אוניברסיטת בר-אילן
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פרופ' רונית שריד, מעבדה לוירולוגיה

הפקולטה למדעי החיים ע"ש מינה ואברד גודמן,
אוניברסיטת בר אילן

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Electrolyzed Water - Summary of Anti-viral Experiments

Assay #1:
Anti-viral efficacy of the electrolyzed water was tested using Herpes simplex virus type 1 (HSV-1).

- 6.5 µl of 150 mM NaCl were added into 1 µl of HSV-1 suspension containing 2,000 plaque forming units (PFU) followed by the addition of 2.5 µl of electrolyzed water (50 or 200 mg/l). This was followed by 2 or 10-min incubation at room temperature.
- 4 µl of 150 mM NaCl were added into 1 µl of HSV-1 suspension containing 2,000 plaque forming units (PFU) followed by the addition of 5 µl of electrolyzed water (50 or 200 mg/l). This was followed by 2 or 10-min incubation at room temperature.
- Infectious viruses were then quantified by assay plaque assay.
- Controls included uninfected cells (mock), viruses that were incubated with Saline and viruses that were incubated with Sodium thiosulfate which is known to neutralize the electrolyzed water.
- Complete inhibition of plaque formation which indicates complete neutralization of HSV-1 was evident in all assay conditions (addition of 2.5 or 5 µl of electrolyzed water at 50 and 200 mg/l and incubation for 2 or 10-min).
- Similar results were obtained in two independent biologic repeats, each including two repeats for each assay condition.
- This provides a Neutralization Index (NI) of >6.3 at 12.5 mg/l upon 2-min incubation.

Assay #2:
Anti-viral efficacy of the electrolyzed water was tested using the human coronavirus OC43 (HCoV-OC43).

- A similar assay as described in Assay#1 was carried but instead we tested the neutralizing efficacy of 8,000 PFU of HCoV-OC43.
- Complete inhibition of plaque formation which indicates complete neutralization of HCoV-OC43 was evident in all assay conditions (addition of 2.5 or 5 µl of electrolyzed water at 50 and 200 mg/l and incubation for 2 or 10-min).
- Similar results were obtained in two independent biologic repeats, each including two repeats for each assay condition.
- This provides a Neutralization Index (NI) of >6.9 at 12.5 mg/l upon 2-min incubation.
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<u>Assay #3</u>:
Anti-viral efficacy of the electrolyzed water was tested using the human coronavirus OC43 (HCoV-OC43) (variations in virus challenge, concentrations of the electrolyzed water and incubation time)

- 1, 2 or 3 µl of HCoV-OC43 suspension, corresponding to 30,000, 60,000 and 90,000 PFU, respectively, were added to 150 mM NaCl.
- 2.5 or 5 µl of 50 mg/l or 2.5 µl of 25 mg/l electrolyzed water were added, followed by 1 or 2-min incubation at room temperature (each assay was in total volume of 10 µl).
- Complete inhibition of plaque formation was obtained when 10,000 PFU were incubated for 2-min with 2.5 or 5 µl of 50 mg/l electrolyzed water (Neutralization index >7 at 12.5 mg/l).
- Complete inhibition of plaque formation was obtained when 30,000 PFU were incubated for 2-min with 5 µl of 50 mg/l electrolyzed water (Neutralization index >7.4 at 25 mg/l).
- Partial inhibition of plaque formation was obtained when 10,000 PFU were incubated for 1-min with 2.5 or 5 µl of 50 mg/ml or with 2.5 µl of 25 mg/l electrolyzed water.
- Partial inhibition of plaque formation was obtained when 30,000 PFU were incubated for 1-min with 2.5 µl of 50 mg/ml.
- The results with 90,000 PFU could not be evaluated.
- <u>This experiment provides NI of >7 at 12.5.mg/ml upon 2-min incubation</u>.

<u>Assay #4</u>:
Anti-viral efficacy of the electrolyzed water was tested using the human coronavirus OC43 (HCoV-OC43).

- This experiment employed a different preparation of HCoV-OC43.
- The experiment was carried as described in Assay #1 yet employed 18,000, 45,000 and 90,000 PFU.
- Use of 2.5 or 5 µl of 50 mg/l electrolyzed water for 2-min.
- Complete inhibition of plaque formation was obtained when 18,000 PFU were incubated with 2.5 or 5 µl of 50 mg/ml electrolyzed water for 2-min (<u>Neutralization index >7.25 at 12.5 mg/l upon 2-min incubation</u>).
- An estimated 70-80% inhibition was evident with 45,000 and 90,000 PFU (<u>Neutralization index > 7.49-7.8 at 12.5 mg/l upon 2-min incubation).</u>

Assay # 5:

Anti-viral efficacy of the electrolyzed water was tested using the human coronavirus OC43 (HCoV-OC43).

- The experiment was carried as described in Assay #1 yet employed 18,000, 45,000 and 90,000 PFU.
- Use of 2.5 or 5 µl of 200 mg/l electrolyzed water for 30 seconds.
- Complete inhibition of plaque formation was obtained when 18,000 PFU were incubated with 5 µl of 200 mg/ml electrolyzed water for 30 seconds (Neutralization index >7.25 at 100 mg/l upon 30 sec incubation).
- An estimated 95% inhibition was evident with 45,000 and 90,000 PFU were incubated with 5 µl of 200 mg/ml electrolyzed water for 30 seconds (Neutralization index > 7.63-7.93 at 100 mg/l upon 30 incubation).
- No inhibition with 2.5 µl of 200 mg/ml electrolyzed water for 30 seconds.