A Guide to the Development of Laboratory Chemical Disinfection Protocols

Purpose:
The act of disinfecting a contaminated surface, tool, piece of equipment, or any other item can significantly reduce the risk of transmitting infection from pathogenic organisms to individuals. Although a relatively simple process, if any one step is done incorrectly, disinfection and ultimately decontamination won’t occur.

The purpose of this guide is to outline how each individual laboratory can implement an effective disinfection program.

Scope
This guidance document provides instruction to those working or performing research with microorganisms or substances containing microorganisms with the potential to cause infection. It only outlines protocols for chemical disinfection, not physical sterilization.

Definitions:
Sterilization refers to the process, either physical (extreme heat and/or pressure), or chemical that destroys ALL forms of life, especially microorganisms.

Disinfection refers to the process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects.

Cide or cidal – The suffix “cide” is applied to a word to convey that it performs a killing action on that particular substance. For instance, bactericides, virucides, and sporicides, are chemical agents that will kill bacteria, viruses, and spores, respectively.

Static - The suffix “static” applied to a word means the agent will inhibit an organism’s growth. For instance, a bacteriostatic, virostatic, or sporostatic chemical agent will merely halt the growth of bacteria, viruses, and spores, respectively, and not kill them.

Chemical Disinfection is the process of applying a chemical agent to eliminate the majority of pathogenic microorganisms on inanimate objects or surfaces.

Decontamination is the process of removing harmful substances from a surface, equipment, tool, or person. These substances could include biological agents, radioactive substances, or even chemical substances.
Developing the Disinfection Protocol for your Work

Step 1 – Materials and/or Organisms
Some disinfectants are ineffective on some microbiological organisms (See Table 1, Appendix 1). Therefore to aid in the selection of the most effective disinfectant, the materials or organisms involved should be itemized and categorized into one of the following broad groups:

- Vegetative bacteria
- Mycobacteria
- Enveloped viruses
- Non-enveloped viruses
- Spores
- Fungi

Use Table 1 to determine which type of disinfectant would be the most effective.

Step 2 – Tools, Surfaces, and Equipment
In step one, the types of disinfectants were narrowed down to only those that were effective against the materials and organisms being used. In this step, itemize all tools, equipment, and surfaces that will be used, including the characteristics of each item. Next, you can select which disinfectant is most compatible with the items you are working with.

Step 3: Develop the protocol to be used
Write your protocol. It does not have to be lengthy, it just has to be clear. At a minimum indicate the following:

- Disinfectant being used
- How the disinfectant is to be applied (sprayed, wiped, soaked, etc...)
- What the disinfectant selected is effective against (meaning what organisms it can be used on)
- Final concentration that will be used
- Contact time needed to achieve disinfection and how the contact time can be achieved (in some cases multiple applications are needed)
Appendix 1 - Disinfectant Properties

Understanding how a disinfectant works, what it works best against, and its application parameters allows one to make the best determination of which disinfectant would be ideal to use in a given situation. The listing below explains the various criteria that must be considered before selecting a disinfectant. Ensure you understand these before selecting a disinfectant. These criteria are listed in alphabetical order below.

**Application Method** – refers to how the product is being applied to the surface or item in question. In most cases, the product will be wiped on a surface, but in cases where it is sprayed, brushed or applied in another manner, characteristics of the disinfectant will have to be considered. For instance, alcohol becomes an even greater fire hazard when sprayed, and will evaporate at a quicker rate – thus altering the expected contact time.

**Concentration** – refers to the strength of the disinfectant. It is important to understand the specific concentration range that is effective against the microorganism being inactivated. Both overly diluted and overly concentrated solutions can have their problems. Overly diluted disinfectants will not be strong enough to inactivate the target material, while overly concentrated materials may pose health risks to individuals using them, and others present in the area. Always use the recommended concentration specific to target materials.

**Contact Time** – can be considered the most important characteristic of a disinfectant. It is important that the appropriate contact time for each disinfectant is considered alongside its drying time. If the disinfectant dries BEFORE the allotted contact time, MULTIPLE APPLICATIONS will be needed to ensure the minimum amount of contact with the disinfectant is achieved. If this is not possible, then make sure to select disinfectants that are active before they completely dry.

**Environmental Conditions** – pH, temperature, relative humidity, and water hardness can all have an impact on the effectiveness of any given disinfectant. It is important to understand how these factors affect the disinfectant chosen. For instance, glutaraldehyde efficacy is dependent upon pH, with the optimum pH being above 7. Similarly, QAC’s work best between 9 – 10. pH can also affect the activity of hypochlorites, phenolics and iodine compounds. Furthermore, most disinfectants work best above 20°C (68 F), but higher temperatures can increase the evaporation rate – thus affecting contact times. Conversely, colder temperatures will reduce the activity of some products. Lastly, water hardness can inactivate or reduce efficacy of certain disinfectants (QAC’s and phenols). The free calcium and magnesium in hard water can also complex with various cleaning compounds, creating residues on surfaces. This can block some actions of the disinfectants.

**Health Hazards** – Most disinfectants can cause irritation to some part of the body – whether it is skin, or mucous membranes, like eyes – and some may even be carcinogenic (aldehydes). Other hazards include flammability (alcohols) or even corrosivity. In some cases, the application of the product will require some level of personal protection, so it is important to understand when this is the case, and what PPE should be used when applying the product.
**Incompatibilities** – Most disinfectants can become inactivated by some sort of contaminant or can become dangerous if mixed with other products. For instance, hypo-chlorites (bleach) can become inactivated in the presence of organic matter, iodine products are inactivated by QAC’s and, mixing bleach with an ammonia based product will evolve chloramine vapours – which are deadly. For these reasons, you must know the compatibility of a disinfectant when you use it on a surface or with other materials.

**Organic Load** – Organic material present on the surface or area to be disinfected can significantly compromise the effectiveness of the disinfectant used. Therefore, in almost every case, the surfaces that require disinfection MUST BE CLEANED AND FREE of any foreign materials (especially organics) before a disinfectant is applied.

**Stability and Storage** – Some disinfectants lose stability quickly after being prepared for use. Especially around heat and light. Most disinfectants will list a shelf life and should be stored in a dark cool place in stock concentrations. Bleach, peroxides and alcohols all quickly degrade or evaporate, thus losing their efficacy.

**Surface Topography** – It is easiest to disinfect a smooth surface – any deviation from that will be difficult. For instance, surfaces that are porous, uneven, or cracked, have hidden areas that cannot easily come in contact with the disinfectant. An earthen floor is a good example of something that is almost impossible to disinfect.

*Table 1 outlines the general characteristics of various types of disinfectants. This table can be used as a general guide to select an appropriate chemical disinfectant based upon the situation, surface, equipment, and microorganism present.*
Table 1: Summary of disinfectant properties, and use parameters.

<table>
<thead>
<tr>
<th>Disinfectant Category</th>
<th>Alcohols</th>
<th>Aldehydes</th>
<th>Halogens: Hypochlorites</th>
<th>Isothiocyanates</th>
<th>Oxidizing Agents</th>
<th>Phenols</th>
<th>Quaternary Ammonium Compounds (QAC)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample Trade Names</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ethyl Alcohol</td>
<td></td>
<td>Formaldehde, Glutaraldehyde</td>
<td>Bleach</td>
<td>Betadine®</td>
<td>Hydrogen Peroxide</td>
<td>One-Stroke Environ®</td>
<td>Roccal®</td>
</tr>
<tr>
<td>Isopropyl Alcohol (IPA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Peroacetic acid</td>
<td>Pho-Tek I®</td>
<td>D-256®</td>
</tr>
<tr>
<td><strong>Recommended Use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Peracetic acid - Cold sterilant to disinfect heat-sensitive equipment</td>
<td>Good for broad spectrum use – chemical sterilization</td>
<td>Sanitization of non-critical surfaces, furniture and walls.</td>
</tr>
<tr>
<td>Cleaning of instruments and skin. Also for disinfecting clean surfaces</td>
<td></td>
<td>Cold sterilant to disinfect heat-sensitive equipment</td>
<td>Disinfecting surfaces contaminated with blood and bodily fluids</td>
<td>Disinfecting semi-critical medical equipment</td>
<td>Oxy-Sept 333®</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Final Concentration for use** | 70 - 80% Ethanol in Water | 2% | 0.5%-2% of NaOCl in solution | 0.025%-0.2% | 3%-6% | 1%-5% | 0.1%-2.0% |
| **Min. Contact time (Enveloped Viruses)** | 10 minutes | 15 minutes | 10 minutes | 10 minutes | 10 minutes | 10 minutes |         |
| **Contact time** | 30 minutes | 30 minutes – at high concentrations | 30 minutes | 20 minutes | 30 minutes |         |         |
| **Shelf Life** | 2 years – undiluted, stored in cool well-ventilated place | 14 days | 3 months – undiluted, 24 hours when diluted | 2 years – undiluted, or when solution loses brown colour. | 2 years – undiluted and sealed 5 days when diluted | 1 year – sealed undiluted container | 2 years – sealed undiluted container |

| **Advantages** | Fast acting | Leaves no residue | Broad Spectrum | Broad Spectrum | Easy to use | Inexpensive | Stable in storage | Broad Spectrum | Non-corrosive | Works in presence of organics | Stable in storage |

| **Disadvantages** | Requires multiple applications | Rapid evaporation | Hardens and swells some rubbers and plastics | Only use in well ventilated areas | Limited shelf life | Highly reactive | Leaves a residue | Inactivated by sunlight | Corrodes metals | Prepare fresh before each use | Most effective above 65 F | Leaves a residue | Most effective at pH 2 to 5 | Requires multiple applications | Stains clothing and surfaces | Inactivated by light | Leaves a residue | Damaging to some metals (Al, Zn, brass) | Inactivated by light and heat | Can cause skin and eye irritation | Easily absorbed by skin | Leaves residue | Odourless, colourless, non-irritating, and deodorizing | Leaves residue |

| **Precautions/Hazards** | Flammable | Skin and eye irritant | Inhalation may cause dizziness | Carcinogenic | Sensitizer | Skin and eye irritant | Evolves toxic gases on contact with acids (chlorine) or ammonia (chloramine) | Skin and eye irritant | Highly toxic to aquatic life – contact ESF for disposal | Corrosive | Rinsing required where direct contact is made | Sensitizer | Corrosive to skin and eyes | Toxic to all life | Skin and eye irritant | Toxic | Sensitizer – can trigger allergic respiratory reactions |

| **Vegetative Bacteria** | Effective | Effective | Effective | Effective | Effective | Effective | Variable | Variable | Not Effective |
| **Mycobacteria** | Effective | Effective | Effective | Limited | Effective | Variable | Variable | Not Effective | Not Effective |
| **Enveloped Viruses** | Effective | Effective | Effective | Effective | Effective | Effective | Variable | Variable | Not Effective |
| **Non-Enveloped Viruses** | Effective | Effective | Effective | Limited | Effective | Variable | Variable | Effective | Not Effective |
| **Fungi** | Not Effective | Effective | Limited – extended contact time | Effective | Variable | Variable | Effective | Effective | Not Effective |
| **Efficacy with Organic matter** | Reduced | Effective | Reduced | Rapidly reduced | Reduced | Inactivated | Effective | Inactivated | |
| **Efficacy with Hardwater** | Effective | Reduced | Reduced | Inactivated | Inactivated | Effective | Inactivated | |

| **Efficacy with soap/detergents** | Effective | Reduced | Inactivated | Inactivated | Effective | Inactivated | |

**Note:** If there is a discrepancy between the guidance given on this table and a specific instruction from a manufacturer (for example - contact time), use the manufacturer’s recommendations.
Appendix 2 – Example Protocol for Blood and Bodily Fluids on a Smooth Surface

Routine Surface Disinfection after working with Human Blood and Bodily Fluids

Purpose
The purpose of this protocol is to outline the process to be used for the disinfection of a surface that will be, and has been, used in the processing of human blood or bodily fluids.

Scope
This protocol only outlines the process used for disinfecting a surface pre and post processing of human blood and bodily fluids. It does not provide guidance on the clean-up of high volumes of spilled blood or bodily fluids. For spills, please refer to the UW Emergency Manual for Work with Biological Materials.

Tools and Materials
- Disinfectant – one that is compatible with the material and effective against blood borne pathogens:
  - 70% - 80% Ethanol (dilution with tap water is ok) – not on rubber surfaces
  - 1:50 dilution
- PPE – gloves, labcoat, safety glasses, closed toed shoes.
- Paper towels

Disinfection Protocol
1. Inspect work area and identify work surface composition. The ideal work surface is smooth (not porous). Surfaces like wood, rock, and earth should be avoided as much as possible. If a wood surface or rock surface must be used, it should be sealed prior to use.
2. Identify contaminant (organism or material).
3. Remove all extraneous materials and items from surface.
4. Using a paper towel and water to wipe the surface to remove any residual dirt – for porous surfaces consider using a non-shedding brush to thoroughly clean.  
   *Note: if the surface is porous, consider working somewhere else!*
5. Dispose of used paper towels in biowaste bin.
6. Select disinfectant that is effective against the contaminant (organism or material).
7. Ensure disinfectant is compatible with the surface:
   a. Do not use bleach on stainless steel surfaces
   b. Do not use alcohol for rubber (and some plastic) surfaces or components
   c. Oxidizing agents will damage some metals – consult manufacturer’s guidance
   d. Iodophors will stain some materials
8. Freshly prepare disinfectant according to manufacturer guidelines.
9. Apply disinfectant according to manufacturer guidelines (usually, disinfectant is sprayed on surface then wiped, or is applied to a paper towel then wiped on surface)

10. Ensure surface remains wet for the duration of the recommended contact time - if disinfectant dries before the required contact time, repeat application of disinfectant.

11. Wipe off residues using a paper towel and water.

12. Inspect area once dry. If a residue remains, repeat with another wipe using a water wetted paper towel.

13. Dispose of used paper towel(s) into bio-hazard waste bin.