alter the cell wall permeability of microorganisms. Phenols can be coal-tar derivatives or synthetic formulations and usually have a milky or cloudy appearance when added to water, as well as a strong pine odor.[6,9,11] Pine-Sol is an example of a phenol found in the home. Phenols are typically formulated in soap solutions to increase their penetrative powers and at 5% concentrations are considered bactericidal, tuberculocidal, fungicidal and virucidal for enveloped viruses.[15] Phenols are not effective against non-enveloped viruses and spores. Phenols do maintain activity in hard water and in the presence of organic matter and have some residual activity after drying. Phenolic disinfectants are generally safe for humans but prolonged exposure to the skin may cause irritation.[6] Concentrations over 2% are highly toxic to all animals, especially cats.

Quaternary Ammonium Compounds

Examples: Roccal®, Zepharin®, DiQuat®, D-256®

Also known as “quats” or QACs, these compounds are cationic detergents that are attracted to the negatively charged surfaces of microorganisms, where they irreversibly bind phospholipids in the cell membrane and denature proteins impairing permeability.[13] QACs can be from different “generations” depending on their chemistry, with later generations being more germicidal, less foaming and more tolerate to organic loads. QACs are highly effective against Gram positive bacteria, and have good efficacy against Gram-negative bacteria, fungi and enveloped viruses. They are not effective against non-enveloped viruses or mycobacteria and are considered sporostatic but not sporocidal.[6,11,15] QACs have some residual effect, keeping surfaces bacteriostatic for a brief time. They are more active at neutral to slightly alkaline pH but lose their activity at pH less than 3.5. QACs are considered stable in storage but are, in general, easily inactivated by organic matter, detergents, soaps and hard water (this may vary with the “generation”). QACs are toxic to fish and should not be discharged into water sources (i.e., streams, ponds, lakes).

Implementing a Disinfection Action Plan

There are several important areas to be addressed in an effective disinfection action plan. These include assessment, cleaning, washing, disinfection, and evaluation.

Once a disinfection plan is devised, all employees should be taught on the proper implementation of the disinfection protocol especially with an emphasis on thorough cleaning. Explain the goals and methods clearly. Post signage to remind employees of protocols.

Assessment

The first step in an effective disinfection protocol involves a thorough assessment of the problem. This includes identifying and evaluating the infectious agent suspected, its mode of transmission, potential areas affected and selection of the proper chemical disinfectant.

Because ill animals come into contact with a wide variety of elements (i.e. barns, stalls, equipment, feed bunks, water trough), all elements in the environment must be considered and assessed for contamination. When in doubt – clean and disinfect it!

Cleaning

After the initial assessment, cleaning is the next step and must be done thoroughly before disinfection![5] It has been estimated that cleaning alone may remove over 90% of bacteria from surfaces.[16]

This “dry” cleaning step involves brushing, scraping, sweeping and removing all bedding, feces, feed, dust and debris from the area. If the area is dusty, moisten the area to control dust and minimize aerosolization.[2] Proper disposal of bedding, gross debris, dirt, and organic material, such as manure, is important and may vary with locale but may include burning, burial or composting.[2,5] Disposal of debris into dumpsters should only occur if there is no zoonotic risk of disease transmission.

The goal is to remove as much organic matter as possible. The presence of organic material can harbor microorganisms for long periods of time as well as protect them from the action of chemical disinfectants.[2] Cleaning is also important since many disinfectants may be inactivated or ineffective in the presence of organic debris or waste.

All personnel should wear protective clothing and rubber boots. If a zoonotic disease is suspected, enhanced personal protective equipment should be used (e.g. gloves, face masks, goggles, or headwear).[5]

Washing/sanitizing

Washing or sanitizing further reduces the number of microorganisms in the area to a safer level.[2] **This is <u>the most crucial step</u> in the disinfection process** and will most likely eliminate the majority of remaining microorganisms, if performed correctly. Efficient cleaning can remove up to 99% of bacteria present.

Soak the area with **hot water** and detergent or other cleaning agent, then wash by wiping, spraying, or scrubbing. Application of washing solution can be improved by use of low pressure (90-120 psi) garden hose applicator. Steam and high pressure washers (200-1000 psi) can be very useful for cleaning porous surfaces.[2] **Caution** should be taken if high pressure spraying is used; this method may further aerosolize microorganisms and further spread of contaminants.[2]

Proceed from the cleanest areas to the dirtest and from the highest level (ceiling) to the lowest (floor). Equipment that can be removed should be brushed and soaked in detergent before disinfection. Pay particular attention to troughs, drinkers, corners and floor drains.[2] These areas can serve as reservoirs for pathogens and should be cleaned and disinfected last.

Although cleaning may appear to remove all debris, biofilm may remain on surfaces and interfere with disinfection efficacy. Biofilm is a complex aggregation of bacteria adhering to surfaces in an exopolysaccharide matrix, resulting in a thin residue remaining after cleaning. These bacteria are highly resistant to disinfection. Surfactant detergents, mechanical scrubbing, brushing and scraping during cleaning help reduce biofilm.[2]

After washing affected areas, thorough rinsing at low pressure should follow for all surfaces to remove any residue. Many disinfectants (i.e., QACs, hypochlorite) can be inactivated by soaps and detergents. Areas should be allowed to dry **before** application of the selected disinfectant to reduce potential dilution of the disinfectant upon application.

Disinfection

Selection of the proper disinfectant will depend on the microorganism suspected, as well as environmental factors (e.g. temperature, pH) and safety issues. **Always read the entire product label** and follow dilution instructions explicitly to ensure that the safest, most effective concentration is applied.

Disinfectants should be applied by spraying, using a low pressure washer a garden-type sprayer.[5] During cold weather, buildings should be heated to approximately 20 ^{o}C (68 ^{o}F) because some disinfectants are ineffective at low temperatures.[5]

To achieve effective disinfection, surfaces must be thoroughly wet; disinfectant at the correct concentration should be applied at a rate of 0.4L/m^2.[17] One U.S. gallon of diluted disinfectant is ordinarily applied to approximately 100-150 square feet (9-14 m^2) of surface area.

Disinfectant should remain for the appropriate contact time, which will vary with the product. Consult the product label. Afterwards, areas should be rinsed thoroughly and allowed to dry before animals are returned to the area.

Special attention should be given to any floor drains that may be present. These areas can serve as a reservoir for pathogens and should therefore be cleaned and disinfected last.

Any items removed from the facilities, including cleaning equipment, should be thoroughly washed, rinsed and disinfected. If this cannot be accomplished, the item should be discarded.[2] Electrical equipment installed in the building should be either removed or covered with waterproof material and disinfected manually at a later stage.

Evaluation

To verify that the disease agent(s) have been destroyed, a follow-up evaluation of the premises should be conducted. While visual inspection of cleanliness is important, bacteriological samples should be obtained to determine the effectiveness of the cleaning and disinfection protocol. Failure of a disinfection program may relate to the selection of an ineffective disinfectant, careless use of an effective disinfectant, or environmental factors, such as temperature, relative humidity.

The timing of sample collection is important. The best time to sample is 2-3 days after disinfection.[29,30] Samples for microbiological testing should not be taken from a wet surface (the disinfectant may still be acting and disinfectant residues may prevent growth of microorganisms in culture media). Therefore the sample area should be allowed to dry before sampling.[23]

Surface samples for small, smooth areas, can be collected by wiping or swabbing a moistened, absorptive medium (sterile swab) across a non-porous surfaces. For larger areas, sterile gauze wipes or swiffers may be used. Commercially available methods include RODAC™ and Petrifilm™ Plates (3M). [19,20]

Photo. of sterile swab. http://www.remelinc.com/products/industry/level2/sanicult.cfm

RODAC (Replicate Organism Detection and Counting) plates (Merck) are specially designed agar plates that can be applied directly to surfaces areas due to their convex shape. They are useful for dry, flat, impervious surfaces. The plates are then incubated 48-68 hours at 25-30 °C (77-86 °F). Visible colonies within areas of the plate are then counted to provide an indication of the degree of contamination. There are RODAC plates also available for the growth and detection of yeast and molds.[21]

Additionally Petrifilm™ Plates (3M) may also be used. These small, flat, sample-ready plates allow on-site microbial testing and are commonly used for food processors. These plates are are available for a variety of specific microorganisms or classes (i.e., aerobic count plates, coliform count plates, environmental *Listeria* plates) as well as yeast and mold counts[22] and can also be used for air sampling, direct contact or swab applications.

Photo: Petrifilm Plates. http://solutions. 3m.com

Other methods of environmental sampling include surface samples collected by high-efficiency particulate air (HEPA) vacuuming and as well as other types of air sampling pumps and filters.[18]

Additionally, each step of the disinfection action plan (assessment, cleaning, washing/sanitizing, disinfection) should be evaluated for problems encountered and usefulness or efficiency of the cleaning or disinfection techniques.[29,30]

Special Considerations

Boot baths

To avoid mechanical transfer of microorganisms by personnel, boot baths should be a standard BRM practice and are extremely important. However, protocols and maintenance of boot baths are commonly neglected and are overlooked, so deserve special mention.

Common problems with boot baths include inadequate removal of organic debris prior to stepping into the disinfectant solution, inappropriate contact time allowed for the disinfectant, and infrequent change of disinfection solution. Incorrect use of boot baths will be a waste of resources and can lead to a false sense of security.

The most overlooked and important step is removal of gross debris from boots **prior** to stepping into a boot bath disinfectant. Studies have shown that removing visible manure from boots prior to stepping into a disinfectant, greatly significantly reduces the number of bacteria present.[24,25] This may either be accomplished by a hose, water and a scrub brush or by a cleaning boots in a preliminary bath filled with dilute detergent.[5,24,25] This process not only reduces the number of microorganisms present, but will also minimize the amount of debris accumulating in the boot bath solution. Therefore, boots should be scrubbed and cleaned of all grossly visible debris prior to a timed soak in the disinfectant boot wash.

Allowing the proper contact time for a disinfectant is just as important for boot baths as it is when cleaning premises and equipment. Additionally, use of a fresh, efficatious solution is also essential. Boot baths should be refilled at least every 2-3 days. For best results, it should be replaced daily.[5] Replacement of the disinfectant solution will also depend on the degree of traffic flow; more frequent replacement will be needed in large or busy areas or when organic debris accumulates in the boot bath.

Phenolic compounds are most commonly used for boot baths, due to their efficacy in the presence of organic matter.[5] However, this should not prevent proper boot washing techniques from being implemented. In fact, cleaning away gross material will help to minimize organic debris accumulation in the boot bath.

Boot bath solutions should also be kept from freezing and protected from rain to avoid over-dilution. The use of rubber boots may better allow compliance with the necessary contact and soak times.[26]

Vehicles

All vehicles (trucks, trailers, etc.) used for transporting animals, their products or by-products or contaminated equipment have the potential to spread disease.[27] This may occur either by the vehicle itself or transfer of material by the wheels. Vehicles allowed to enter farms should be limited and when possible unload outside the farm.

Vehicles should also be cleaned and disinfected between farms using steps similar to those described for buildings and equipment. Primary cleaning should be done first to remove as much organic debris as possible. Debris should be brushed or scraped from the vehicle.[28] Washing of the vehicle should follow. High pressure spraying equipment (i.e., 200 - 1000 psi) may help to clean wood pores, cracks and crevices. The vehicle should always be cleaned from top to bottom. Also be sure to wash the underside of fender wells and the vehicle frame. Apply an appropriate disinfectant with a low pressure sprayer and allow its proper contact time to elapse. Iodophor disinfectants are commonly used for vehicle disinfection and are considered to have a broad spectrum of antimicrobial activity. They are also considered more environmentally safe than phenolic disinfectants and more stable than hypochlorite-based compounds.[5] The interior of the vehicle should also be thoroughly cleaned and disinfected prior to leaving the affected farm.[28]

Foot and Mouth Disease (FMD)

The FMD virus can survives in lymph nodes and bone marrow at neutral pH, but is destroyed in muscle when is pH <6.0 (i.e., after rigor mortis). The virus is also susceptible to elevated pH (i.e., >9.0).[33] The virus can persist in contaminated fodder and the environment for up to one month, depending on the temperature and pH conditions. Sodium hydroxide (2%), sodium carbonate (4%), and citric acid (0.2%) are effective disinfectants. FMDV is resistant to iodophors, QACs, low concentrations of sodium hypochlorite, and phenols.[33,34]

The USDA has approved several disinfectants for field use in the event of a FMD outbreak.

Product	Dilution	Comments
5.25% Sodium hypochlorite* (NaOCl) (household bleach)	3%	Inactivated by organic soiling; unstable in warm sunny conditions.
Acetic acid*	4-5%	Vinegar is a 4% solution of acetic acid.
Potassium peroxymonosulfate and sodium chloride*	1%	e.g. Virkon-S
Sodium carbonate* (soda ash)	4%	The solution is mildly caustic, but can dull paint and varnished surfaces.
Sodium hydroxide* (NaOH) (lye)	2%	This solution is highly caustic. Use protective rubber clothing, gloves and safety glasses. **Warning: Always add the lye to the water.** Never pour the water over the lye.

* *U.S. Department of Agriculture. Appendix III – Disinfectants for Foot-and-Mouth Disease, Field Use* In: *National Emergency Response to a Highly Contagious Animal Disease, Executive Summary, March 30, 2001. Accessed at http://www.aphis.usda.gov/oa/pubs/fco412.pdf.*

Prions

Prions, the etiologic agents of bovine spongiform encephalopathy and scrapie, are exceptionally resistant to disinfectants, heat, ultraviolet radiation, ionizing radiation and formalin, especially if it is in tissues, dried organic material or at a very high titer.[5,31,32] High concentrations of sodium hypochlorite (2% available chlorine) or heated strong solutions of 2-N sodium hydroxide are reported to inactivate these unconventional infectious agents.[5,32] These agents also exhibit exceptional thermal stability and have prolonged survival when exposed to dry heat at 160°C (320 °F). Autoclaving at 138°C (280 °F) for 18 minutes was considered effective for these agents, but is not considered reliable.[5]

These recommended decontamination measures will reduce titers but may be incompletely effective if dealing with high titer material, when agent is protected within dried organic matter, or in tissue preserved in aldehyde fixatives. Rendering at 133°C (271 °F) at 3 bar pressure for a minimum of 20 minutes is used in Great Britain in order to dispose of the infected carcasses.[31,32]

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