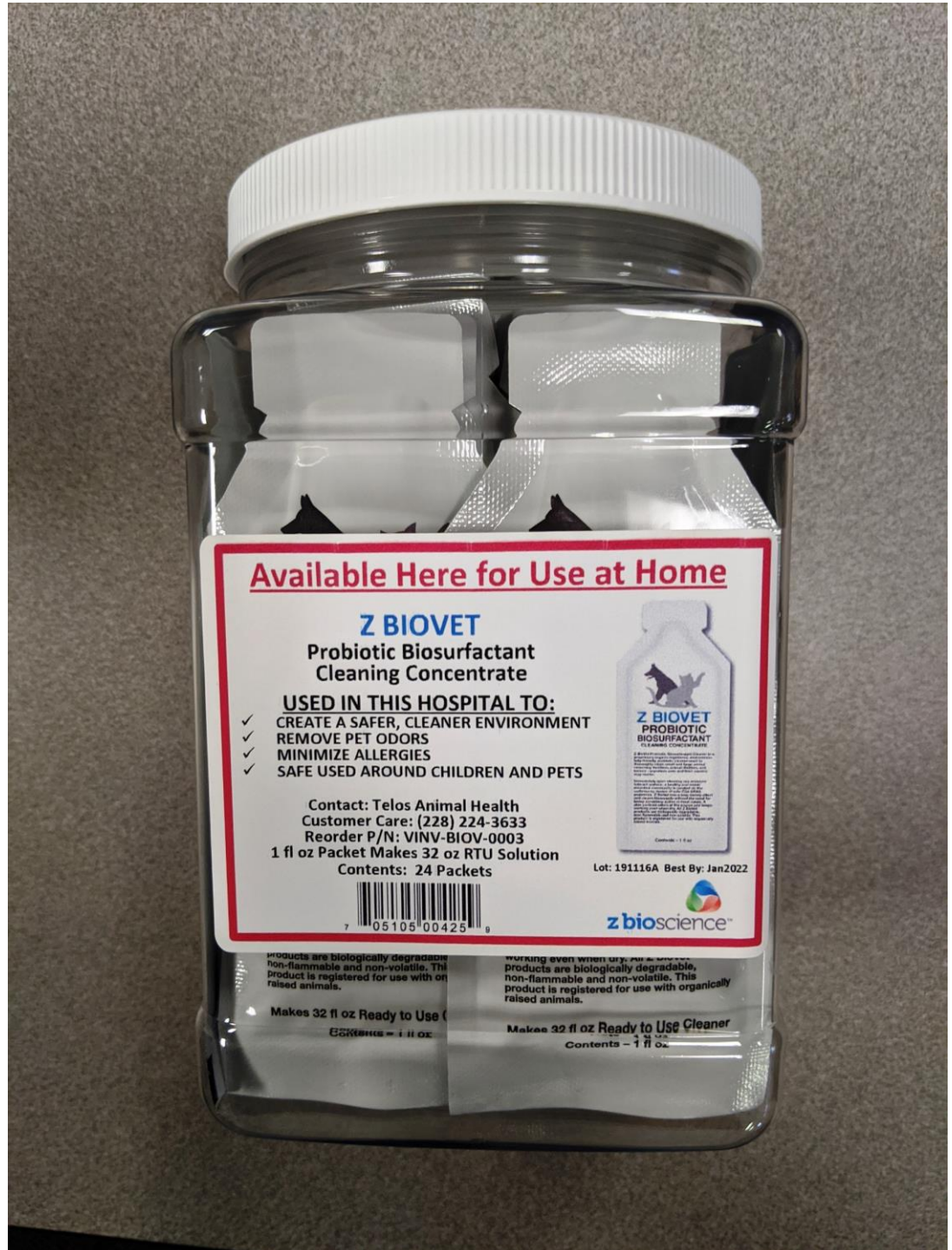
Telos Animal Health

"Trust, Experience, and Innovation"


Z BIOVET
PROBIOTIC
BIOSURFACTANT
CLEANING CONCENTRATE
Z BioVet Probiotic Biosurfactant Cleaner is a proprietary organic registered, environmentally-friendly probiotic cleaner used to thoroughly clean small and large animal veterinary facilities, animal shelters, and homes - anywhere pets and their owners may reside.
Immediately upon cleaning any moisture tolerant surface, a healthy and stable microbial community is created on the surfaces by means of safe FDA GRAS probiotics. Z BioVet has a long lasting effect and cleans thoroughly without the need for heavy scrubbing action in most cases. It also controls odors at the source and keeps working even when dry. All Z BioVet products are biologically degradable, non-flammable and non-volatile. This product is registered for use with organically raised animals.
Contents – 1 fl oz


Available Here for Use at Home
Z BIOVET
Probiotic Biosurfactant
Cleaning Concentrate
USED IN THIS HOSPITAL TO:
✓ CREATE A SAFER, CLEANER ENVIRONMENT
✓ REMOVE PET ODORS
✓ MINIMIZE ALLERGIES
✓ SAFE USED AROUND CHILDREN AND PETS
Contact: Telos Animal Health
Customer Care: (228) 224-3633
Reorder P/N: VINV-BIOV-0003
1 fl oz Packet Makes 32 oz RTU Solution
Contents: 24 Packets
7 05105 00425 9
Lot: 191116A Best By: Jan2022
z bioscience
Makes 32 fl oz Ready to Use Cleaner


Z BIOVET
PROBIOTIC
BIOSURFACTANT
CLEANING CONCENTRATE
Directions: Before cleaning with Z BioVet Cleaner, remove excess and loose organic materials from heavily soiled areas.
Shake packet before mixing.
1. Prepare Ready to Use Solution – Add the entire contents of this packet to 32 oz of potable tap water. If possible, use warm water between 100°F and 140°F.
2. Spray all surfaces completely; for vertical surfaces, spray starting at bottom and moving to top. Wipe surfaces before the solution dries.
3. An alternate method of application is to spray the surface as noted in #2 above, allow it to reside for 10 minutes and rinse with potable water.
Discard unused diluted solution within 7 days.
Ingredients: Complies with OSHA 29 CFR XVII-1910.1200 Section (i) "Trade Secrets". Contains no hazardous components under current OSHA definitions. May contain ionic and non-ionic surfactant, non-GMO GRAS microorganisms, enzymes.
Caution: Do not store in direct sunlight or in temperatures above 110°F or allow this product to freeze.
Protection Notice: Keep away from children. May be irritating to eyes. For eye contact, wash thoroughly with water for several minutes. Do not ingest. If swallowed – show container to physician. If allergy prone wear gloves.
These statements have not been evaluated by the FDA. This product is not intended to diagnose, treat, cure or prevent any disease.
800 South Ohio Street
Tuscola, IL 61953
800.500.9551
www.zbioscience.com
www.zbiovet.com
FDA Reg: 14347519134
ZB/BVP/001c
Lot: 191116A
Best By: Jan 2022
z bioscience
true clean technology™


Z BIOVET
PROBIOTIC
BIOSURFACTANT
CLEANING SOLUTION
1 Gallon (3.79L)

100% NATURAL

Safer Method
Clean and Control
Pathogenic Organisms

z bioscience™
true clean technology™

Telos Animal Health
"Trust, Experience, and Innovation"

Z BIOVET

Probiotic Biosurfactant Cleaner
Specially formulated for the Animal Health Industry

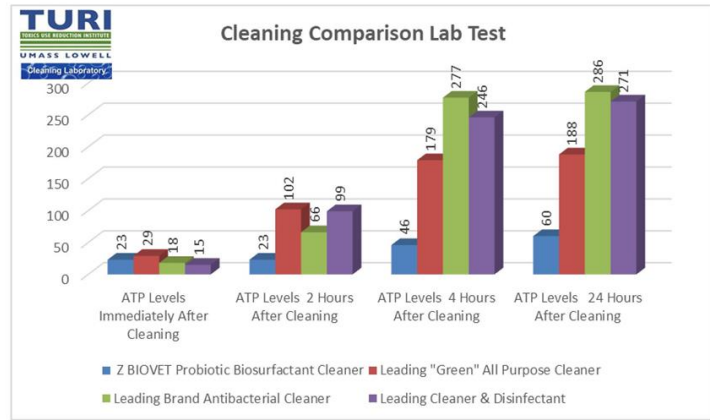
Critical Performance Qualities	Disinfectants (UnSustainable)	Z BIOVET (Sustainable)
Ongoing Odor Elimination	NO	YES
Ongoing Cleaning & Protection (up to 3 days)	NO	YES
Work When Dry and Wet	NO	YES
Surface Motility	NO	YES
Cumulative, Ongoing Benefits	NO	YES
Safe for Animals and Clinic Personnel	NO	YES
Surface Safe / pH Neutral	NO	YES
Addresses Biological Contamination (Biofilm)	NO	YES

Disinfectants kill both good and bad bacteria

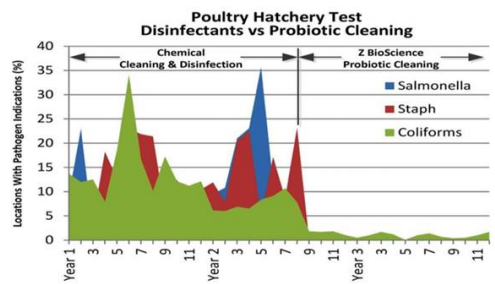
Z BIOVET - Adds Beneficial Biology (Probiotics) while consuming *ALL* of Pathogens' food sources for Superior Cleaning & Protection

Without food sources, Pathogens starve and die

Telos Animal Health Introduces Z BIOVET – A specially formulated Probiotic Cleaner for the Animal Health Industry from the makers of Z Bioscience



All four tested products produced levels below 30 immediately after cleaning. **HOWEVER**, just 4 hours after cleaning, the surface cleaned using Z BIOVET was the only one to maintain an acceptable level of cleaning. The leading brand antimicrobial, 'green', and disinfectant products all failed.



The data set represented in this chart is from 100 plate count tests every day for 3 years (a very robust and comprehensive data set). It also demonstrates the longevity of the ongoing efficacy of Z BioScience's Probiotic Cleaners against pathogens.

We have a lot more success stories that are similar to the two graphs above. Call us at (228) 224-3633 or come visit us at TelosAH.com

z bioscience™
true clean technology™

Telos Animal Health
"Trust, Experience, and Innovation"

All areas within a veterinary hospital that have a continuous and ongoing need for both cleaning and sanitation.

The practice of hygienic procedures in veterinary hospitals is necessary to control the spread of potential infectious organisms such as;

Ecoli

Salmonella

Pseudomonas

MRSA

These organisms and many others can can unintentionally contaminate the surface areas within a veterinary hospital where they can be spread via physical contact with hands, feet or paws from;

PET TO PET
PET TO HOSPITAL STAFF
PET TO PET PARENT

When pet derived odors occur in veterinary hospitals, the real cause of these odors is the presence of unwanted bacteria that are colonizing within the source of the odor itself.

As a result, ongoing hygienic procedures are necessary in order to control odors that can occur within the hospital itself, due to the confinement of pets and their waste along with unwanted colonizing bacteria.

Z BIOVET BIOSURFACTANT PROBIOTIC CLEANING SOLUTION

A revolutionary "two in one" solution that cleans and protects through the utilization of billions of beneficial probiotic bacteria that goes to work immediately.

Following application, Z BioVet continues to work up to 3 full days, populating the surface where it is applied, with a healthy and stable microbial community that is completely safe and natural.

Cleans all the way down to the microbial level.

THROUGH A METHOD OF COMPETITIVE EXCLUSION, CLEANS ANY MOISTURE TOLERATE SURFACE DOWN TO THE MICROBIAL LEVEL, WHILE ALSO CONTROLLING OFFENSIVE ODORS

100% ORGANIC.

COMPETITIVE EXCLUSION

Z BioVet does not kill pathogens with drugs or chemicals, but rather inhibits them by competitive exclusion.

1. The probiotics rapidly colonize surfaces with good bacteria.
2. They ingest the available food and other resources so pathogens have no source of energy and are thereby unable to get a foothold.
3. Since pathogens require a source of food, when that is taken away they are inhibited, starve and die.

INGESTION DOWN TO THE MICROBIAL LEVEL

Z BioVet ingests all of the components that keep biofilm alive. Biofilm is usually invisible. In spite of surface areas appearing to be clean, they could be crawling with salmonella, MRSA or other pathogenic organisms.

ADENOSINE TRIPHOSPHATE (ATP)

Bacteria and the support system in which they survive leave a marker known as adenosine triphosphate or ATP. ATP is the energy currency that stores energy and powers the cells of bacteria. It can be thought of as cellular gasoline. Virtually all cells including bacteria contain ATP at different levels. Consequently, the lower the ATP level, the cleaner the surface and the less likely it is to harbor biofilm and pathogens.

HYGIENA LUMINOMETER

Measuring the presence of ATP is determined through the use of a Hygiena Luminometer. An ATP reading of 30 or less is an indication that it is unlikely that there is surface contamination that would support pathogen growth. On the other hand an ATP reading of 30 or greater, would indicate a probability of something harmful being on the surface and the probability increases as the ATP reading increases. When using Z BIOVET Biosurfactant Cleaner it achieves better ATP readings on the initial cleaning than most products with harmful chemicals. It has been shown that ATP readings remain near the post test levels for 24, 48, and even 72 hours following use of Z BIOVET Biosurfactant Cleaner.

SAFETY

Z BioVet is very safe to handle. It is hypoallergenic, pH neutral and environmentally friendly. It simply makes a veterinary hospital cleaner, while being healthier for pets, pet parents and hospital staff. Z BioVet is 100% organic. It cleans and controls pathogens naturally. Thus, it becomes a far safer method to clean and control pathogenic organisms.

PROBIOTIC BACTERIA PICTURED IN BACKGROUND

Comparison of Common Methods Used to Control the Spread of Pathogens and Odors Inside Veterinary Hospitals

CHEMICAL DISINFECTANTS

All chemical disinfectants require the presence of water in order to be effective. When dry they no longer work.

Prior to using a chemical disinfectant, it is recommended that all surfaces be thoroughly cleaned first with soap or detergent and then rinse with potable water.

Z BIOVET BIOSURFACTANT CLEANER

Z BioVet Biosurfactant Cleaner DOES NOT require the presence of water in order to be effective and continues to work while dry.

Z BioVet Biosurfactant Cleaner DOES NOT require a pre-cleaning prior to use.

CHEMICAL DISINFECTANTS REQUIRE A DWELL TIME EXPOSURE IN ORDER TO BE EFFECTIVE

Hand spray surface areas and wipe dry with a paper towel. This is very commonly done on Exam Tables following each pet examination. This is an effective use of cleaning if the required dwell time is utilized. Once the area is dried, disinfectants no longer work because they require the presence of water in order to be effective.

Floor mopping all ground surfaces within the hospital are to help clean and sanitize the foot traffic from both people and their pets. This is an effective use of cleaning if the required dwell time is utilized. Once the area is dried, disinfectants no longer work.

Z BIOVET BIOSURFACTANT CLEANER DOES NOT REQUIRE A DWELL TIME EXPOSURE IN ORDER TO BE EFFECTIVE

Hand spray surface areas and wipe dry with a paper towel. A dwell time is not required. It does not require the presence of water to be effective and it continues to work up to 3 days following the initial application.

Floor mopping all ground surfaces within the hospital to help clean and sanitize the foot traffic from both people and their petsA dwell time is not required. It does not require the presence of water to be effective and it continues to work up to 3 days following the initial application.

Directions for Use

Rescue Instructions from label

Animal Housing Facilities in Veterinary Clinics: Dilute at 2.0 – 8.0 oz. of product per gallon of water (1:16 – 1:64).

For cleaning and disinfecting the following hard non-porous surfaces:
feeding and watering equipment, utensils, instruments, cages, crates, kennels, catteries, etc.

1. Remove all animals and feeds from premises, animal transportation vehicles, crates, etc.
2. Remove all litter, droppings, and feces from floors, walls, and surfaces of facilities occupied or traversed by animals.
3. Empty (or cover) all troughs, racks and other feeding and watering appliances
4. Thoroughly clean all surfaces with soap or detergent and rinse with potable water.
5. Saturate all surfaces (floors, walls, cages and other washable hard, non-porous environmental surfaces) by spraying with use solution until thoroughly wet. To disinfect, all surfaces must remain wet for 5 minutes when using a 1:16 (8.0 oz per gallon of water) dilution for bactericidal, fungicidal and *virucidal efficacy. If using a 1:64 (2.0 oz per gallon of water) dilution, allow 5 minutes for *viruses and 10 minutes for bacteria.

Clorox Pro Quaternary Commercial Solutions All-Purpose Disinfectant Cleaner1

This page contains information on Clorox Pro Quaternary Commercial Solutions All-Purpose Disinfectant Cleaner1 for **veterinary use**.
The information provided typically includes the following:

- Clorox Pro Quaternary Commercial Solutions All-Purpose Disinfectant Cleaner1 Indications
- Warnings and cautions for Clorox Pro Quaternary Commercial Solutions All-Purpose Disinfectant Cleaner1
- Direction and dosage information for Clorox Pro Quaternary Commercial Solutions All-Purpose Disinfectant Cleaner1

Clorox Pro Quaternary Commercial Solutions All-Purpose Disinfectant Cleaner1

Active Ingredients

Octyl decyl dimethyl ammonium chloride
Dioctyl dimethyl ammonium chloride
Didecyl dimethyl ammonium chloride
Alkyl (50% C14, 40% C12, 10% C16) dimethyl benzyl ammonium chloride
OTHER INGREDIENTS:
TOTAL:

KEEP OUT OF REACH OF CHILDREN

DANGER

FIRST AID: Have the product container or label with you when calling a poison control center or doctor or going for treatment. **IF IN EYES:** Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if

present, after the first 5 minutes, then continue rinsing eye. Call a poison control center or doctor for treatment advice. **IF ON SKIN OR CLOTHING:** Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call poison control center or doctor for treatment advice. **IF SWALLOWED:** Call poison control center or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by the poison control center or doctor. Do not give anything by mouth to an unconscious person. **IF INHALED:** Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably by mouth-to-mouth, if possible. Call a poison control center or doctor for further treatment advice.

NOTE TO PHYSICIAN: Probable mucosal damage may contraindicate the use of gastric lavage.

Directions For Use

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

DISINFECTION - To disinfect inanimate, hard, nonporous surfaces, add 1 ounce of this product per gallon of water. Apply solution with a mop, cloth, sponge, hand pump trigger sprayer or low-pressure coarse sprayer so as to wet all surfaces thoroughly. Allow to remain wet for 10 minutes, then remove excess liquid. For sprayer applications, spray 6-8 inches from surface, rub with brush, sponge or cloth. Do not breathe spray mist. For heavily soiled areas, a precleaning step is required. Prepare a fresh solution for each use.

Clorox® Pro Quaternary All-Purpose Disinfectant Cleaner$_1$ is not to be used as a terminal sterilant/high-level disinfectant on any surface or instrument that (1) is introduced directly into the human body, either into or in contact with the bloodstream or normally sterile areas of the body, or (2) contacts intact mucous membranes but which does not ordinarily penetrate the blood barrier or otherwise enter normally sterile areas of the body.

PRECAUTIONARY STATEMENTS: HAZARDS TO HUMANS AND DOMESTIC ANIMALS.

DANGER: KEEP OUT OF REACH OF CHILDREN. CORROSIVE.

Causes irreversible eye damage and skin burns. Do not get in eyes, on skin or on clothing. May be fatal if absorbed through skin. Harmful if swallowed. Wear goggles or face shield, rubber gloves and protective clothing. Remove contaminated clothing and wash before reuse. Wash thoroughly with soap and water after handling and before eating, drinking, chewing gum, using tobacco or using the toilet.

STORAGE AND DISPOSAL: DO NOT CONTAMINATE WATER, FOOD OR FEED BY STORAGE OR DISPOSAL. PESTICIDE STORAGE - Store in a dry place no lower in temperature than 50°F or higher than 120°F. **CONTAINER HANDLING -** Nonrefillable container. Do not reuse or refill this container. Offer for recycling if available or puncture and dispose of in a sanitary landfill, or by incineration, or, if allowed by state and local authorities, by burning. If burned, stay out of smoke. Triple rinse container promptly after emptying. Triple rinse as follows: Empty the remaining contents into application equipment or a mix tank and drain for 10 seconds after the flow begins to drip. Fill the container 1/4 full with water and recap. Shake for 10 seconds. Pour rinsate into application equipment or a mix tank or store rinsate for later use or disposal. Drain for 10 seconds after the flow begins to drip. Repeat this procedure two more times. **PESTICIDE DISPOSAL -** Pesticide wastes are acutely hazardous. Improper disposal of excess pesticide, spray mixture or rinsate is a violation of Federal law. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency or the Hazardous Waste representative at the nearest EPA Regional Office for guidance.

DIRECTIONS FOR USE

It is a violation of federal law to use this product in a manner inconsistent with its labeling.

Nolvasan Solution final use dilutions may be applied by wiping, mopping, or spraying on the inanimate surface. It may also be used in fogging (wet misting) operations as an adjunct either preceding or following regular cleaning and disinfecting procedures. Fog (wet mist) until the area is moist using automatic foggers according to manufacturer's directions.

When applying by wiping, mopping, or spraying: Applicators or other handlers must wear long-sleeve shirt and long pants, socks plus shoes, and protective gloves.

When applying by wet-mist fogging: Applicators and other handlers exposed to the fog during wet-mist fogging applications and until the fog has dissipated and the enclosed area has been thoroughly ventilated must wear: Long sleeve shirt and long pants, protective gloves, socks plus shoes, and a full face respirator with a canister approved for pesticides (MSHA/NIOSH approval number prefix TC-14-G).

Do not apply this product in a way that will contact workers or other persons, either directly or through drift. Only protected handlers may be in the area during application.

Entry Restrictions: Thoroughly ventilate buildings, vehicles, and closed spaces following application. Do not enter, allow other persons to enter, house livestock, or use equipment in the treated area until ventilation is complete and the liquid chlorhexidine diacetate has been absorbed, set or dried.

For entry into fogged areas before ventilation is complete and the fog has completely dissipated, absorbed, set, or dried, all persons must wear: Long sleeve shirt and long pants, protective gloves, socks plus shoes and a full face respirator with a canister approved for pesticides (MSHA/NIOSH approval number prefix TC-14-G).

User Safety: Follow manufacturer's instructions for cleaning/maintaining personal protective equipment. If there are no such instructions for washables, use detergent and hot water. Keep and wash personal protective equipment separately from other laundry.

Users should wash hands before eating, drinking, chewing gum, using tobacco, or using the toilet. Users should remove clothing immediately if pesticide gets on or inside it, then wash both skin and clothing thoroughly and put on clean clothes. Users should remove personal protective equipment immediately after handling this product. Wash the outside of gloves before removing. As soon as possible, wash skin and clothing thoroughly and change into clean clothes.

Veterinary or Farm Premises

1. Remove all animals and feed from premises, vehicles and other equipment.
2. Remove all litter and manure from floors, walls and surfaces of barns, pens, stalls, chutes and other facilities and fixtures occupied or traversed by animals.
3. Empty all troughs, racks and other feeding and watering appliances.
4. Thoroughly clean all surfaces with soap or detergent and rinse with water.
5. Saturate all surfaces with the recommended disinfecting solution for a period of 10 minutes.
6. Immerse all halters, ropes and other types of equipment used in handling and restraining animals, as well as forks, shovels and scrapers used for removing litter and manure.
7. Thoroughly scrub all treated feed racks, mangers, troughs, automatic feeders, fountains and waterers with soap or detergent, and rinse with potable water before reuse.

For use in federally inspected meat, poultry, rabbit and egg establishments

1. All food products and packaging material must be removed from the room or carefully covered and protected.
2. Remove any loose dirt, litter, etc., that might be lying on floor or attached to the equipment.
3. Thoroughly clean all surfaces with soap or detergent and rinse with water.
4. Saturate all surfaces with the recommended disinfecting solution for a period of 10 minutes.
5. Expose or soak all equipment and/or utensils with the recommended disinfecting solution for a period of 10 minutes.
6. After disinfection all equipment and/or utensils must be thoroughly rinsed with potable water before operations are resumed.

For dipping teats as an aid in controlling bacteria that causes mastitis

Immediately after the cow is milked dip each teat into the dipping solution. Teat dipping should start one week before the cow freshens. When drying off a cow the teats should continue to be dipped once a day for 3 to 4 days. Udder and teats of the cow must be thoroughly washed before milking.

Product No.: 1N0L402
8N0L402 Rev.: 04/15

FPO - UPC 90%
7 26287 10625 8

13933903

Nolvasan® Solution

Chlorhexidine diacetate

Disinfectant

Bactericide Virucide†

For Animal Premises Use Only

Active Ingredient:	
Chlorhexidine (1,1'-Hexamethylenebis [5-(p-chlorophenyl) biguanide]) diacetate	2%
Other Ingredients	98%
	100%

The product contains the active ingredient at 0.168 pounds per gallon.

KEEP OUT OF REACH OF CHILDREN

DANGER

SEE SIDE PANEL FOR ADDITIONAL PRECAUTIONARY STATEMENTS

1 Gallon (3.7 L)

zoetis

Recommended Concentration For Use

I. †For disinfection of inanimate objects to aid in control of canine distemper virus, equine influenza virus, transmissible gastroenteritis virus, hog cholera virus, parainfluenza-3 virus, bovine rhinotracheitis virus, bovine viral diarrhea virus, infectious bronchitis virus, Newcastle virus, Venezuelan equine encephalitis virus, equine rhinopneumonitis virus, feline rhinotracheitis virus, pseudorabies virus, equine arteritis virus and canine coronavirus — 3 ounces (6 tablespoonfuls) per gallon of clean water. Nolvasan Solution has been shown to be virucidal *in vitro* against rabies virus (CVS strain) in laboratory tests when used as directed above.

II. For disinfection of veterinary or farm premises — 1 ounce (2 tablespoonfuls) per gallon of clean water.

III. For use in federally inspected meat, poultry, rabbit and egg establishments — 1 ounce (2 tablespoonfuls) of Nolvasan Solution to each gallon clean water.

IV. For dipping teats as an aid in controlling bacteria that causes mastitis. Make up a final dipping solution by putting 32 ounces (one quart) of Nolvasan Solution in a clean gallon container, adding 6 ounces of glycerin and then adding clean potable water until you have a total volume of one gallon.

Not effective against *Pseudomonas aeruginosa* or gram-positive cocci on inanimate surface."

"According to A.O.A.C. Use Dilution Test Method.

PRECAUTIONARY STATEMENTS HAZARDS TO HUMANS (AND DOMESTIC ANIMALS)

Prolonged or frequently repeated skin contact may cause allergic reaction in some individuals.

Remove contaminated clothing and wash before reuse.

DANGER

Corrosive: Causes irreversible eye damage. Wear protective eyewear (Goggles, face shield or safety glasses.) Harmful if swallowed or absorbed through skin or inhaled. May be fatal if inhaled. Avoid breathing spray mist.

Avoid contact with skin or clothing and do not swallow. Wear protective gloves when handling or applying.

FIRST AID

IF IN EYES: Hold eye open and rinse slowly and gently with water for 15-20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing. Call a poison control center or doctor for treatment advice.

IF SWALLOWED: Call a poison control center or doctor immediately for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to by a poison control center or doctor.

IF ON SKIN: Take off contaminated clothing. Rinse skin immediately with plenty of water for 15-20 minutes. Call a poison control center or doctor for treatment.

IF INHALED: Move person to fresh air. If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth, if possible. Call a poison control center or doctor for further treatment.

ENVIRONMENTAL HAZARDS

Do not discharge effluent containing this product into lakes, streams, ponds, estuaries, oceans, or other waters unless in accordance with the requirements of a National Pollutant Discharge Elimination System (NPDES) permit and the permitting authority has been notified in writing prior to discharge. Do not discharge effluent containing this product to sewer systems without previously notifying the local sewage treatment plant authority. For guidance contact your State Water Board or Regional Office of the EPA. Do not contaminate water by cleaning of equipment or disposal of waste.

STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal. Protect from freezing.

Pesticide Storage: store in a cool, dry place inaccessible to children.

Container Disposal: Nonrefillable container. Do not reuse or refill this container. Offer for recycling if available. Triple rinse container promptly after emptying. Triple rinse as follows: Fill container 1/4 full with water and recap. Shake for 10 seconds. Follow Pesticide Disposal instructions for rinsate disposal. Drain for 10 seconds after the flow begins to drip. Repeat procedure two more times. Then offer for recycling or reconditioning. If not available, puncture and dispose of in a sanitary landfill.

Pesticide Disposal: pesticide wastes are acutely hazardous. Improper disposal of excess pesticide, spray mixture, or rinsate is a violation of Federal Law. If these wastes cannot be disposed of by use according to label instructions, contact your State Pesticide or Environmental Control Agency, or the Hazardous Waste representative at the nearest EPA Regional Office for guidance.

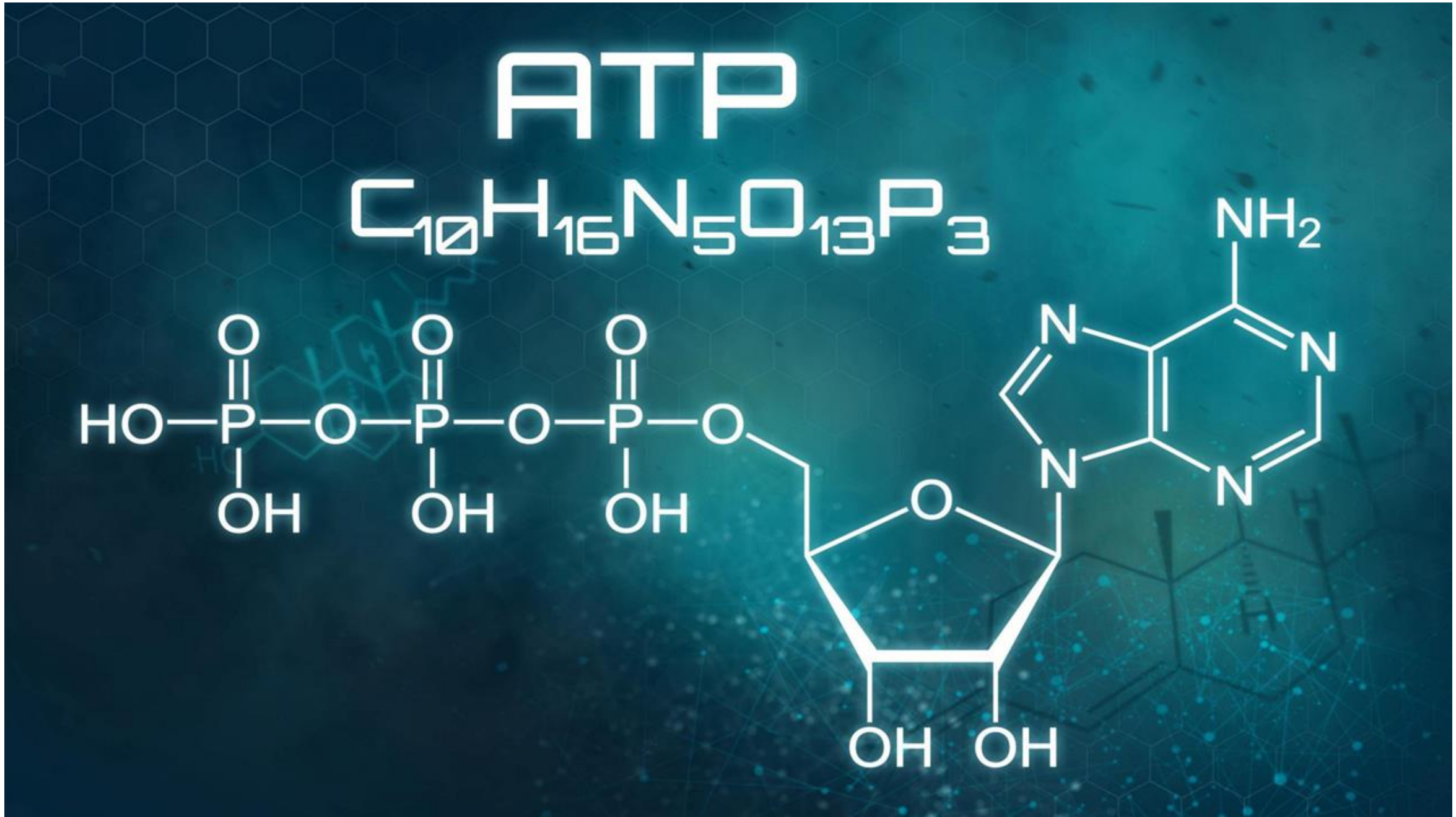
For further information contact Zoetis Inc. at 1-888-963-8471

EPA Est. No. 68223-MN-001
EPA Reg. No. 1007-99

Distributed by: Zoetis Inc.
333 Portage Street
Kalamazoo, MI 49007

Lot:
Exp. Date:

IMPRINT AREA


ATP
C10H16N5O13P3
NH2
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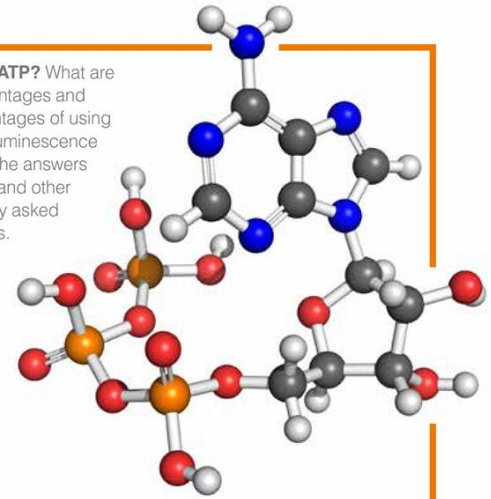
What is ATP? What are the advantages and disadvantages of using ATP bioluminescence assay? The answers to these and other frequently asked questions.



A Q&A on ATP Bioluminescence Assay

Adenosine triphosphate (ATP) is the molecule used for energy storage by all types of living cells (animal, plant, bacterial, yeast, and mold). ATP transfers energy within living cells to power the enzymes needed for cellular functions. After cell death, ATP is broken down by autolysis within a few minutes.

What is ATP bioluminescence assay? ATP bioluminescence assay cell detection was first developed in the 1950s by NASA scientists who were interested in finding life (living cells) on other planets. In the food industry, it is a technique used to measure the cleanliness of a surface. ATP bioluminescence detects the amount of ATP, which is an indirect measurement of the amount of organic/food residue on a surface that has the potential to support microbial growth and also microbial biomass. In simple terms, it measures the dirt or filth on a surface indicating the need for cleaning and sanitizing.

ATP bioluminescence assay is probably the most widely used technique in the food industry for hygiene monitoring and cleaning validation. It was created mainly to validate the cleaning on a production surface before the use of the sanitizer.

What are the advantages of ATP bioluminescence assay? It is simple, highly sensitive, cost effective, rapid (compared to conventional methods which take days), and provides real-time results within minutes. It saves water used for rinsing and optimizes sanitizer use.

What is the ATP bioluminescence assay principle? All living cells (animals, plants, bacteria, yeast, and mold) contain ATP. It is based on the firefly's ATP luminescent reaction. The firefly has two chemical compounds, Luciferin and Luciferase, that react with the insect's ATP to produce bioluminescence light. The ATP collected from a surface reacts with Luciferin/Luciferase compounds present in the sample swab to create bioluminescence light.

The amount of bioluminescence light is measured by the Luminometer and is expressed in Relative Light Units (RLU). RLU numbers are directly proportional to the amount of ATP, and therefore the amount of organic/food residue or microbial biomass on the sampled surface.

What are other uses of ATP bioluminescence assay? ATP bioluminescence assay also are used to:

- Detect microbial load in raw milk (cfu/ml).
- Assess microbiological quality of beef and pork carcasses and minced meat (cfu/g).
- Monitor microbiological activity in indoor air (cfu/ml).
- Monitor sanitary conditions in clinical settings.
- Monitor yeast and bacteria in beverages and fruit juices.
- Monitor cleanliness (bio-burden) of NASA spacecraft (to limit terrestrial microbes being transferred to other planetary bodies) and to detect life (living cells) on other planets.
- Monitor water quality.
- Verify cleaning (whether equipment is clean enough to go for production).

What are the disadvantages of ATP bioluminescence assay? The disadvantages of the assay are that:

- It does not easily distinguish ATP from microorganisms, animals, and plants.
- Luminescence from food can affect the actual ATP bioluminescence readings.
- The presence of detergents, sanitizers, or other chemicals also can affect the readings.
- It is not very sensitive for spore detection since the level of ATP is very low in spores.
- It does not substitute using traditional microbiological analysis. **AIB**

The author is Director of Microbiology and Food Safety Education, AIB International.

AIB now offers basic microbiology training for food plant personnel, environmental monitoring program assistance, kill step validation assistance, and specialized microbiological consulting. To schedule micro consultation at your facility, contact Food Safety Education at 800-633-5137 or fse@aibonline.org.

NASA TECHNICAL NOTE

NASA TN D-7680

NASA TN D-7680

ADENOSINE TRIPHOSPHATE (ATP) AS A POSSIBLE INDICATOR OF EXTRATERRESTRIAL BIOLOGY

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16. Abstract

The ubiquity of adenosine triphosphate (ATP) in terrestrial organisms provides the basis for proposing the assay of this vital metabolic intermediate for detecting extraterrestrial biological activity. If an organic carbon chemistry is present on the planets, the occurrence of ATP is possible either from biosynthetic or purely chemical reactions. However, ATP's relative complexity minimizes the probability of abiogenic synthesis.

A sensitive technique for the quantitative detection of ATP has been developed using the firefly bioluminescent reaction. The procedure has been used successfully for the determination of the ATP content of soil and bacteria. This technique is also being investigated from the standpoint of its application in clinical medicine.

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CONTENTS

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INTRODUCTION

Among the vast array of questions which man seeks to answer in his investigations of the upper atmosphere and outer space, none is more challenging and provocative than that pertaining to the existence of life outside terrestrial boundaries. Finding a satisfactory answer to this question is the purpose of intensive studies by several investigators.

Basic to these studies is the establishment of the parameters which define life. A definition amenable to operational manipulation is one which characterizes life as the capacity of a molecular complex to perform certain functional activities including metabolism, growth, and reproduction. If this definition is valid, it follows that a rational life-detection system must be capable of measuring phenomena related to these criteria.

To detect life is not difficult in many instances, especially in the presence of visible manifestations such as characteristic form, movement, or reproduction. It becomes increasingly difficult, however, as the size of the organism becomes microscopic; it becomes even more difficult as the number of organisms in a sample decreases. It is in the latter instances that classical life-detection techniques begin to depend upon one of the manifestations mentioned above: reproduction. Although adequate in many laboratory situations, detection techniques based on reproduction have the requirement of long time periods and rigorously controlled conditions. Another factor to be considered is that, in order to detect life beyond the surface of earth, techniques amenable to remote operation must be employed.

DETECTION OF THE METABOLIC INTERMEDIATE ATP

It is to satisfy these requirements that one begins to consider the detection of certain vital metabolic intermediates as a means of life detection. The metabolic intermediate would have to fulfill certain requirements; it would have to be ubiquitous and specific for living organisms, of sufficient complexity to render a spontaneous abiogenic synthesis unlikely, and amenable to sensitive detection. A compound that fulfills these requirements is adenosine triphosphate (ATP). The structure of ATP is shown in Figure 1.

ATP, the primary storage form of biochemical energy, is a prime example of a relatively complex organic molecule present on earth. It is so specifically and ubiquitously associated with biological reactions that its presence is accepted as a positive indication of the present or past existence of a terrestrial organism. This correlation provides one strong rationale for the

use of an assay for this compound as a means of detecting biological activity in extraterrestrial material.

This assay takes on additional significance as the result of the work of several investigators[1-4] who have demonstrated the abiogenic synthesis of a number of complex biological intermediates, including ATP, under conditions approximating those believed to be the primordial earth environment. These investigations suggest that ATP was a component of the earth's prebiotic chemistry. Therefore, its detection in an extraterrestrial sample (devoid of biological activity as confirmed by other detection techniques) could indicate an evolutionary chemistry similar to earth's, but not yet culminating in the emergence of life.

The likelihood of finding extraterrestrial ATP is dependent upon the fundamental requirement for its synthesis: carbon-based chemistry. Carbon in the form of CO_2 has been detected in the atmosphere of Mars[5] and Venus,[6] and the Jovian atmosphere contains abundant quantities of methane.[7] Findings of compounds hydrolyzable to amino acids in lunar soil samples have also been reported.[8] Examination of meteorites has revealed the presence of hydrocarbons and derivatives including amino acids.[9,10] Of great significance are the recent radio telescope observations revealing the presence of an array of carbon compounds of low molecular weight in interstellar clouds.[11] (Interstellar clouds are the matter from which planets are formed.) These compounds include formaldehyde, hydrogen cyanide, ammonia, formic acid, formamide, and methanol; all are implicated in chemical and biological evolution on earth.

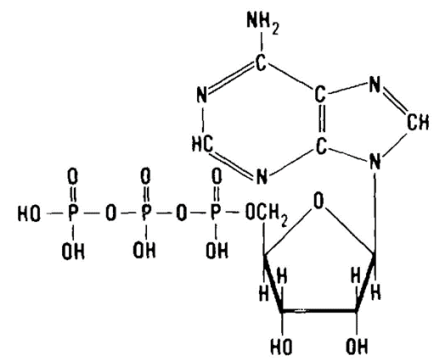

Figure 1. Chemical structure of adenosine triphosphate.

The occurrence of these intermediates in interstellar dust and meteorites throughout the galaxy strongly suggests that life anywhere in the galaxy would have a chemical makeup similar to that of terrestrial life and would quite likely involve ATP. It can be assumed that an extraterrestrial carbon-based chemistry would be subject to the same thermodynamic restraints that govern the permutations of terrestrial carbon compounds and thus, under similar environmental conditions, could follow a chemical evolutionary pathway leading to the synthesis of ATP. This assay will be of particular value in conjunction with life-detection experiments involving metabolism and growth, as described by Levin et al.[12] and Vishniac,[13] and also in experiments involving analysis of organic compounds.

THE ANALYSES OF ATP

In the analyses of extraterrestrial material for ATP, at least four results are possible: (1) positive detection of ATP (along with positive proof of life by other detection systems), meaning that biological activity was present and resembled terrestrial life with respect to this compound; (2) positive indications of life in the absence of ATP, which point to life basically different from that found on earth; (3) detection of ATP in the absence of any life detected by other systems could mean that no life existed as detectable by the techniques employed and that the ATP was of abiogenic origin; and (4) the absence of ATP together with negative results from other life-detection systems, indicating either the absence of life or the presence of an exotic life form undetectable by the procedures used. However, in consideration of (3) above, our investigations with terrestrial soils show that, under certain conditions, ATP may remain stable for indefinite periods after it is released from a dead cell.[14] Thus, detection of ATP in the absence of other positive determinations of life might mean that the compound was present as a result of abiogenic synthesis or as the trace of a life form long since dead. ATP detection could also mean that the chemical evolution had not yet reached the stage for the presence of life.

In order to realize the full potential of ATP as a monitor for the presence of life in space, it becomes necessary to select and develop a method with which it can be assayed with the highest degree of sensitivity, accuracy, and rapidity.

There are two general techniques by which ATP may be measured. The first technique requires its isolation in the pure state, after which it can be assayed by means of ultraviolet spectrophotometry.[15] The second technique employs an enzyme system in which ATP is a substrate. The latter technique enjoys the advantage of not requiring that ATP be in a pure state, thus providing measurement procedures with simplicity and speed that cannot be approached by the first technique.

The choice of an enzyme system for ATP assay is dictated mainly by the degree of sensitivity of the assay methods for the products formed during the reaction between ATP and the enzyme. In most of the enzymatic assays of ATP at very low concentrations, the primary enzyme is coupled with one in which a pyridine nucleotide is reduced at a rate proportional to the ATP concentration. Reduced pyridine nucleotide, which has a very high extinction coefficient, is then assayed at 340 nm in the ultraviolet spectrophotometer.

Another enzymatic reaction requiring ATP, which appears to have far greater potential, is the bioluminescent reaction occurring in fireflies. The reaction mechanism has been well established by the excellent work of McElroy[16] and his associates. In brief, the light emission in firefly bioluminescence results from the steps shown below.[17]

$$E + LH_2 + ATP \overset{Mg^{++}}{\rightleftharpoons} E \cdot LH_2 \cdot AMP + PP$$

$$E \cdot LH_2 \cdot AMP + O_2 \rightarrow E + AMP + CO_2 + light + T$$

where E = firefly luciferase, LH_2 = reduced luciferin, ATP = adenosine triphosphate, AMP = adenosine monophosphate, PP = pyrophosphate, and T = thiazolinone.

The quantity of light emitted during the reaction is a function of the concentration of luciferase, luciferin, ATP, and O_2. Therefore, in the presence of excess luciferase, oxygen, and luciferin, the total emitted light is a direct function of the concentration of ATP. It also has been shown that, under appropriate conditions, the peak light intensity is a linear function of the quantity of ATP, thus allowing the assay to be made in a much shorter period of time.[18]

The assay procedure that has been developed[18] consists of injecting either standard ATP solutions or cellular extracts containing ATP into a cuvette containing the enzyme system (purified luciferase, synthetic luciferin, magnesium ion, and buffer).

Figure 2 is a schematic of a basic instrumentation system for performing the assay. The system consists of a rotary reaction chamber coupled to a photomultiplier tube. A section of the rotary chamber is cut out to accommodate a 6- by 50-mm glass cuvette. Immediately above the cuvette holder is a small injection port through which ATP is injected by needle and syringe into the enzyme solution. The signal from the photomultiplier tube is amplified, and the dc signal from the amplifier can be observed by a variety of means (recorder, oscilloscope, and so forth). Commercial instruments that are available for observing the dc signal include photometers made by American Instrument Co., E. I. DuPont De Nemours & Co., and JRB, Inc.

A concentration curve showing maximum intensity as a function of ATP concentration is shown in Figure 3. Although not shown, instrumentation of sufficient sensitivity has been developed by this laboratory to allow the detection of $1 \times 10^{-8}\mu g$ of ATP.[19]

Another obstacle of some magnitude that will confront any planetary-lander experiment is the effect of dry heat sterilization: a current NASA requirement. This is a difficult requirement for much of the hardware to meet and would seem impossible for an enzyme and necessary cofactors to meet. However, a technique has been developed in this laboratory that prevents a loss of biological activity by firefly luciferase and luciferin during heating.[20]

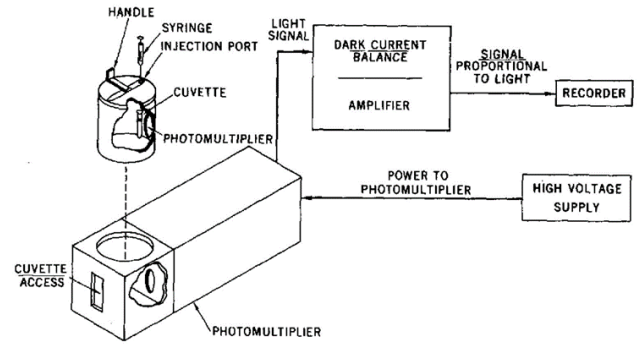

Figure 2. Light measuring instrumentation.

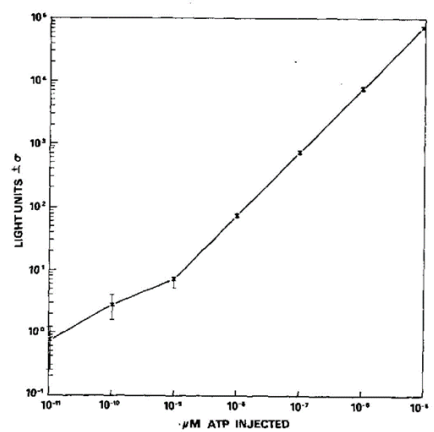

Figure 3. The initial peak height as a function of ATP concentration.

The necessary conditions include encapsulation in a molecular filtration gel, complete removal of H_2O by ultrahigh vacuum, and exposure to sterilization temperature in the complete absence of oxygen. Approximately 40 percent of the activity was retained after exposure of the enzyme system to a temperature of 408 K (135°C) for 36 hours.

The feasibility of the use of this assay for the sensitive quantitative detection of ATP in living organisms has been demonstrated with a variety of bacteria as shown in the following list. The amount of ATP per cell in the listed micro-organisms was determined by the firefly luciferase assay.

Organisms	μg ATP/cell ($\times 10^{-10}$)	Organisms	μg ATP/cell ($\times 10^{-10}$)
Bacillus cereus	1.10	*Micrococcus lysodeikticus*	1.30
Bacillus coagulans	1.70	*Mycobacterium phlei*	1.90
Bacillus globigii	5.40	*Mycobacterium smegmatis*	8.90
Brevibacterium helvolum	0.37	*Pseudomonas aeruginosa*	1.00
Enterobacter aerogenes	0.28	*Pseudomonas flourescens*	3.10
Erwinia carotovora	0.44	*Proteus vulgaris*	1.80
Escherichia coli	1.00	*Sarcina lutea*	0.37
Flavobacterium arborescens	1.50	*Serratia marcescens*	1.00
Gaffkya tetragena	0.61	*Staphylococcus aureus*	0.64
Klebsiella pneumoniae	5.00		

In order to carry out this analysis, a technique for the quantitative extraction of ATP from bacterial cells was developed[18] that involves *n*-butanol. As in situ biological investigations of other planets will most certainly use surface samples, studies designed to establish the necessary conditions for compatibility of the assay technique with soil samples have been conducted. The ATP content of a variety of soil types from many parts of the world has been measured. An excellent correlation between soil ATP and microbial count was obtained in most cases.[14]

While adequate instrumentation is available for the use of this technique on earth, extraterrestrial investigations will require automated instrumentation that is capable of performing all of the assay steps on remote demand. Progress toward the realization of such instrumentation has been reported.[21]

A number of investigations at various stages of development are being carried out by us in collaboration with two hospital centers. The goals of these studies include the development of rapid automatable techniques for the detection of infection in biological fluids, rapid antibiotic susceptibility tests, viral detection techniques based on host-cell ATP changes, and techniques for monitoring kidney transplants. Progress made in the use of this technique for detection of bacteria in urine is exemplified in Table 1 which shows the correlation between colony count and count by ATP. The discrepancies between the luciferase method

and colony count are possibly due to various causes: trace amounts of nonbacterial ATP, the necessity of assuming an average value for ATP content per bacterium, the inability of some bacteria to produce colonies in the growth environment, and the presence of ATP within bacteria that are not dividing. Colony count technique requires 24 to 48 hours, while the count by the luciferase procedure requires 1 hour.

SUMMARY

In summary, the progress in instrumentation and analytical procedure allows us to view the ATP assay of extraterrestrial matter as a practical experiment. In concert with other exobiological experiments, the results from such an assay could provide insight into the biological status of other celestial bodies in our solar system.

An important ramification of the methodology developed for extraterrestrial ATP detection is its possible usefulness for a variety of applications on earth. As has been described, ATP is a constituent of all living organisms, thus making it an excellent monitor for microorganisms such as bacteria and is also a sensitive indicator of the physiological state of any living cell.

Table 1

Bacterial Counts per ml from Clinical Urine Specimens Comparing the Luciferase Centrifugation Procedure with the Agar Pour Plate Method.

Specimen Number	Luciferase	Pour Plate
1	3.2×10^8	$>10^7$
2	3.1×10^4	4×10^3
3	$<10^3$	$<10^2$
4	$<10^3$	8×10^3
5	7.0×10^3	1×10^5
6	$<10^3$	$<10^2$
7	9.4×10^3	2×10^2
8	$<10^3$	$<10^2$
9	3.1×10^7	$>10^7$
10	3.1×10^7	$>10^7$
11	$<10^3$	$<10^2$
12	2.7×10^4	$<10^2$
13	8.4×10^7	$>10^7$
14	3.2×10^4	9×10^2
15	2.1×10^8	8×10^6
16	1.3×10^7	1×10^7
17	1.6×10^7	$>10^7$
18	8.6×10^4	4×10^4
19	$<10^3$	1×10^2
20	2.4×10^4	$<10^2$
21	3.1×10^6	5×10^6
22	5.5×10^3	1×10^3

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Greenbelt, Maryland May 21, 1973
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How Reliable Are ATP Bioluminescence Meters in Assessing Decontamination of Environmental Surfaces in Healthcare Settings?

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Abstract

Background: Meters based on adenosine triphosphate (ATP) bioluminescence measurements in relative light units (RLU) are often used to rapidly assess the level of cleanliness of environmental surfaces in healthcare and other settings. Can such ATP measurements be adversely affected by factors such as soil and cleaner-disinfectant chemistry?

Objective: This study tested a number of leading ATP meters for their sensitivity, linearity of the measurements, correlation of the readings to the actual microbial contamination, and the potential disinfectant chemicals' interference in their readings.

Methods: First, solutions of pure ATP in various concentrations were used to construct a standard curve and determine linearity and sensitivity. Serial dilutions of a broth culture of Staphylococcus *aureus*, as a representative nosocomial pathogen, were then used to determine if a given meter's ATP readings correlated with the actual CFUs. Next, various types of disinfectant chemistries were tested for their potential to interfere with the standard ATP readings.

Results: All four ATP meters tested herein demonstrated acceptable linearity and repeatability in their readings. However, there were significant differences in their sensitivity to detect the levels of viable microorganisms on experimentally contaminated surfaces. Further, most disinfectant chemistries tested here quenched the ATP readings variably in different ATP meters evaluated.

Conclusions: Apart from their limited sensitivity in detecting low levels of microbial contamination, the ATP meters tested were also prone to interference by different disinfectant chemistries.

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Competing Interests: The authors have the following interests: This study was funded by Virox Technologies Inc. Two of the authors (Faraz Ahmadpour and Nicole Kenny) are employed by Virox Technologies Inc. The third author, Navid Omidbakhsh, used to be an employee of Virox Technologies at the time of submitting this manuscript, but not anymore. The authors hold the following patents, which are owned by Virox Technologies: Hydrogen peroxide disinfectant with increased activity US 6,346,279, US 6,803,057 (CIP), EP 1139762 B1, CA 2344471, AU 741104, AT225128, BR9915987, DE69903347, JP3350526. Enhanced activity hydrogen peroxide disinfectant US 7,632,523, NZ0534352A, AU3245498 AA, CA2475327, EP1473998A1. Enhanced activity hydrogen peroxide disinfectant 13/410737. Hydrogen Peroxide Disinfectant Containing a Cyclic Carboxylic acid and/or an Aromatic Alcohol US 7,354,604, EP1562430B1, CA2503627AA, AU 2003302067, JP 4813059. Hydrogen Peroxide Disinfectant Containing a Cyclic Carboxylic acid and/or an Aromatic Alcohol EP 1 955 593 B1. Low foaming hydrogen peroxide solutions for organic soils US 6,686,324, CA 23661741, CA 2454437. Hydrogen peroxide-based skin disinfectant 13/022683, NZ 550744, AU2005244462, CA 2564763. Antimicrobial compositions 60/955,991, WO 2009021336, EP2182811A1. Concentrated Hydrogen Peroxide disinfecting solutions US 8,591,958 B2, CA 2,733,644, 09817139.0, EP232900281, NZ 591314, 2011-528154. Virox is the creator and manufacturer of cleaning and disinfecting chemicals based on our patented accelerated hydrogen peroxide technology. Virox is not directly or indirectly associated with the sale or support ATP meters. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

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Introduction

Several types of pathogens can readily survive on high-touch environmental surfaces in healthcare and other settings [1–4] as a result these surfaces may act as vehicles for the spread of a variety of nosocomial pathogens [3,5]. In 2002 in the US, 5% of all patients acquired such infections and of these, the mortality rate was nearly 6% [6–11]. In the United States alone, the cost of such hospital-acquired infections (HAIs) is estimated to be between 5 and 29 billion dollars annually [9,12–14].

To limit the impact of HAIs, routine cleaning and disinfection of high-touch environmental surfaces in healthcare facilities is crucial for infection control [2,4,15–19]. In addition, it is imperative to ascertain that the decontamination procedures in such facilities are optimal. While the widely used practice of visual inspections may be sufficient for aesthetic purposes, it does not

provide quantitative feedback on the effectiveness of the decontamination process [7,20,21]. While culture-based approaches provide quantitative results, they cannot provide immediate feedback and antibody- or PCR-based techniques have limited applications such as in the food industry where immediate availability of the results may be less crucial [2,13].

ATP bioluminescence meters, which measure the concentration of ATP as relative light units (RTU) in organic material and living cells [16], are widely used in food and beverage industries because of their ease of use and fast turn-around of results. Such meters are increasingly being used in healthcare facilities as well.

This study evaluated four leading ATP bioluminescence monitoring systems for their accuracy and linearity in detecting ATP values, detection limits for microbial count, correlation with plate-counting using *Staphylococcus aureus* and the quenching and enhancement effect of various disinfectant chemistries.

Materials and Methods

Test Materials

ATP bioluminescence meters Kikkoman Lumitester PD-20 from Luminultra Technologies Ltd. (with LuciPac Pen swabs), EnSURE Hygiene Meter – ATP-205 from Hygiena/Scigiene Corporation (with ATP3000 SuperSnap swabs), Clean-Trace NG Luminometer UNG2 from 3M Company (with Surface ATP - UXL100 swabs), and Charm novaLUM from Charm Sciences Inc. (with PocketSwab Plus ATP swabs).

ATP standard solution. Adenosine 5′-triphosphate, disodium salt (ATP.2Na) from Enzo Life Sciences.

Microorganism. Staphylococcus aureus (ATCC 6538).

Culture medium. 4% Tryptone soya agar (TSA) plates (Oxoid Microbiology Products; Nepean, Ontario).

Disinfectants Tested

Table 1 shows the list of tested disinfectants in this study. They were selected because they are sold for the decontamination of environmental surfaces in healthcare settings. In addition to the commercial products, a few antimicrobial active ingredients were also used in this study to compare their results with actual disinfectant formulations.

Methods

First, the ATP luminometer meters were tested for their linearity in reading standard ATP solutions. A 0.1 molar solution of ATP standard powder was prepared in autoclaved deionized (DI) water, followed by serial 10-fold dilutions from 10^{-2} to 10^{-10}. 10 µL of each dilution was pipetted directly onto the swab tip using positive displacement tips. This was done to avoid the variability resulting from the difference of swab-to-swab efficiency in picking up the organic load from the surface. Each meter

Table 1. Tested disinfectants, their active ingredients, and manufacturers.

Product	Chemical Ingredients as listed on the Label	Manufacturer, Location
CaviCide	Isopropyl alcohol, 17.2%; 2-butoxyethanol, 1–5%; Diisobutyl –phenoxy-ethoxy-ethyl-dimethyl-benzyl ammonium chloride, 0.28%	Metrex; Orange, CA
CleanCide	Citric acid, 0.6%	Wexford Labs, Inc.; Kirkwood, MO
Ultra Clorox Bleach (1:10 dilution)	Sodium hypochlorite, 5–8%	The Clorox Company; Oakland, CA
PCS 1000	Sodium hypochlorite, 0.1%	Process Cleaning Solutions Ltd.; Peterborough, ON
Sani-Cloth Plus	Isopropanol, 10–20%; 2-butoxyethanol, 1–4%; Benzyl-C12–18-alkyldimethyl ammonium chlorides <0.125%, C12–18-alkyl [(ethylphenyl) methyl] dimethyl chlorides, <0.125%	Nice-Pak Products Inc.; Mooresville, IN
Clorox Hydrogen Peroxide Wipes	Hydrogen peroxide, 1.4%;	The Clorox Company; Oakland, CA
Clorox Clean-up disinfectant	Sodium hypochlorite, 1.84%	The Clorox Company; Oakland, CA
Isopropyl alcohol	Isopropyl alcohol, 70% v/v	VWR International, LLC.; Mississauga, ON
Hydrogen peroxide	Hydrogen peroxide, 0.5% w/w	Arkema Inc.; Philadelphia, PA
BTC 50 (1:125 dilution)	Alkyl dimethyl benzyl ammonium chloride (C12–18) 50–51.5%, Ethanol 5–5.5%	Stepan Company; Northfield, IL
Accel TB	Hydrogen peroxide, 0.5%	Virox Technologies Inc.; Oakville, ON
Accel PREVention RTU	Hydrogen peroxide, 0.5%	Virox Technologies Inc.; Oakville, ON
Virox 5 RTU	Hydrogen peroxide, 0.5%	Sealed Air Corporation; Elmwood Park, NJ
Sporicidin	Phenol, 1.58%, sodium phenate, 0.06%	Sporicidin by Contec Inc.; Spartanburg, SC

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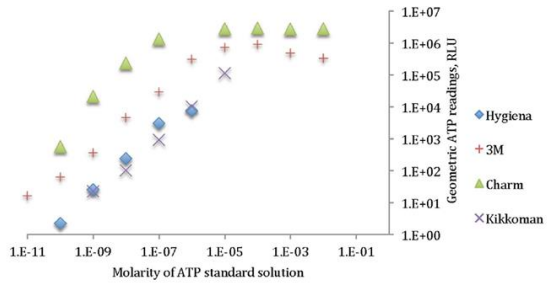

Figure 1. Linearity in ATP readings for 4 different ATP meters.
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measured ATP and reported the data in RLU. Later, serial dilutions of *S. aureus* were prepared from a freshly thawed stock culture. A 10 µL volume of each serial dilution (10^0 to 10^8) was separately pipetted directly on the tip of each swab and the readings were recorded. To correlate the RLU reading with the actual CFU, 900 µL of 10^{-9} and 10^{-10} dilutions of the bacterial suspension were separately plated on TSA in triplicates and incubated for 24 hours at 36±1°C. Any chemical interference through quenching or enhancement of bioluminescence was tested by placing 10 µL of the appropriate dilution of ATP standard solution onto the tip of a swab followed by placement of 10 µL of the test disinfectant. The baseline ATP solution concentrations used above were individually determined for each of the luminometers, selecting the aliquot with ATP concentration that fell between the ATP meters' true maximum and minimum detection limits based on their obtained linearity standard curves. Also, the volume of dispensed disinfectant on the swabs, 10 µL, was determined by testing the average volume of water required to keep 50% of a 10 cm×10 cm hard non-porous surface (a typical surface area dimension recommended by ATP meter manufacturers to be swabbed) wet for 3 minutes. The calculated average volume required was 80 µL in ambient room temperatures. This volume was reduced to 10 µL to compensate for the evaporation of the volatile ingredients.

To account for the repeatability of the results, all the tests have been performed in triplicates.

Statistical Analysis

Microsoft Excel was used in this study to determine correlation, R^2, between mean readings. A log transformation of the RLU and CFU values were used since the original distribution is highly skewed with a long tail towards the higher values. Therefore, geometric mean is used for these calculations.

Results

Figure 1 shows the linearity between the geometric mean of the ATP readings versus the molarity of ATP standard solution.

As can be seen, none of the ATP meters provided a linear relationship between ATP readings and the actual concentration of the ATP throughout the whole test range. Based on the results, approximately 6 logs of ATP reading RLUs is the highest difference observed in reading the same ATP concentration among different ATP meter brands. It can be noticed, however, that at some selected regions, the readings are almost linear; for example, for Hygiena, if the ATP reading at the 10^{-6} molarity is not considered, the rest of the data are completely linear (R^2 of 0.99952 compared to 0.98591 for the dataset including 10^{-6} molarity data point). Table 2 shows the correlation of ATP values to the ATP readings both at logarithmic scales.

Figure 2 shows CFUs of *S. aureus* versus the geometric mean of the ATP readings for each ATP meter.

The detection limit of each ATP meter is displayed in Figure 2, as well as the smallest detectable number of the test organism on the swab. Table 3 shows the exact values of CFU at which each bioluminator was able to detect. It also demonstrates the correlation between RLU reading to CFUs.

Figures 3 to 6 show the quenching/enhancement effect of each disinfectant on the ATP readings.

In Figures 3 to 6, the horizontal line represents the average ATP reading for the control sample, which is a dilution of the standard ATP solution and is specified in the caption of each figure. The error bars show the standard deviation for the three measurements at each point. Instances in which the bars which do not reach the horizontal line (even with their error bar) indicate that the disinfectant has significantly quenched the ATP readings.

Table 2. Correlation between ATP amount and ATP reading values in logarithmic scales for 4 different ATP meters.

	Charm	Hygiena	3M	Kikkoman
Correlation	0.8230	0.9827	0.9228	0.9966

doi:10.1371/journal.pone.0099951.t002

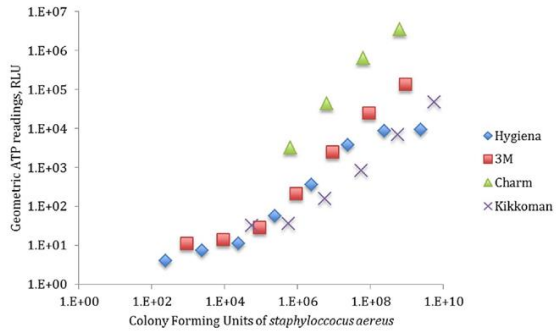

Figure 2. Geometric mean of ATP readings for various dilutions of *S. aureus* CFUs.
doi:10.1371/journal.pone.0099951.g002

Discussion and Concluding Remarks

In spite of the wide acceptance of ATP measurement technology there are gaps in our knowledge concerning the true reliability of the approach to assessing the cleanliness of environmental surfaces in healthcare and other settings. A correlation between RLU and colony forming units (CFU) has been reported previously [15,17–22]. In some studies, ATP meters have not been examined for their correlation with the actual microbial count, and have only reported RLU values as a measure of surface cleanliness [16,17,23]. Other studies suggest a loose correlation between the RLU values and the actual counts [1]. Further studies have also shown the interaction of detergents and disinfectants in RLU readings [6,8,10,11,16,17,23] and some include comparisons of different bioluminescent meters [1,12,14]. Carrick *et al* compared four different ATP meters and their swab units and found poor detection and linearity when the surfaces are swabbed. They also reported that the swabbing units are unreliable at picking up total surface ATP. In a study by Carmen and colleagues, two of the three tested ATP luminometer failed verification, which means that they both need modifications by their manufacturers. In this study, four of the market leading ATP meters were used. Disinfectant chemistries used in this study are the most widely used products in North America. They include quaternary ammonium chlorides, phenol, sodium hypochlorite, isopropanol, citric acid and hydrogen peroxide. Furthermore, individual active ingredients of these disinfectants were tested in parallel to see the interference of the whole formulation versus the active ingredient(s).

Our analyses demonstrate that the higher the concentration of ATP or *S. aureus* on the swab tip, the higher the ATP reading values; in other words there is a strong positive correlation between true concentrations and RLU readings. These results support earlier studies showing such a correlation [6,8,10,11,15,17–19] [12,14,20,21]. The observed correlations were slightly higher for the standard ATP solutions than those derived from *S. aureus*. This slight lower RLU value correlation can be explained by the fact that a single bacterial cell of a specific strain does not always produce/release the same amount of ATP molecules at a given time.

The detection limit test also showed that there could be a significant difference in the level of bacteria detectable by each device. For example, one device required 6.17×10^5 CFU on the swab in order to detect an RLU value of greater than zero. Minimum detection limit values among different brands varies at up to 2 logs of ATP standard dilution. The same for maximum ATP concentration true detection varies at up to 2 logs as well. Therefore, in actual testing, an ATP reading of zero by swabbing may be misleading since the surface may in fact contain at least 10^2 CFU bacteria. It should be noted that the detection limit results in this study are based on *S. aureus*, while in real life, many other bacteria may be present in the environment and therefore the lower limit of bacterial detection varies very more widely.

Comparing Figures 1 and 2, we see that the detection limits of each luminometer for the bacterial ATP and the standard ATP solution are completely different. For example, Figure 1 shows that 3M detects ATP at 10^{-11} molarity, Charm and Hygiena detect it at 10^{-10} molarity and Kikkoman at 10^{-9} molarity, therefore 3M can detect the least concentration of ATP among these four bioluminescence meters, while by examining Figure 2, it can be seen that Hygiena is the most sensitive unit among the four in detecting *S. aureus* by showing a lowest detection limit of 2.4×10^2 CFU followed by 3M (8.98×10^2 CFU), Kikkoman (5.6×10^4CFU) and Charm (6.2×10^5 CFU). The only conformance between these

Table 3. The minimum CFU of *S. aureus* that was detected for each ATP meter.

	Charm	Hygiena	3M	Kikkoman
Least detected CFU count	6.17E+05	2.40E+02	8.98E+02	5.60E+04
Correlation of RLU readings to plate counting (both in logarithmic scales)	0.9955	0.97737	0.9746	0.95634

doi:10.1371/journal.pone.0099951.t003

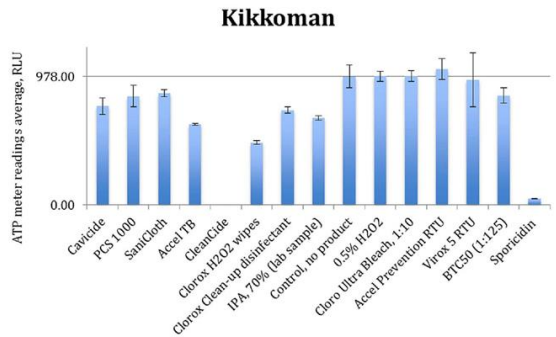

Figure 3. Quenching and enhancement effects of various disinfectant chemistries on Kikkoman luminometer readings, the control was ATP standard solution with 10^{-7} molarity.
doi:10.1371/journal.pone.0099951.g003

two sets of data is the detection limit for Charm, which is the last in both cases.

Chemical disinfectants seem to significantly affect the ATP readings of all four tested units. Data in Figures 3 to 6 are summarized in Table 4.

These data were generated based on the deviation of the average ATP readings from the control sample. The values in this table represent the percentage deviation from the control sample. Since the majority of the tests led to quenching, the quenching values are shown in positive while enhancements are demonstrated as negative.

These results clearly show that each chemistry has a unique effect in either quenching or enhancing the ATP readings. Some formulations (Sporicidin and CleanCide) show the highest quenching among all tested chemicals. The 3M-meter is also shown to be the most susceptible to disinfectant chemistries. Comparing 0.5% hydrogen peroxide (in DI water) with disinfectant products containing hydrogen peroxide (0.5–1.4%), we observe that other ingredients (inerts) present in these formulations are almost entirely responsible for the interaction. Comparing Accel TB, Accel PREVention RTU and Virox 5 RTU shows that although they all have 0.5% hydrogen peroxide as actives, their different inert ingredients can have a profound distinctive effect in the interference.

CleanCide (0.6% Citric acid) and Sporicidin (phenol based) have the most quenching effect among all the products. The CleanCide data are in conformance with findings of Mubiru [15,17–19,24] and that citric acid interferes with ATP determination by bioluminescence. Phenol was not tested in this study separately and therefore it is not possible to conclude whether Sporidicin interference comes from phenol or the inert ingredients in the formulation. Cavicide and Sani-Cloth plus are both combinations of quaternary ammonium compounds, 2-butoxy ethanol, and isopropanol, with close concentration ranges. These

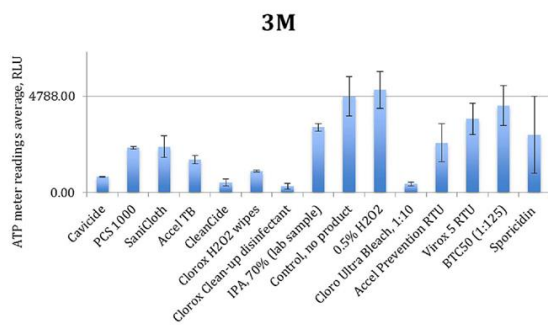

Figure 4. Quenching and enhancement effects of various disinfectant chemistries on 3M luminometer readings. the control was ATP standard solution with 10^{-8} molarity.
doi:10.1371/journal.pone.0099951.g004

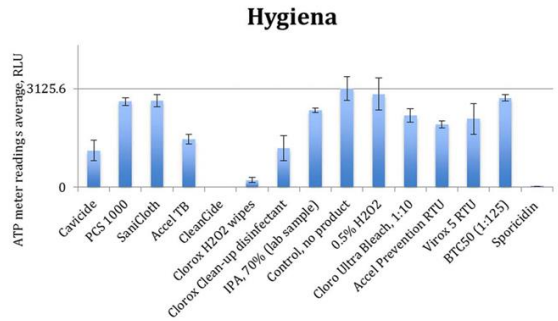

Figure 5. Quenching and enhancement effects of various disinfectant chemistries on Sciegiene luminometer readings, the control was ATP standard solution with 10^{-7} molarity.
doi:10.1371/journal.pone.0099951.g005

show almost identical quenching results with Cavicide to have more quenching effect on Hygiene and Charm units. This could be due to using different types quaternary ammonium compounds and/or using different types of inert chemicals. BTC 50, another Quat based disinfectant was also tested here to examine the effect of an exemplary quaternary ammonium compound. It shows mild quenching on Kikkoman and Charm and no effect on Hygiene and 3M. If it is used in a disinfectant formulation however, it may show different interaction, due to the effect of the other ingredients in its formulation.

By examining Table 1, it can be seen that healthcare disinfectants contain high levels of active ingredients. They also have other inert ingredients, which are usually not disclosed on their materials safety data sheets. Therefore swabbing a surface which has already been treated with a disinfectant has the potential to introduce high levels of residual chemicals to the swab and, subsequently, to the ATP measuring device. In food processing facilities, on the other hand, the chemical exposure will be significantly lower as FDA requirements (21 CFR 178.1005 & 1010 and similar guidelines) significantly limit the level of chemicals in food sanitizing and disinfecting solutions. This, results in much less chemical interaction, which could be the reason why not much chemical interaction is reported in ATP bioluminescence meters in these applications. It should be noted however that in this study, the disinfectant was directly applied to swab for the interaction test, while in real life situations, the disinfectant will be applied to the surface first, and in most part it will dry before swabbing. Therefore for volatile active ingredients such as alcohols or hydrogen peroxide, the actual chemical interaction may be less than the test results here, but for those non-volatile active ingredients, such as quaternary ammonium compounds or citric acid, the chemical interaction should be more or less the same if the surface is properly swabbed.

In summary, these results suggest that ATP meters cannot be relied upon to evaluate the effective disinfection of a healthcare surface and in particular, cannot be used as a tool to compare the effectiveness of disinfection between different disinfectants. These units have a number of limitations in detecting the true number of organisms on the surface, which can lead into false confidence in surface disinfection. Furthermore the cleaning/disinfecting chem-

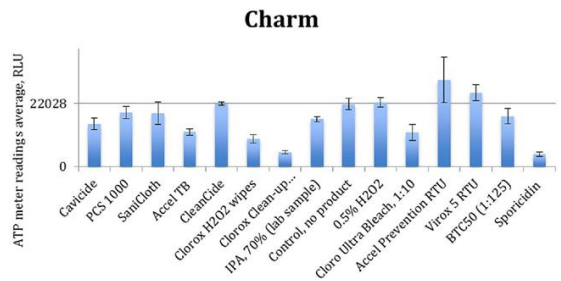

Figure 6. Quenching and enhancement effects of various disinfectant chemistries on Charm luminometer readings, the control was ATP standard solution with 10^{-9} molarity.
doi:10.1371/journal.pone.0099951.g006

Table 4. Quenching and enhancing summary for tested disinfecting chemistries versus each tested ATP unit.

Product	Kikkoman	3M	Hygiene	Charm
CaviCide	23.18%	83.67%	62.80%	31.61%
PCS 1000	15.34%	53.49%	12.94%	13.71%
Sani-Cloth Plus	13.16%	52.23%	12.05%	14.78%
Accel TB	37.08%	65.69%	51.44%	44.48%
CleanCide	99.86%	89.64%	99.90%	-0.43%
Clorox Hydrogen Peroxide Wipes	51.40%	77.95%	92.83%	55.34%
Clorox Clean-up disinfectant	26.28%	93.54%	60.40%	77.02%
IPA, 70%	32.34%	32.20%	22.07%	24.24%
0.5% H2O2	−0.10%	−6.79%	5.43%	−2.80%
Ultra Clorox Bleach (1:10)	−0.20%	91.18%	27.35%	45.34%
Accel PREVention RTU	−5.69%	48.29%	36.11%	−38.80%
Virox 5 RTU	2.69%	23.34%	30.73%	−17.88%
BTC 50 (1:125)	14.79%	9.54%	9.54%	19.34%
Sporicidin	95.16%	40.14%	99.10%	80.15%

Enhancing is shown in negative values while quenching is in positive values.
doi:10.1371/journal.pone.0099951.t004

istry residues can have a very high impact in the ATP readings, and therefore again can result in more false confidence. As of now, there have been no reports of scientific publications that specifically studied the quenching phenomena for its true cause. Our assumption on the mechanism of chemical quenching points to two main directions: either the chemicals react with the ATP molecules and make them no longer available by breaking/masking the ATP molecule, or perhaps the chemicals enter the luciferase activity chamber and adversely affect the enzymatic pathway for fluorescence generation. In this study, all the test solutions such as ATP standard solutions, inoculum and disinfectant chemistries were pipetted into the swab, and therefore the efficiency of each swab was not studied here. Furthermore, only one type of Gram-positive bacterium was tested here to obtain more definitive and reliable conclusions. Further studies should involve the use of both Gram-positives and Gram-negatives to expand on this study's finding. Needless to say, testing viral contaminations with ATP meters would result futile as viral cells do not contain or produce ATP molecules on their own, raising another concern on the limitations of the ATP bioluminescence technology in healthcare use.

Our findings suggest that introducing ATP meters to healthcare facilities, as a disinfection validation tool is not a reliable choice. The limitations of ATP luminometers clearly show that the units are not reliable in confirming proper removal of disease causing agents at healthcare settings. Inaccurate bioluminescence results causing false confidence on surface disinfection can ultimately jeopardize public health and rise infection control costs at hospitals and healthcare facilities.

We should note that our findings are based on one bacterium namely *S.aureus*, on four ATP meters and fourteen disinfectant products. All tests were performed in controlled laboratory conditions. To avoid variability in the recovery, the inoculum was directly applied to the swabs, and as such our tests did not involve sampling of environmental surfaces.

Our findings, in conjunction with the available literature, can help healthcare infection control practitioners make more educated decisions about the methods they choose to evaluate the microbial cleanliness of healthcare surfaces.

Author Contributions

Conceived and designed the experiments: NO FA NK. Performed the experiments: FA. Analyzed the data: NO. Contributed reagents/materials/analysis tools: FA NO. Wrote the paper: NO FA NK.

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Competitive Challenge Measured Objectively with ATP Luminometer

Initial ATP Testing

Challenge 3 Products

- Z BioVet Probiotic Cleaner
- Quaternary Ammonium
- Accelerated Hydrogen Peroxide

ATP Results

- 0 tor 30 RLU Highly Effective Cleaning
- 31 to 79 RLU Effective Cleaning
- 80 to 299 RLU Fail/Needs Minor Improvement
- 300 to 599 Fail/Needs Major Improvement
- 600 or Above Fail/Ineffective

Supporting Studies for ATP Pass/Fail

- In a recent five-year study conducted by North Tees and Hartlepool Hospitals, data showed that by monitoring cleaning performance with the Hygiena SystemSURE Plus system, these two hospitals experienced a 20% increase in Pass scores. In this study, Pass scores were categorized as any score below 100 RLU. During this time, the hospitals also experienced a 35.24% reduction in reported post-48 hour C. difficile infections. (Hygiena, 2012) Mulvey, et al validated the Hygiena SystemSURE Plus ATP system and reported "An ATP benchmark value of 100 relative light units [RLU] offered the closest correlation with microbial growth levels <2.5 CFU/cm2" (Mulvey, 2011)

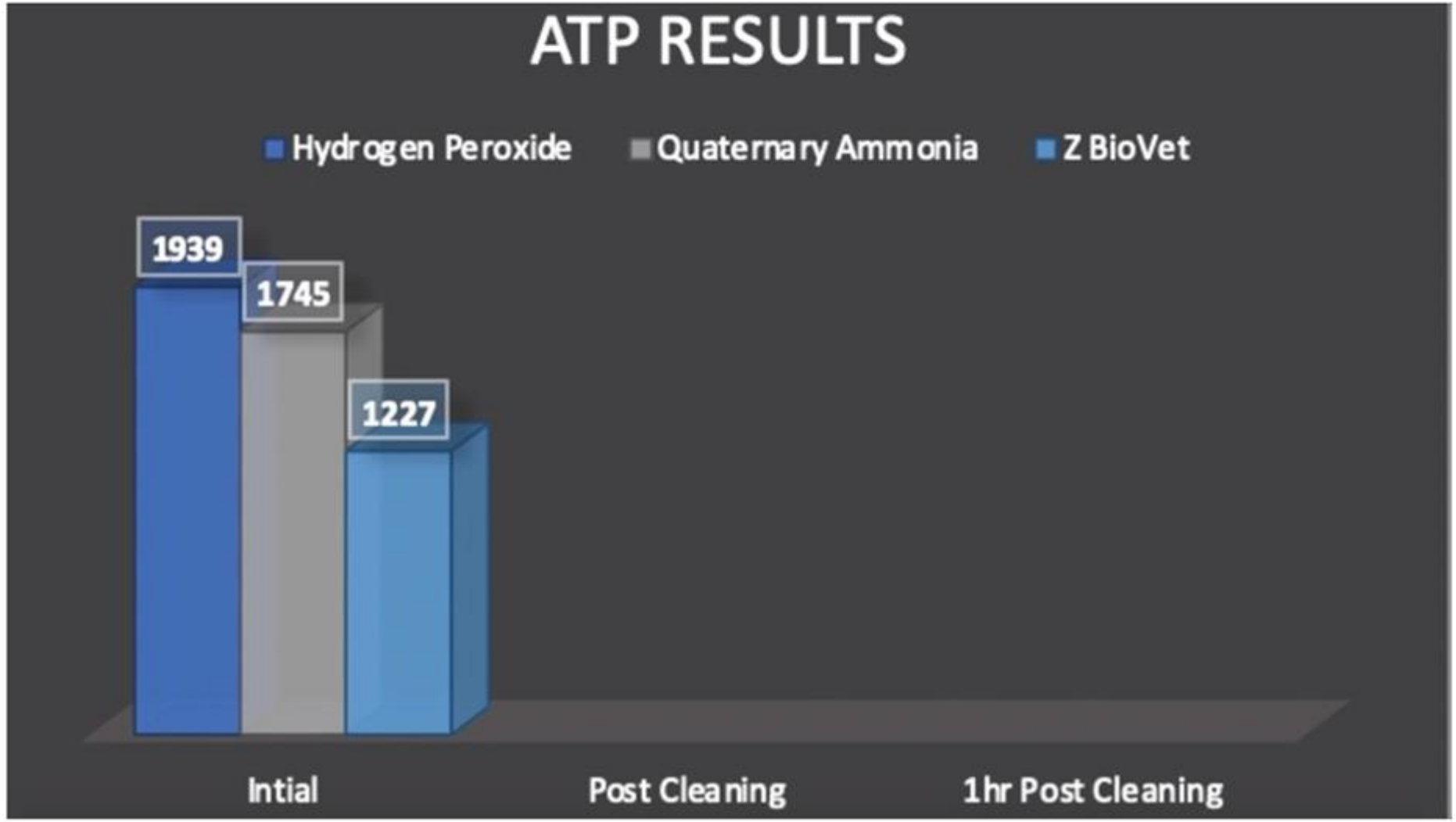
Initial ATP Results on Orthopedic Surgery Table

Accelerated H2O2

Quaternary Ammonium

Z BioVet Probiotic Cleaner

Initial ATP Results on Orthopedic Surgery Table



Cleaning According to Directions For Use

5 Minute Dwell Time Observed

Accelerated H2O2

Quaternary Ammonium

No Dwell Time Required

Z BioVet Probiotic Cleaner

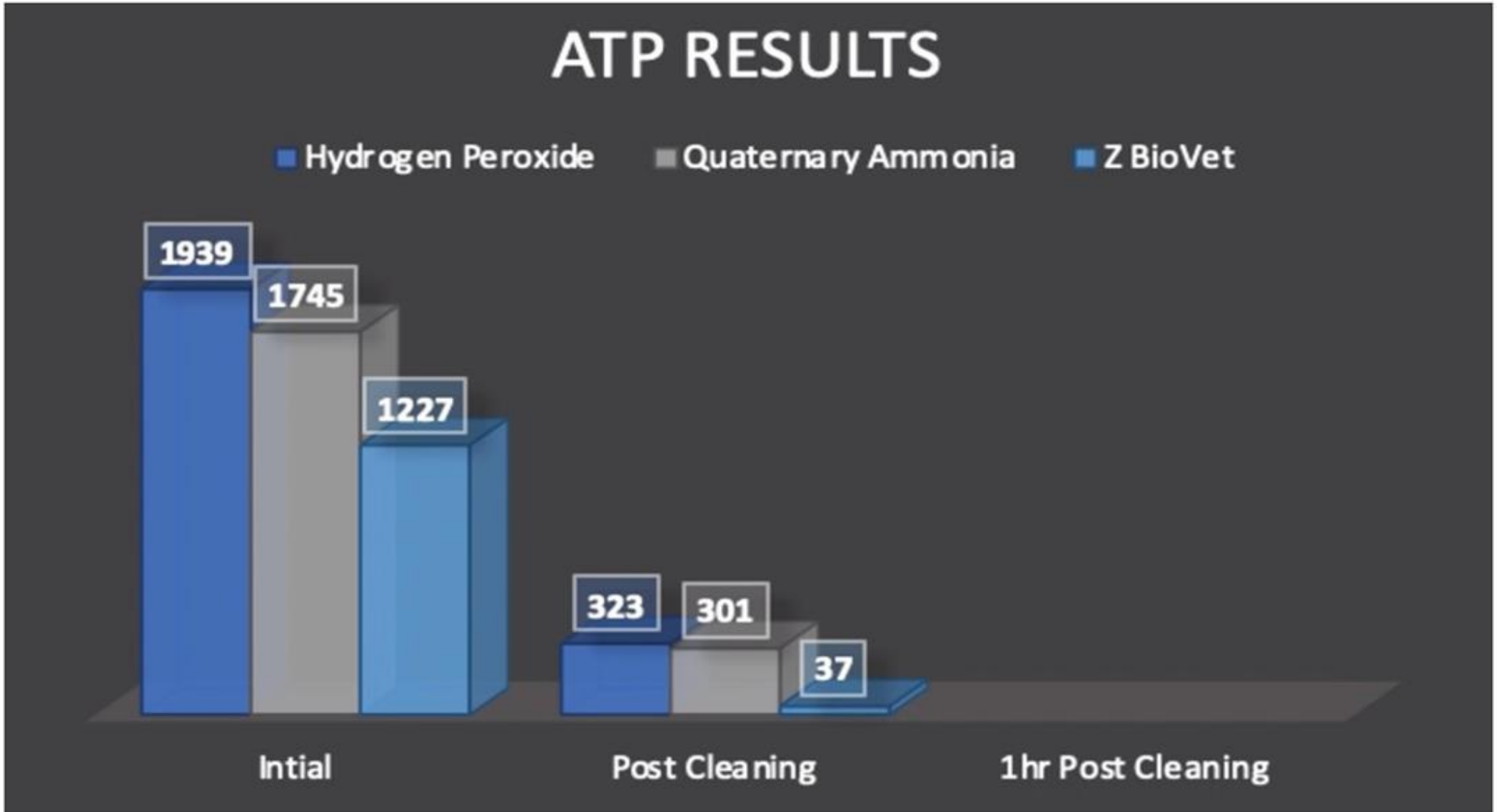
Post Cleaning ATP Results

Accelerated H2O2

Quaternary Ammonium

Z BioVet Probiotic Cleaner

Post Cleaning ATP Results



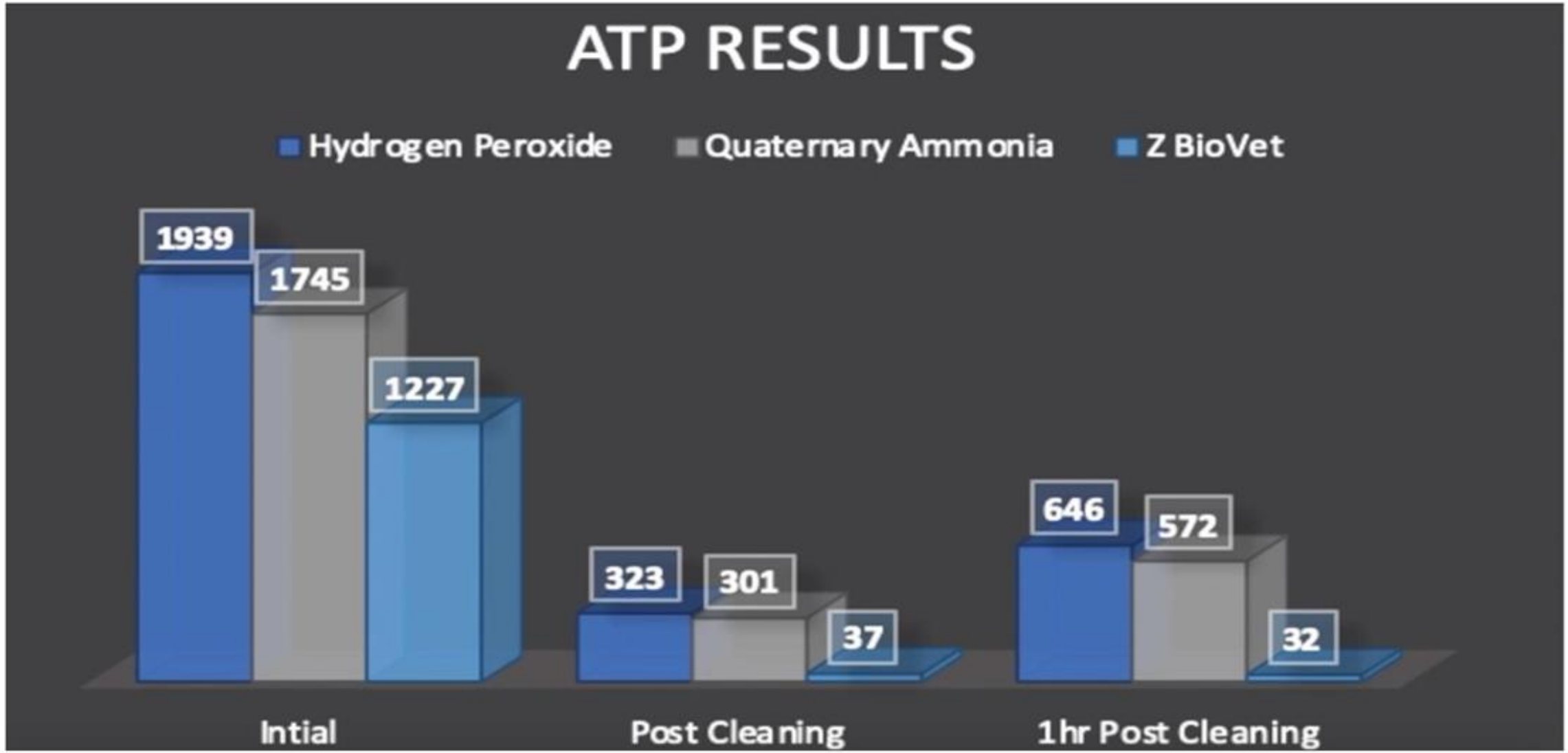
1 Hour Post Cleaning ATP Results

Accelerated H2O2

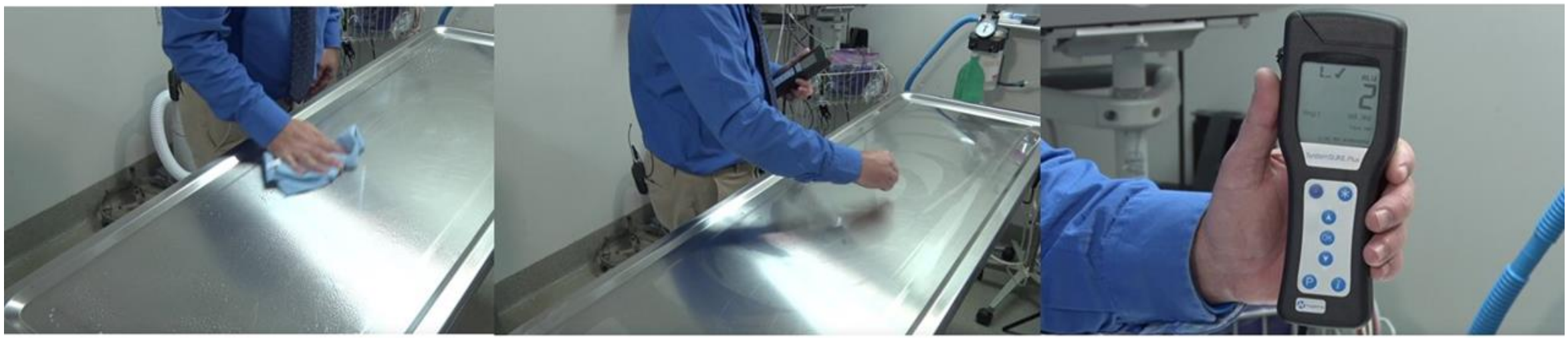
Quaternary Ammonium

Z BioVet Probiotic Cleaner

1 Hour Post Cleaning ATP Results



Whole Table Cleaned With Z BioVet




Ecoli Bacteria
Salmonella Bacteria
Pseudomonas Bacteria
Methycillin Resistant S. Aureus Bacteria
MRSA

CHEMICAL DISINFECTANTS

Claim as high as a 99.9999% kill rate

- **Also kills 99.9999% of good bacteria**
- **The remaining 0.0001 percent can colonize rapidly and come back strong.**

6 LOG DISINFECTANT

Applied to a colony of pathogen bacteria with a population of 1,000,000

1 CFU would be left

When a 99.9999% kill rate (6 Kill Log) dissenfectant is applied to a colony of pathogen bacteria with a population of 1,000,000, 1 CFU would be left. The following calculations show 2 different replication rates. Staph bacteria replicates at approximately every 20 minutes.The staph bacteria will have repopulated to over 1,000,000 bacteria in only 7 hours and could be more resistant to future treatments

# of bacteria	Replicating every 10 minutes	# of minutes		# of bacteria	Replicating every 20 minutes	# of minutes
1		10				
2		20		1		20
4	hour 1	30			hour 1	
8		40		2		40
16		50				
32		60		4		60
64		70				
128		80		8		80
256	hour 2	90			hour 2	
512		100		16		100
1,024		110				
2,048		120		32		120
4,096		130				
8,192		140		64		140
16,384	hour 3	150			hour 3	
32,768		160		128		160
65,536		170				
131,072		180		256		180
262,144		190				
524,288		200		512		200
1,048,576	hour 4	210			hour 4	
2,097,152		220		1,024		220
4,194,304		230				
8,388,608		240		2,048		240
16,777,216		250				
33,554,432		260		4,096		260
67,108,864	hour 5	270			hour 5	
134,217,728		280		8,192		280
268,435,456		290				
536,870,912		300		16,384		300
1,073,741,824		310				
2,147,483,648		320		32,768		320
4,294,967,296	hour 6	330			hour 6	
8,589,934,592		340		65,536		340
17,179,869,184		350				
34,359,738,368		360		131,072		360
68,719,476,736		370				
137,438,953,472		380		262,144		380
274,877,906,944	hour 7	390			hour 7	
549,755,813,888		400		524,288		400
1,099,511,627,776		410				
2,199,023,255,552		420		1,048,576		420
4,398,046,511,104		430				
8,796,093,022,208		440		2,097,152		440
17,592,186,044,416	hour 8	450			hour 8	
35,184,372,088,832		460		4,194,304		460
70,368,744,177,664		470				
140,737,488,355,328		480		8,388,608		480
281,474,976,710,656		490				
562,949,953,421,312		500		16,777,216		500
1,125,899,906,842,620	hour 9	510			hour 9	
2,251,799,813,685,250		520		33,554,432		520
4,503,599,627,370,500		530				
9,007,199,254,740,990		540		67,108,864		540