TUMI GENOMICS

# Tool Sterilization to Prevent Viroid Transmission

## A Comprehensive Literature Review

To determine the most effective method of tool sterilization to prevent mechanical transmission of Hop Latent Viroid, we conducted a comprehensive literature review of studies investigating viroid disinfection treatments.

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# Review Summary

Fifty three different chemicals and treatments were evaluated in this review for the ability to prevent viroid transmission in the ten referenced studies. Among these, five tested treatments showed 100% effectiveness at viroid deactivation. While most of these chemicals are shown to be effective against specific viroids (Table 1), one chemical, household bleach (sodium hypochlorite – NaClO), shows broad effectiveness across numerous studies, different plant species and multiple viroids.

Based on this observation, we strongly recommend the use of 10-20% household bleach (0.5%-1% sodium hypochlorite) for at least 60 seconds to disinfect cutting tools, equipment and surfaces in order to limit the spread of Hop Latent Viroid in cannabis grow facilities

Bleach is also highly effective for deactivation of most other cannabis pathogens including, fungus, mold, oomycetes, bacteria. Careful evaluation of conditions applied in each referenced study indicates that bleach is an effective disinfectant when used at a range of 10% to 20% concentration and for various incubation times. This quality provides flexibility and room for human error when employed as a broad disinfection SOP (Standard Operating Procedure) in large facilities.

Because the effective concentration in dilute bleach fluctuates over time, mixing a fresh bleach solution at the start of each day or when the solution becomes saturated with plant material is recommended. However, studies indicate that 20% bleach solutions can remain effective for up to 30 days at room temperature (Li et. al., 2015) allowing for flexibility.

Several common disinfection practices are not effective at viroid decontamination, including alcohol and heat treatment, or even the combination of these two.

It should be noted that several common disinfection practices are not broadly effective at viroid decontamination. While alcohol (ethanol or isopropanol) can be effective at deactivating other plant pathogens, viroids are not deactivated by alcohol, and treatment of cutting tools with only alcohol may increase transmission of viroid infection (Table 4 and Matsuura et. al., 2010). Viroids also show tolerance to heat treatment in multiple studies. Infectiousness persisted following alcohol dip and flaming, propane flame treatment and prolonged heat incubation of contaminated tools (Table 2 and Table 3). Therefore, we recommend not relying solely on heat treatment as a regular disinfection practice for prevention of viroid transmission.

# Tool Sterilization Recommendation

To prevent mechanical transmission of viroids in cannabis facilities, a dilute bleach solution should be used to disinfect tools, surfaces and gloved hands between plants.

**Diluting Bleach Procedure**

1. **Prepare the Area**
   Clean the work surface and prep a mixing container and/or spray bottle

2. **Prepare the Substances**
   You can use household bleach and tap water

3. **Mix the Substances**
   Wearing rubber gloves, mix concentration.


- 10% dilution is 9 parts water to 1 part bleach

  1/4 cup bleach and 2 1/4 cups water
  (62 ml bleach and 562 ml water)

  1 cup bleach and 9 cups water
  (250 ml bleach and 2250 ml water)

- 20% dilution is 19 parts water to 1 part bleach

  3/4 cup bleach and 16 cups water
  (177 ml bleach and 3785 ml water)

## Tools should remain in the bleach solution for 60 seconds

All tools should remain immersed in the bleach solution for 60 seconds or more to allow complete deactivation. Decontaminated tools can be rinsed in clean water prior to use. DO NOT mix bleach and alcohol as this creates toxic gas. Bleach is corrosive to metal tools so prolonged incubation is not recommended. Bleach should be stored in air-tight plastic containers and kept in a cool (50°F - 70°F), dry area away from sunlight. Bleach exposed to light or temperatures above 70OF degrades faster. The bleach solution should be mixed fresh at the start of each day and replaced if the solution becomes saturated with plant material. Dilute bleach in a spray bottle can be used periodically to disinfect gloved hands.

Bleach should not be stored with incompatible chemicals such as strong acids, ammonia, or alcohol. The shelf life of bleach when stored correctly is about six months. Old bleach solutions can be disposed off down the drain with ample amounts of water.

# Solutions

## Eliminates Viroid Transmission

- House hold bleach (5%–25%)
- 2%–37% formaldehyde + 2% sodium hydroxide
- 2%–3% Menno Florase (9% w/v benzoic acid)
- 2% Virkon S (20.4% potassium peroxymonosulfate, 1.5% sodium chloride)"
- NaOH 0.5% pH 13

## Possible Eliminates Viroid Transmission

- 95%–96% alcohol followed by flame
- 20%–100% fresh or powdered milk
- 6%–23% Hydrogen peroxide

## Does Not Eliminate Viroid Transmission

- 0.5%–1% Virkon S
- 1%–5% formaldehyde
- 1%–10% Bromodine
- 2% detergent
- 1% or 10% Triodine
- 1% Roccal
- 0.1%–10% sodium hydroxyide
- 2%–10% trisodium hypochlorite
- 70%–95% ethyl alcohol
- Diesel fuel
- 20% Phisohex
- 3% formalin
- 10% Borax
- 1%–50% Lysol Vinegar/oil/water (4:1/3:12)
- 20% diethyl ether
- 2% trisodium orthophosphate
- Incyte
- Alcide LD
- Exspor
- 0.781% Octave
- 1% MENNOTER forte
- 0.52% GREENSHIELD
- 0.977% StorOx
- 0.5% – 1% Virkon S
- Electrolyzed acid water
- 0.1 N hydrochloric acid
- 70% isopropanol
- 2% sodium hydroxide + 2% formaldehyde
- Chlorine Dioxide 3ppm–15ppm
- NaCO3 0.5% pH 11
- NaHCO3 pH 8.15

## Literary Summary Data

The tables below summarize results from published scientific reports investigating the efficacy of various chemicals and treatments for removal of viroid contamination from tools. Only studies where effectiveness was determined by inoculation of clean plants with sterilized tools are included in this summary. Tables indicate the concentration(s) of each chemical used in the studies, details of the treatment applied and the percent transmissibility that remained after disinfection.

## Table 1: Highly Effective Solutions/Treatments for Viroid Decontamination

Note that household bleach was 100% effective at stopping viroid transmission in every study for every tested viroid.

| Solution/ Treatment | Treatment Details | Transmissibility Following Treatment | Viroids Tested | References |
|---|---|---|---|---|
| House hold bleach (5%-25%) NOTE: Concentrations below 3% were not fully effective in numerous studies | 10 seconds for more in bleach solution (60 seconds recommended) | Varied, but treatment 0% infection rate in all studies tested | ASBVd, CEVd, PSTVd, TCDVd, HSV, CSVd | Thi Thu (2018), Desjardins et. al (1987), Garnsey and Whidden (1971), Roistacher et at. (1969), Sigh et. al. (1989), Li et. al (2015), Mackie et. al (2015), Matsuura et. al. (2010), Singh et. al (1989) |
| 2%-37% formaldehyde + 2% sodium hydroxide | Razor blade dipped in viroid extract and then used to slash seedling | 0% infection rate (12 plants tested) | ASVBd | Desjardins et. al (1987), Garnsey and Whidden (1971) |
| 2%-3% Menno Florase (9% w/v benzoic acid) | 10-60 second incubation | 0% infection rate (12 plants tested) | PSTVd | Timmermann et. al. (2001) |
| 2% Virkon S (20.4% potassium peroxymonosulfate, 1.5% sodium chloride) | 10-60 second dip | 0% infection rate (27 plants tested) | PSTVd | Li et. al (2015) |
| NaOH 0.5% pH 13 | 15 minute exposure time | 0% infection rate – 8 plants tested | TCDVd | Thi Thu (2018) |

## Table 2: Solutions/Treatments with Conflicting Evidence of Effectiveness for Viroid Decontamination

| Solution/ Treatment | Treatment Details | Transmissibility Following Treatment | Viroids Tested | References |
|---|---|---|---|---|
| 95%-96% alcohol followed by flame | TCDVd- 0% infection rate 2-3 second dip followed by flame (8 plants tested) | CEVd- 95%-100% infection rate after 2-3 second dip followed by flame (34 plants tested, two studies) | TCDVd, PSTVd, CEVd | Thi Thu (2018), Garnsey and Shidden (1971), Roistacher et at. 1969 |
| 20%-100% fresh or powdered milk | PSTVd- 0% infectivity after 1 minute incubation – 10 plants tested (Mackie et. al.) | PSTVD and CEVD- 11%-38% infectivity rate after 2-60 seconds incubation with 20%-100% powdered milk and 7% infection rate with 100% fresh milk- 16 plants tested (CEVd) | CEVd, PSTVd | Garnsey and Shidden (1971), Li et. al. (2015), Mackie et. al. (2015) |
| 6%-23% Hydrogen peroxide | ASVBd- 0% infectivity after dip in solution- 12 plants tested | PSTVd – Various level of infectivity in combination with other acids (see Bioside, Sanidate, Octave and Vortexx in Table 3) | ASVBd, PSTVd | Desjardins et. al (1987), Li et. al. (2015) |

## Table 3: Physical treatments that are not effective at viroid decontamination

| Solution/ Treatment | Treatment Details | Transmissibility Following Treatment | Viroids Tested | References |
|---|---|---|---|---|
| Flame - propane torch (4-6 seconds) | Six seconds of flame resulted in average temperature of 222℃ (432℉) | 61% infection rate (36 plants tested) | CEVd | Roistacher et at. 1969 |
| Heat treatment | 10 minutes at 140℃ (284℉) and 10 minutes at 100℃ ( 212℉) NOTE: 160 ℃ (320℉) for 10 minutes was effective. | 90℃-140℃ (194℉ - 284℉) infection rate ranged between 7% and 61%. | HSV, CSVd | Takahashi and Yaguchi (1984), Hollings and Stone (1973) |
| Ultraviolet Radiation | 10-30 minute treatment | 17%-38% infection rate (29 plants tested per condition) | CSVd | Hollings and Stone (1973) |
| Sonication | 5-15 minutes | 30% infection rate (29 plants tested per condition)* | CSVd | Hollings and Stone (1973) |

*Infection rate of untreated ~38%

## Table 4: Chemical treatments that are not effective at viroid decontamination

| Solution/ Treatment | Treatment Details | Transmissibility Following Treatment | Viroids Tested | References |
|---|---|---|---|---|
| 0.5%-1% Virkon S | 2-60 second dip | 33%-100% infection rate (67 plants tested- two studies) | PSTVd | Mackie et. al (2015), Li et. al (2015) |
| 1%-5% formaldehyde | 2-30 second dip in chemical | 12.5%-81% infection rate (278 plants tested- Both studies combined) | CEVd, CSVd | Garnsey and Shidden (1971), Hollings and Stone (1973) |
| 1%-10% Bromodine | 2-3 second dip or 2 minute dip (rinsed) | 38% infection rate (24 plants tested). Note- 10% concentration for 2 minutes stopped transmission (8 plants tested). | CEVd | Garnsey and Shidden (1971) |
| 2% detergent | 2-3 second dip | 70% infect rate (20 plants tested). | CEVd | Garnsey and Shidden (1971) |
| 1% or 10% Triodine | 2-3 second dip | 75% infection rate (16 plants tested) | CEVd | Garnsey and Shidden (1971) |
| 1% Roccal | 2-3 second dip | 75% infection rate (8 plants tested) | CEVd | Garnsey and Shidden (1971) |
| 0.1%-10% sodium hydroxyide | 2-3 second dip, 5 minute or 10 minute treatment | 54% infection rate for 2-3 second dip (28 plants tested), 0% infection rate for 5-10 minute incubation (17 plants tested- two studies) | CEVd, HSV | Garnsey and Shidden (1971) Takahashi and Yaguchi (1984) |
| 2%-10% trisodium hypochlorite | Several seconds | 25% (139 plants tested, two studies) | CEVd | Roistacher et at. 1969 |
| 70%-95% ethyl alcohol | 1-60 second dip | 65%-100% infection rate (30 plants tested, two studies) | CEVd, PSTVd | Roistacher et. al. (1969), Mackie et. al (2015) |
| Diesel fuel | 2-3 second dip | 100% infection rate (8 plants tested) | CEVd | Garnsey and Shidden (1971) |
| 20% Phisohex | Several seconds | 29% infection rate (14 plants tested) | CEVd | Roistacher et. al. (1969) |
| 3% formalin | Several seconds | 50% infection rate (14 plants tested) | CEVd | Roistacher et. al. (1969) |
| 10% Borax | Several seconds | 64% infection rate (14 plants tested) | CEVd | Roistacher et. al. (1969) |
| 1%-50% Lysol | 2-60 second dip | 22%-100% infectivity rate (41 plants tested, two studies) | CEVd, PSTVd | Roistacher et. al. (1969), Li et. al (2015) |
| Vinegar/oil/water (4:1/3:12) | Several seconds | 100% infection rate (9 plants tested) | CEVd | Roistacher et. al. (1969) |
| 2% trisodium orthophosphate | 2-3 second dip | 0.5% infection rate (18 plants tested) | CSVd | Hollings and Stone (1973) |
| 20% diethyl ether | 2-3 second dip | 25% (139 plants tested) | CSVd | Hollings and Stone (1973) |
| Incyte | Various times depending on equipment | 100% infection rate (11 plants tested) | PSTVd | Singh et. al (1989) |

| Solution/ Treatment | Treatment Details | Transmissibility Following Treatment | Viroids Tested | References |
|---|---|---|---|---|
| Alcide LD | Various times depending on equipment | Between 5-20 plants tested, variable infectivity depending on equipment | PSTVd | Singh et. al (1989) |
| Exspor | Various times depending on equipment | Between 5-20 plants tested, variable infectivity depending on equipment | PSTVd | Singh et. al (1989) |
| 0.781% Octave | 10-60 second dip | 18.5% infectivity (27 plants tested) | PSTVd | Li et. al. (2015) |
| 1% MENNOTER forte | 10-60 second dip | 18.5% infectivity (27 plants tested) | PSTVd | Li et. al. (2015) |
| 0.52% GREENSHIELD | 10-60 second dip | 26% infectivity (27 plants tested) | PSTVd | Li et. al. (2015) |
| 0.977% StorOx | 10-60 second dip | 26% infectivity (27 plants tested) | PSTVd | Li et. al. (2015) |
| 0.5%- 1% Virkon S | 10-60 second dip | 37% - 67% infectivity (18-27 plants tested) | PSTVd | Li et. al. (2015), Mackie et. al. (2015) |
| 0.4% KleenGrow | 10-60 second dip | 44% infectivity rate (18 plants tested) | PSTVd | Li et. al. (2015) |
| 0.11% Greenhouse Guardian | 10-60 second dip | 41% infectivity rate (27 plants tested) | PSTVd | Li et. al. (2015) |
| 0.195% Vortexx | 10-60 second dip | 50% infectivity (18 plants tested) | PSTVd | Li et. al. (2015) |
| 0.078% BioSide | 10-60 second dip | 56% infectivity rate (27 plants tested) | PSTVd | Li et. al. (2015) |
| 0.382% SaniDate | 10-60 second dip | 63% infectivity rate (27 plants tested) | PSTVd | Li et. al. (2015) |
| 0.1% DES-O-GERM | 10-60 second dip | 67% infectivity rate (18 plants tested) | PSTVd | Li et. al. (2015) |
| 2.5%- 10% trisodium phosphate | 2-60 second dip | 16.7%-70% infection rate (69 plants tested, 3 studies) | TCDVd, PSTVd, CEVd | Matsuura et. al. (2010), Li et. al. (2015), Garnsey and Shidden (1971) |
| 10% FARMcleanse | 1 minute dip | 100% infection rate (10 plants tested) | PSTVd | Mackie et. al. (2015) |
| Electrolyzed acid water | 15 minute dip | 75% infection rate (48 plants tested) | TCDVd | Matsuura et. al. (2010) |
| 0.1 N hydrochloric acid | 15 minute dip | 62.5% infection rate (48 plants tested) | TCDVd | Matsuura et. al. (2010) |
| 70% isopropanol | 15 minute dip | 95.8% infection rate (48 plants tested) -NOTE- water dip alone resulted in 78.1% infection rate. | TCDVd | Matsuura et. al. (2010) |
| 2% sodium hydroxide + 2% formaldehyde | 15 second dip | 4.2% infection rate (48 plants tested) | TCDVd | Matsuura et. al. (2010) |
| Chlorine Dioxide 3ppm-15ppm | 15 minute exposure time | 100% infection rate (36 total plants tested) | TCDVd | Thi Thu (2018) |
| NaCO3 0.5% pH 11 | 15 minute exposure time | 50% infection rate (8 plants tested) | TCDVd | Thi Thu (2018) |
| NaHCO3 pH 8.15 | 15 minute exposure time | 50% infection rate (8 plants tested) | TCDVd | Thi Thu (2018) |

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This comprehensive report reviews 10 papers with over 50 experiments conducted to determine the most effective methods of tool and equipment sterilization to prevent the transmission of Hop Latent Viroid (HLVd) in cannabis.

At TUMI Genomics we recommend consistent and periodic testing to effectively control for HLVd.

Additional publications include Standard Operating Procedures (SOPs) that will help professional growers to maintain a clean operation.

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