



PROJECT REPORT

Project Title:

Evaluation of the “Anolyte Water” or “Neutral Electrolyzed Water” Disinfectant Solution on Inactivation of Avian Influenza Virus and Other Avian Viruses

Submitted To:

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Summary of Laboratory Finding

The Avian Virology Section of the Animal Diagnostic Laboratory at Penn State University has conducted a research study on the “Anolyte Water” or “Neutral Electrolyzed Water” disinfectant solution on inactivation of avian influenza virus and other important avian viruses. The NTS NatureTech Solutions© (Lancaster, PA 17601) has provided newly produced and fresh Anolyte Water solutions for this research study.

Preliminary results indicated that the Anolyte Water solution effectively inactivated or killed a variety of avian respiratory and enteric viruses tested including avian influenza virus (AIV), paramyxoviruses (PMV) and Newcastle disease virus (NDV, or PMV-1), infectious bronchitis virus (IBV), fowl adenovirus (FAV), avian reovirus, and pigeon herpesvirus. Effective dilutions of the Anolyte Water were found between 1:2 and 1:5 with sterile distilled water or deionized water. Laboratory findings are summarized as the following.

1. Effects of the Anolyte Water solution on inactivation of avian influenza virus (AIV)

The Anolyte Water solution was tested against 10 avian influenza virus (AIV) subtypes of H1N1, N2N2, H3N2, H4N8, H5N2, H5N3, H5N9, H6N8, H7N2 and N9N1 at various dilutions of stock Anolyte Water and different reaction times with these AIV subtypes.

The Anolyte Water solution at 1:2 -to- 1:5 dilutions: each of the 10 AIV subtypes was 100% inactivated within 10 min, 15 min, 30 min, 60 min and 120 min of reaction times tested.

2. Effects of the Anolyte Water solution on inactivation of Paramyxoviruses (PMV) and Newcastle disease virus (NDV, or PMV-1)

The Anolyte Water solution at 1:2 -to- 1:5 dilutions: PMV type 1 (PVM-1) or Newcastle disease virus (NDV) and PMV type 2 (PVM-2) were 100% inactivated within 10 min, 15 min, 30 min, 60 min and 120 min of reaction times tested.

3. Effects of the Anolyte Water solution on inactivation of infectious bronchitis virus (IBV)

The Anolyte Water solution at 1:2 -to- 1:5 dilutions: Five IBV serotypes of Mass, Conn, Ark, Del, and PA strains were 100% inactivated within 10 min, 15 min, 30 min, 60 min and 120 min of reaction times tested.

4. Effects of the Anolyte Water solution on inactivation of fowl adenovirus (FAV)

The Anolyte Water solution at 1:2 -to- 1:5 dilutions: Four FAV strains of M2, KR5, C229, and LA/SPF/C were were 100% inactivated within 5 min 10 min, 15 min, 30 min, 60 min and 120 min of reaction times tested.

5. Effects of the Anolyte Water solution on inactivation of avian reovirus

The Anolyte Water solution at 1:2 -to- 1:5 dilutions: A selection of 10 field isolates of avian reovirus were used for the inactivation test, and each of the 10 reovirus isolate was 100% inactivated within 5 min, 10 min, 15 min, 30 min, 60 min and 120 min of reaction times tested.

6. Effects of the Anolyte Water solution on inactivation of pigeon's herpesvirus.

The Anolyte Water solution at 1:2 -to- 1:5 dilutions with distilled water: A selection of 10 field isolates of pigeon herpesvirus were used for the inactivation test, and each of the 10 herpesvirus isolates was 100% inactivated within 5 min, 10 min, 15 min, 30 min, 60 min and 120 min of reaction times tested.

The inactivation tests on avian respiratory viruses of AIV, PMV, and IBV:

Each preparation of a mixture of the Anolyte Water solution and an avian virus was inoculated into 9-11-day old specific-pathogen-free (SPF) embryonating chicken eggs (ECE) for testing the inactivation result or if any residual life virus. A minimum of 10-15 min was required for preparation of test procedure in ECE.

Virus titers of the avian respiratory viruses of AIV, PMV and IBV used in this study were measured from $10^{5.0}$ - $10^{6.5}$ of ELD₅₀ dose/ml (ELD = embryo lethal dose)

The inactivation tests on avian enteric viruses of FAV, avian reovirus and herpesvirus:

Each preparation of the Anolyte Water solution and virus mixture was inoculated into chicken embryo cell cultures or LMH cell cultures for testing the inactivation result or if any residual life virus. A minimum of 5-10 min was required for preparation of test procedure in cell cultures.

Virus titers of the avian enteric viruses of FAV, avian reovirus and herpesvirus used in this study were measured from $10^{4.5}$ - $10^{6.5}$ of CLD₅₀ dose/ml (CLD = cell culture lethal dose).

Table 1. Neutral Electrolyzed Water on Inactivation of Avian Virus
Avian Influenza Virus (AIV), Newcastle disease virus (NDV), infectious bronchitis virus (IBV), fowl adenovirus (FAV), avian reovirus, and avian herpesvirus

Avian Respiratory Virus	Neutral Electrolyzed Water at 1:2 –to- 1:5 dilutions			
	10-15 min	30 min	60 min	120 min
<u>AIV subtypes</u>				
H1N1	100%	100%	100%	100%
H2N2	100%	100%	100%	100%
H3N2	100%	100%	100%	100%
H4N8	100%	100%	100%	100%
H5N2	100%	100%	100%	100%
H5N3	100%	100%	100%	100%
H5N9	100%	100%	100%	100%
H6N8	100%	100%	100%	100%
H7N2	100%	100%	100%	100%
H9N2	100%	100%	100%	100%
<u>PMV</u>				
PMV-1 (NDV)	100%	100%	100%	100%
PMV-2	100%	100%	100%	100%
<u>IBV strains</u>				
Mass	100%	100%	100%	100%
Conn	100%	100%	100%	100%
Ark	100%	100%	100%	100%
Del	100%	100%	100%	100%
PA	100%	100%	100%	100%
Avian Enteric Virus	Neutral Electrolyzed Water at 1:2 –to- 1:5 dilutions			
	5-10 min	30 min	60 min	120 min
<u>FAV</u>				
M2	100%	100%	100%	100%
LA/C	100%	100%	100%	100%
C229	100%	100%	100%	100%
KR5	100%	100%	100%	100%
<u>Avian Reovirus</u>				
10 field isolates	100%	100%	100%	100%
<u>Avian Herpesvirus</u>				
10 field isolates	100%	100%	100%	100%

100% = virus was completely inactivated