Changes in this version:

* New SOP template
* General update

## INTRODUCTION/PURPOSE

This is a procedure including a detailed risk assessment for the use of HMC, HV-25 Autoclave. The autoclave is being used for sterilisation of liquids and dissolving agar, as well as the destruction of biological agents prior to waste disposal.

## NECESSARY SAFETY EQUIPMENT

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| --- | --- | --- |
|  |  |  |
|  | Heat protective |  |

## Chemical and biological hazards

### Chemicals

Remove if not applicable

| **Chemical information** | **Health -, Precautions - and Emergency planning** |
| --- | --- |
| **2% CTAB Buffer**  [A4150 (Applichem lifescience) supplied by VWR](https://app.ecoonline.com/app/documents/msds/1007108/15607359_286_d5dacd23af5e4da1f5b5ad60d399115f.pdf)  http://mnhms-dev.net/wp-content/uploads/2015/04/Helsefare.jpg | H319: Causes serious eye irritation  P280: Wear protective gloves/ eye protection/ face protection. P305+P351+P338: IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing P337+P313: If eye irritation persists get medical advice/attention. |

### Biological Agents

Remove if not applicable

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| --- | --- |
| **Biological agent** | **Laboratory hazards, necessary precautions and emergency planning** |
| *[Neisseria gonorrheae](https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/neisseria-gonorrhoeae.html)*  Risk Group 2  ilderesultat for biohazards | Containment Level 2 facilities is required  Reported laboratory acquired infections:   * Gonococcal conjunctivitis (eye) * Cutaneous infections (skin)   The bacteria are mostly known for causing Genital gonorrhoeae  Wear safety goggles, lab coat and gloves.  Avoid contact with contaminated gloves  Wash hands after work  If exposed seek medical assistance. |

## Special cautions necessary due to reproductive toxicity:

Generally, it is not recommended to work with a chemical that has carcinogenic or reproductive effects if you are planning to be or are pregnant. If a chemical is proven to pass into breast milk it is not recommended to perform procedure if you are breast feeding.

If you are working with Class II biological agents that may cause infections, you should consider the risks using the relevant PSDS and other relevant documentation.

Planning pregnancy (men and women): Not recommended to perform this procedure.

Pregnant: Not recommended to perform this procedure.

Breast feeding: None.

## PROCEDURE and risk assessment

**Necessary equipment:**

HMC- HV-25 Autoclave

Distilled water

*We always wear lab coat when working in the lab.*

**Autoclave can be used for**

* Autoclaving buffers and solutions
* Tools of glass, ceramics, metal or/and rubber that withstand high temperature, high pressure steam and abrupt de-pressurisation during the exhaust process.
* Autoclaving solutions for disposal, e.g. media, etc.

**Do not use this autoclave to:**

* Solid waste
* Waste disposal bags

**IMPORTANT:**



* Pay attention to quantity of water level in the working chamber. It should be just below the basket.
* Check the water level in this container (exhaust container). Min and max level are indicated.
* Use container caps that are loose fitting and allow the passage of air. Container may break if venting is not possible.
* Do not pile specimens: Over-packed chamber may result in incomplete sterilisation

**Basic operation:**

1. **Pour water into the exhaust bottle.**
   1. Pour water into the bottle through the water filling port.
   2. Fill water to the reference line level
   3. Check to make sure that the water level is at **LOW level (the lowest water level),** if not remove access water by facing the filling and drain port downwards.
   4. Check to see that the drain valve is closed.
   5. Load the bottle into the area. (Error message (ErE) will appear if the bottle is not in the correct position. )
   6. Steam is apt to emit from the exhaust port located at the rear of the body. Please a drain bottle to the exhaust hose, ensure that the hose top is about 5 cm into the drain bottle.
2. **Turning on power switch** 
   1. Turn on the power switch at the front of the body.
3. **Pouring water** 
   1. Slide the open/close lever to the UNLOCK side
   2. Pour water through the opening of the chamber until you can see water through the hole at the centre of the heater cover.   
      HV-25 require 1.5 L or more.
4. **Loading substance**
   1. Place the substance to be sterilised ont the chamber
   2. Close lid and slide the open/close lever to **LOCK**
5. **Selecting Mode (process)**
   1. Select program by pressing the mode switch repeatedly until program is selected

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| --- | --- | --- |
| **Mode** | **Name** | **Description** |
| **1** | LIQ | Sterilizing of agar medium (warmed to prevent coagulation after sterilization |
|  | Sterilisation temperature | 121°C |
|  | Sterilisation time | 20 min |
|  | Warming temperature | - |
|  | Exhaust | 10% |

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| --- | --- | --- |
| **2** | LIQ | Sterilizing of agar medium (warmed to prevent coagulation after sterilization |
|  | Sterilisation temperature | 121°C |
|  | Sterilisation time | 20 min |
|  | Warming temperature | 50°C |
|  | Exhaust | 10% |
| **3** | SOLID | Sterilizing of tools of glass, ceramics, metal or/and rubber that withstand high temperature, high pressure steam and abrupt de-pressurisation during the exhaust process. |
|  | Sterilisation temperature | 121°C |
|  | Sterilisation time | 20 min |
|  | Warming temperature | - |
|  | Exhaust | - |
| **4** | AGAR | Dissolution of Agar, (Max= 2 litre agar medium) |
|  | Dissolution temperature | 100°C |
|  | Dissolution time | 20 min |
|  | Warming temperature | 50°C |
|  | Exhaust | 10% |

1. **Changing Set Values:** 
   1. Exhaust set value:
      1. to a small value to gradually exhaust
      2. to 0°C to natural cooling
   2. Sterilization of liquid: The larger volume the longer delay time per flask. In case there is 3 liter of water in a flas it take nearly 30 minutes (delay time) to reach the set sterilization temperature. You should set the sterilisation time 30 minutes linger than desired to cope with this: Set sterilisation time 50 minuttes = Dealy time (30 minutes) + desired sterilisation time (20 minutes)
      1. 3 Litres- add 30 minutes per flask. Set sterilisation time = 50 minutes
      2. 2 litres- add 25 minutes per flask. Set sterilisation time = 45 minutes
      3. 1 litres- add 20 minutes per flask. Set sterilisation time = 40 minutes
      4. 0.5 litres- add 15 minutes per flask. Set sterilisation time = 35 minutes
   3. Dissolution of coagulated agar medium: The larger volume the longer dissolution time per flask.
      1. 2 litres- add 60 minutes per flask at 100°C
      2. 1 litres- add 20 minutes per flask at 100°C
      3. 0.5 litres- add 15 minutes per flask at 100°C
2. **Starting Operation**
   1. Check that the exhaust bottle is between **HIGH** and **LOW**
      1. If too high remove liquid
      2. If too low add more water
   2. Select program, then press **START/STOP**
3. **Unloading**
   1. Confirm that the display shows “OPEN” blinking and the gauge for pressure in the chamber reads “0MPa” before opening the lid
   2. Slide the open/close lever to the UNLOCK side.
   3. Check that there are no spills in the chamber, note that salt water and much salinity causes corrosion of the chamber and piping.
   4. Turn off the power.

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| **Part of procedure** | **Unwanted scenarios** | **Necessary precautions** | **S\*K (Probability\*Consequence)** |
| 8 | Steam may gush out of the opening and cause injury. | Keep the face and hands away from chamber when lifting the lid after operation are complete.  Wear thick gloves. | 2\*1 |

## WASTE DISPOSAL

When making this solution some waste is generated. It is important to access the potential risk this on the environment and how this waste should be handled.

| **Waste** | | **Volume** | **Disposal method** | **Environmental risk** |
| --- | --- | --- | --- | --- |
| 1 | Media waste |  | All media waste should be disposed of in the hazardous waste |  |
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## REFERENCES