



Ascertaining the Frequency of Reapplication of Viral Shield Self-Disinfecting Coating on Inanimate Surfaces of Mass Rapid Transport, Buses, Taxis, Manufacturing Plants, Etc. Using ATP Luminometer

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Abstract

Visual inspection, the most common, if not only an evaluation used onboard mass rapid transport, buses, taxis, schools, restaurants, etc. before Covid-19 Pandemic was found to be wholly unreliable in the measure of surface contamination. Adenosine Triphosphate Tests (ATP) is recommended as an effective measure with benchmark of <100 RLU for inanimate surfaces coated with Legionella-X Viral Shield. RLU-relative light units are a measurement to indicate how soiled a surface is; the lower count being optimal.

Legionella-X Viral Shield, a self-disinfecting coating has passed the JIS Z 2801:2010/A1:2012 Test Method for Antibacterial Activity and Efficacy with 99.9998% inactivation efficacy up to 60 days. However, the laboratory testing may differ greatly from field application, this journal covers the use ATP Luminometer [1,2,3,4,5] to ascertain the reapplication of Viral Shield on inanimate surfaces onboard mass rapid transport trains,

buses, taxis, train stations, restaurants, elevators, schools, etc. As the duration of residual inactivation efficacy of self-disinfecting coating depends on the integrity of the coating. It is essential to ascertain the frequency of re-applying Viral Shield on the specific inanimate surfaces onboard public transports. This technical journal emphasizes the common inanimate surfaces onboard trains, buses and taxi that are likely to be contaminated by bacteria where special reapplication of self-disinfecting coating is needed more frequently than other surfaces. It also covers the rationale of using an ATP Luminometer and why it has been adopted for use. The technical journal briefly covers the description of ATP, its functions, and its mechanism. It also provides guidelines for reapplication of Legionella-X Viral Shield onboard public transport and the parameters and used.

Keywords:

Legionella-X Viral Shield, JIS Z 2801:2010/A1:20012, Residual Inactivation Efficacy, Covid-19 Virus, ATP Luminometer, ATP, ADP.

Introduction

Legionella-X Viral Shield has passed the JIS Z 2801:2010/A1:2012 Test Method for Antibacterial Activity and Efficacy [6] with 99.9998% inactivation against positive and negative-gram bacteria up to 60 days in a world renown laboratory. However, the duration of its residual inactivation efficacy depends largely on the integrity of the physical coating of Legionella-X Viral Shield.

In the field tests, the residual inactivation effect of Legionella-X Viral Shield depends on the volume of traffic of commuters, the number of contaminated hands touching an inanimate surface etc.

The authors of this journal feel that it is essential to establish a baseline for reapplication of self-disinfecting coating to prevent any false sense of security onboard mass rapid trains, buses, taxis, etc. coated with the said coating. To contain the spread of Covid-19, it is absolutely essential to ensure that no guesswork is allowed or take for granted that the inanimate surfaces coated with self-disinfecting will provide all necessary protection of commuters using said public transport systems against any cross contamination infections.

In view of the above, Magna and its' Think Tank Group feel it is unquestionably necessary to ascertain the frequency of reapplication of Legionella-X Viral Shield onboard all public transport, restaurants, schools, elevators, bank teller machines etc. using an ATP Luminometer to ascertain whether an inanimate surface coated with Legionella-X Viral Shield needs to be reapplied before 60 days instead of just relying on laboratory test results of 60 days inactivation efficacy.

The measurement of Adenosine Triphosphate (ATP) is widely used in food and beverage processors to quickly assess the cleanliness of surfaces as Adenosine Triphosphate (ATP) is present in all organic materials and is a unit used in all living cells [14,15].

All living organisms respire [16,17]. Respiration is the biochemical process in which the cells of an organism obtain energy by combining oxygen and glucose, resulting in the release of carbon dioxide, water, and ATP (the currency of energy in cells).

Cells need and use the energy that is formed through this process to assist with life processes for organisms to survive and reproduce [18,19]. Oxygen and carbon dioxide are the main gases involved in aerobic respiration. They carry out gas exchange in a different way to mammals [20].

As ATP is the main carrier of energy used for all cellular activities, when it is hydrolyzed and converted to adenosine diphosphate (ADP), energy is released. The removal of one phosphate group releases 7.3 kilocalories per mole, or 30.6 kilojoules per mole, under standard conditions. This energy powers all reactions that take place inside the cell. ADP can also be converted back into ATP so that the energy is available for other cellular reactions [21,22,23,24,25,26]

What is Adenosine triphosphate

Adenosine triphosphate (ATP) [7,8,9,10,11,12,13] is an organic compound that provides energy to drive many processes in living cells, e.g. muscle contraction, nerve impulse propagation, and chemical synthesis. Found in all known forms of life, ATP is often referred to as the "molecular unit of currency" of intracellular energy transfer. Adenosine triphosphate (ATP), energy-carrying molecule found in the cells of all living things, captures chemical energy obtained from the breakdown of food molecules and releases it to fuel other cellular processes.

How Adenosine triphosphate (ATP) Works

As mentioned above, Adenosine triphosphate (ATP) is a molecule that carries energy within cells. It is the main energy currency of the cell, and it is a product of the processes of photophosphorylation [28,29,30,31] (adding a phosphate group to a molecule using energy from light), cellular respiration, and fermentation. All living things use ATP. In addition to being used as an energy source, it is also used in signal transduction pathways for cell communication and is incorporated into deoxyribonucleic acid (DNA) during DNA synthesis. It is made up of the molecule adenosine (which itself is made up of adenine and a ribose sugar) and three phosphate groups. It is soluble in water and has a high energy content due to having two phosphoanhydride bonds connecting the three phosphate groups. The structural of ATP is shown in diagram 1 below.

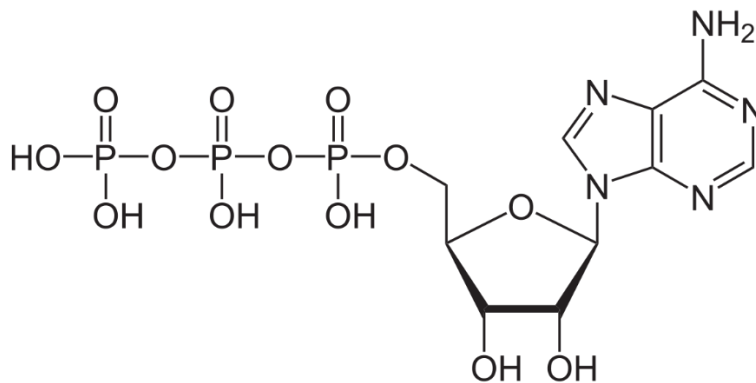


Diagram 1

This is a structural diagram of ATP

ATP is a nucleotide that consists of three main structures: the nitrogenous base, adenine; the sugar, ribose; and a chain of three phosphate groups bound to ribose. The phosphate tail of ATP is the actual power source which the cell taps.

ATP is an unstable molecule which hydrolyzes to ADP (Adenosine diphosphate) inorganic phosphate [32,33,34] when it is in equilibrium with water. Adenosine diphosphate (ADP), also known as adenosine pyrophosphate (APP), is an important organic compound in metabolism and is essential to the flow of energy in living cells. ATP contains one more

phosphate group, whereas, ADP. AMP contains one fewer phosphate group, with the chemical formula: $C_{10}H_{15}N_5O_{10}P_2$.

ATP is produced through several different methods. Photophosphorylation is a method specific to plants and cyanobacteria. Photophosphorylation occurs during cellular respiration.

ATP is also formed from the process of cellular respiration in the mitochondria of a cell. The processes cells use to make energy in the form of ATP. This can be through aerobic respiration which requires oxygen or anaerobic respiration, which does not. Aerobic respiration produces ATP along with carbon dioxide and water from glucose and oxygen [35,36,37,38].

Anaerobic respiration uses chemicals other than oxygen [39,40], and this process is primarily used by archaea and bacteria that live in anaerobic environments. Fermentation is another way of producing ATP that does not require oxygen; it is different from anaerobic respiration because it does not use an electron transport chain. Yeast and bacteria are examples of organisms that use fermentation to generate ATP.

If a cell needs to spend energy to accomplish a task, the ATP molecule splits off one of its three phosphates, becoming ADP (Adenosine di-phosphate) + phosphate. The energy holding that phosphate molecule is now released and available to do work for the cell. When one phosphate group is removed by breaking a phosphanhydride bond in a process of hydrolysis, energy is released and ATP is converted to ADP adenosine diphosphate, like wise energy is also released when a phosphate is removed from ADP to form adenosine monophosphate,

In view of the above, ATP can be used as indicator for the measurement of microbiological activity on inanimate surfaces. Hence, using ATP Luminometer for quick measurement of the presence of microorganism seems the most appropriate method for onsite testing.

The ATP's mechanism is herein briefly described below:

Chemically, ATP is an adenine nucleotide bound to three phosphates. There is a lot of energy stored in the bond between the second and third phosphate groups that can be used to fuel chemical reactions [41,42,43,44].

When a cell needs energy, it breaks this bond to form adenosine diphosphate (ADP) and a free phosphate molecule [45]. In some instances, the second phosphate group can also be broken to form adenosine monophosphate (AMP) [46,47]. When the cell has excess energy, it stores this energy by forming ATP from ADP and phosphate. See diagram 2 and 3.

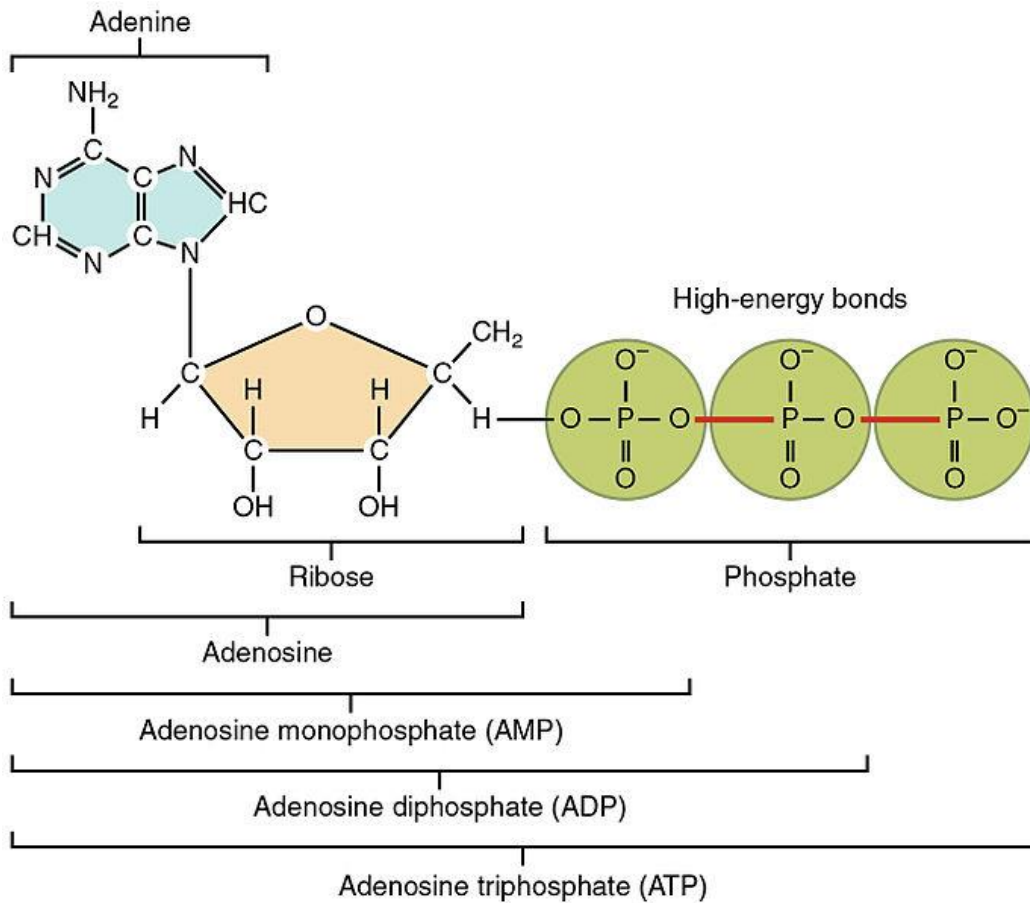


Diagram 2

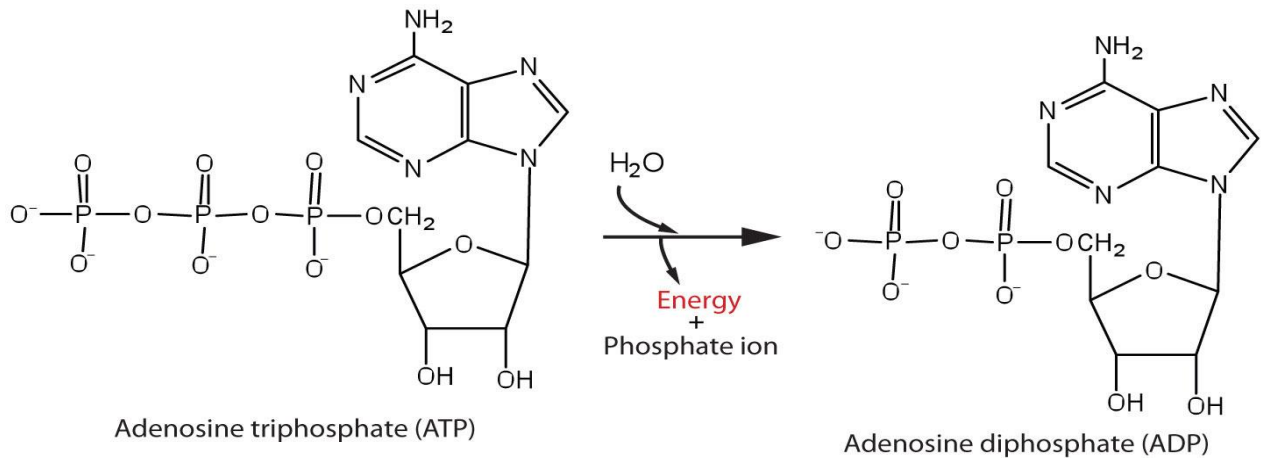


Diagram 3

While many methods exist for evaluating cleanliness, ATP bioluminescence is the only method that combines quantitative data collection with scientific measurement and still delivers speedy result.

ATP is a general indicator for the presence of living cells [48] ATP can be measured in a sensitive way, using firefly extracted from *Photinus pyralis*. The light emission is in the range between 500 to 700 nm wavelength [49] and the assay requires the presence of the luciferase, luciferin, magnesium, and oxygen shown in diagram 4. The measured amount of light is proportional to in the sample. In optimum conditions 1 proton of light is produced by 1 molecule of ATP

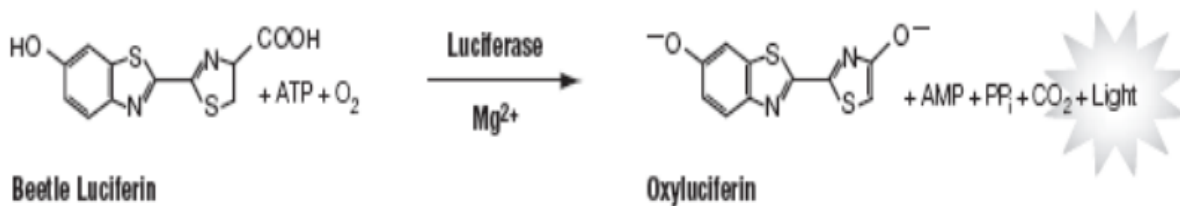
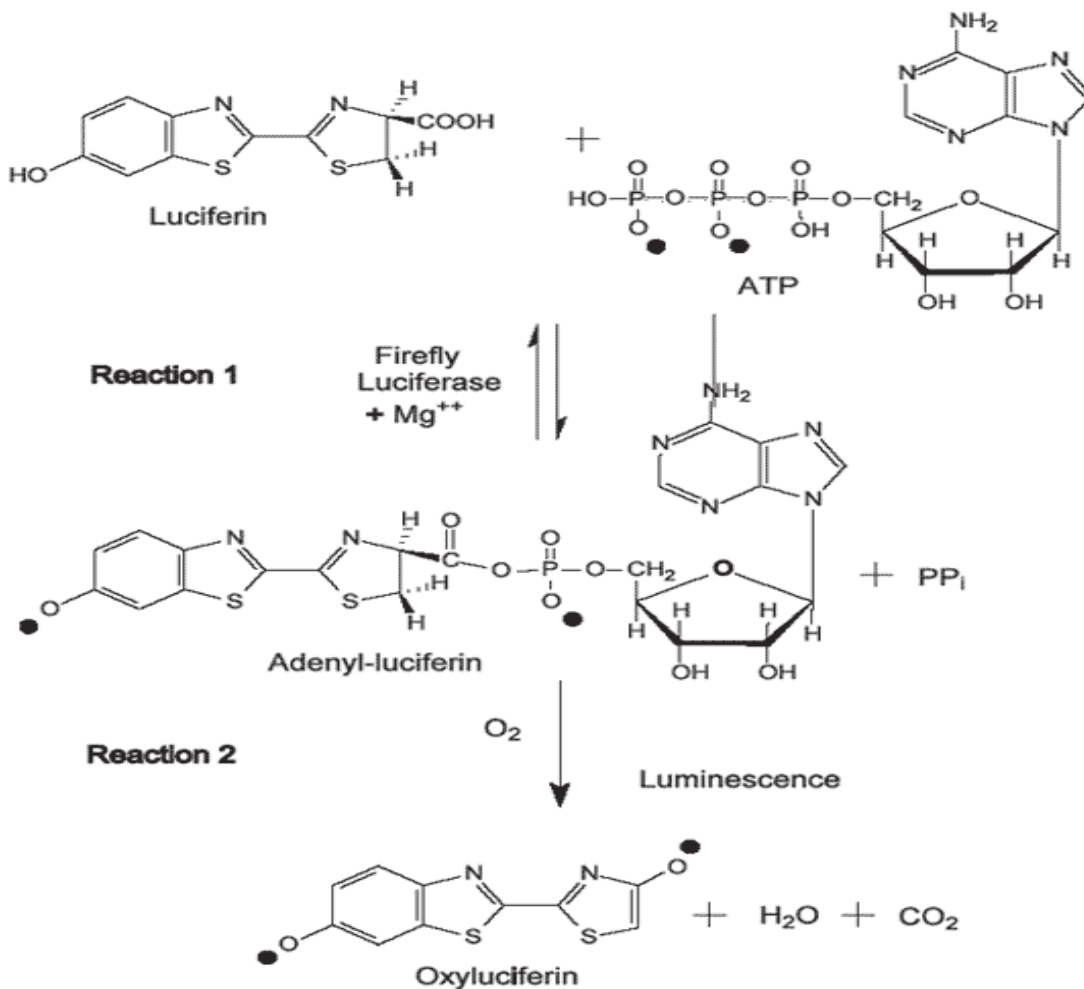


Diagram 4



Based on the above findings, the rationale of using an ATP Luminometer to ascertain the cleanliness of an inanimate surface coated with Legionella-X Viral Shield self-disinfecting disinfectant and its reapplication has hence been established.

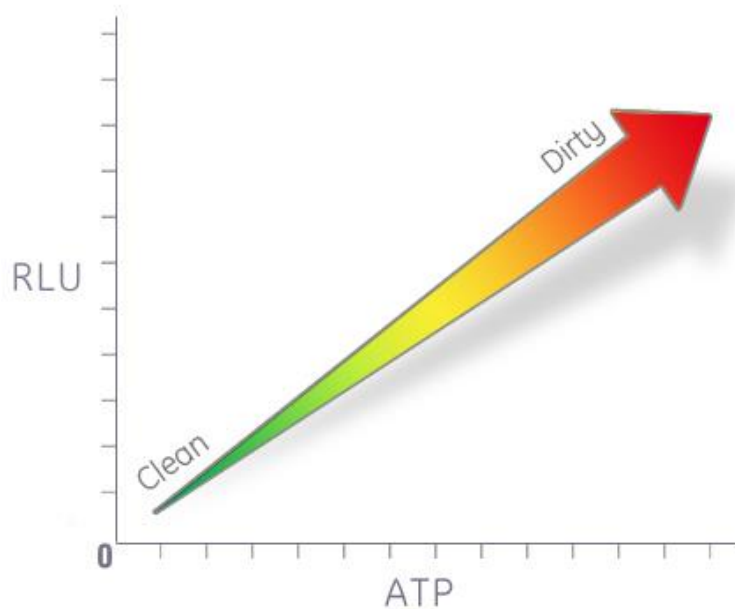
ATP is measured in RLU's (relative light units).

ATP systems use relative light units (RLU) as the unit of measure for adenosine triphosphate (ATP). Though the ratio of RLU to ATP varies per manufacturer, the greater the ATP, the higher the RLU. The cut-off scores for acceptable or unacceptable RLU scores are called thresholds, or limits. RLU limits enable users to categorize RLU test results as Pass, Caution, or Fail.

A Pass score is indicated by a check mark and means the surface was thoroughly cleaned and is safe for production.

A Caution result is indicated by an exclamation point. At your discretion, locations with a Caution result may be re-cleaned, recoated and retested or monitored for future problems.

A Fail score is indicated by an X and should be re-cleaned, recoated and retested until a Pass or Caution score is achieved.



Appended below are general baseline guidelines for each industry.

RLU limits for food and beverage industries.

The default limits of 10 and 30 RLU are based on years of experience in food and beverage industries and published third-party studies [50,51].

Any score of 10 RLU or less is a Pass. Scores from 11 to 30 RLU are a Caution. Any score greater than 30 RLU is a Fail.



Some users prefer to eliminate the Caution area and set both Pass and Fail limits to 10. Any score of 10 RLU or less is a Pass. Any score greater than 10 RLU is a Fail.



Guidelines for Inanimate Surfaces for Mass Rapid Transport, Buses, Taxis, Hospitals, Clinics and Public Places suggested by Luminometer Manufacturers as shown in diagram 5.

Studies concluded that a benchmark of <100 RLU was the most effective measure of cleanliness while using the Hygiena ATP system [56]. Researchers felt that although this technology works well, the relative variability in RLU scores resulting from different ATP systems makes creating a cleaning standard difficult outside specific ATP manufacturers and models.

The ATP benchmark of <100 RLU was found to be an effective measure of surface cleanliness and was recommended for use in other studies as well. The studies concluded that a benchmark of <100 RLU was the most effective measure of cleanliness while using the Hygiena ATP system [56].

ATP benchmark of <100 RLU was a more accurate measure of cleanliness while using the Hygiena ATP system [56].

ATP Levels of Clean for General Surfaces		
Ultra Clean	Sterile surfaces and food preparation area.	RLU: 0-10
Very Clean	Critical touch points.	RLU: 11-30
Good Clean	Floor requirement and typical microfibre performance.	RLU: 31-100
Somewhat Dirty	Caution: Surface should be cleaned and has some risk of contamination from disease-causing bacteria.	RLU: 101-200
Dirty	Warning: Surface need cleaning and has a medium risk of contamination from disease-causing bacteria.	RLU: 201-500

Very Dirty	Danger: Surface need cleaning and has a medium to high risk of contamination from disease-causing bacteria.	RLU: 501-1,000
Filthy	Danger: Surface need cleaning and has a high risk of contamination from disease-causing bacteria.	RLU: >1,000

Diagram 5

High-Risk Areas for Mass Rapid Transport, Buses, Taxis, Hospitals, Clinics and Public Places

All high-touch surfaces are high-risks areas that are handled frequently throughout the day by numerous people. These surfaces include doorknobs, light switches, phones, sink faucets, and toys.

The high-risk areas for Mass Rapid Transport and buses are handholds such as; hanging strap a strap suspended from the ceiling (often with a handle or a loop) grab handle a pivoted, rigidly-mounted, or suspended handle often mounted above eye level of standing passengers, handrails – rigid rails running horizontally below the ceiling. See diagram 6 below.



Diagram 6

Studies reviewed that the highest risk of infection resulting from contaminated surfaces is in the category of high-frequency touch areas. In a study of these areas it was recommended that a standard be first applied to the following areas:

Bed Controls, Phone and call buttons, Light switches, Sink tops, Flush handles, Bed rails Tray tables, IV poles, Doorknobs and door levers, and Monitor touch screens.

Customized RLU limits to fit your specific needs including manufacturing facilities.

Setting custom RLU limits for locations in your facility depends on the following factors:

Surface type.

Easy to clean surfaces, such as stainless steel or new equipment may have lower RLU limits. Hard to clean equipment such as conveyor belts or aged equipment, may have higher RLU limits.

Contact type.

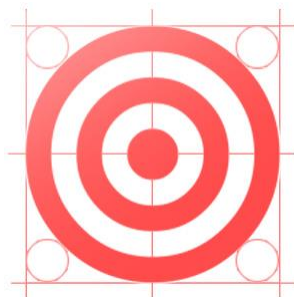
Surfaces that come into direct contact with products, such as conveyor belts or hoppers, should have more strict, lower RLU limits. Indirect contact surfaces, such as control buttons or the sides of a conveyor belt, are less critical and may have higher RLU limits.

Product type.

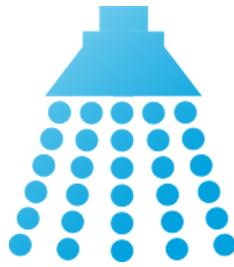
Manufacturing equipment for short shelf-life and ready to eat (RTE) products should be held to a higher standard of cleanliness and should have lower RLU limits. Manufacturing equipment for longer shelf-life or cooked products may have higher RLU limits since the cleaning requirement is not as strict and potential contamination is less dangerous.

Calculating custom limits for your locations.

To calculate custom limits for locations in your facility, follow these four steps: Identify, Clean, Test, Calculate.



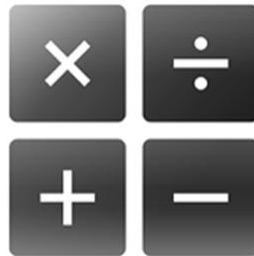
Identify test points in your facility. These are usually outlined in HACCP, GMP, or SSOP programs.



Clean locations to the highest standard of clean. This may include a total production line breakdown.



Test each location 5-10 times. For large locations, such as a conveyor belt, tests can be collected from different 4x4 inch areas on the belt. For smaller areas, such as a fill nozzle, tests must be collected over several days after cleaning.



To calculate the Pass RLU Limit, calculate the average of the RLU scores.

The average is your Pass Limit for that location.

To calculate the Fail Limit, use one of the following two methods:

1. Multiplication Method: Multiply the average by 3.
2. Standard Deviation Method (preferred for statistical accuracy):

Calculate the standard deviation of RLU scores.

Multiply the standard deviation by 3.

Add this result to the average to get the Fail RLU.

Conclusion

Visual Assessment

Researchers conducting a study of a 1300-bed hospital tested a random sample of high-touch surfaces after cleaning, using each of the three methods. These researchers found a relationship between cleaning failures assessed visually and microbiologically. This relationship rendered a p-value of less than 0.05, which indicated, in this study, that visual failures result in microbiological failures. They also found that of the 80% of high-touch surfaces that passed visual assessment, only 19% were found to microbiologically clean [52].

Another study by Griffith produced similar results where 82% percent of the surfaces passed the visual inspection, but only 30% were found to be microbiologically clean [53]. In fact, every study reviewed, that attempted to measure the efficacy of visual cleaning inspection, concluded that this inspection method was an unreliable and ineffective measure of surface cleanliness. Because of the unreliability of visual assessments, researchers agreed that visual assessments should be only used as the first stage in an integrated monitoring program.

ATP Testing

A study performed in a UK teaching hospital used visual, ATP, and ACC inspections to monitor and evaluate current and revised cleaning programs. The study used an ATP benchmark of <500 RLU to determine if a surface was contaminated or not. This method of assessment revealed promising results for the use of ATP testing to improve cleaning results. The ATP test revealed that after revised cleaning practices were applied, not only did the results show a higher degree of consistency, but they also showed 95% of surfaces passed as sufficiently clean. Researchers recommended that a benchmark of <250 RLU might be a better benchmark for the hospital environment [54].

The Malik study used the recommended ATP benchmark of <250 RLU to measure the efficacy of cleaning [55]. Cleanliness was measured in one medical and one surgical ward in a teaching hospital through ATP and ACC tests. Researchers found that a sample taken before and after routine cleaning showed that current cleaning practices reduced contamination by 32.4% and increased contamination in some areas by 10%.

Routine cleaning practices were found to be ineffective at removing MRSA. Researchers also found that ATP benchmark of <100 RLU was a more accurate measure of cleanliness

while using the Hygiena ATP Luminometer had the highest correlation with contamination according to ACC benchmark indicating microbiological growth of $<2.5\text{cfu}/\text{cm}^2$ [55]. Researchers concluded that this recommended change in the ATP benchmark will most likely continue to be reduced as ATP tests gain more accuracy.

The studies concluded that a benchmark of <100 RLU was the most effective measure of cleanliness while using the Hygiena ATP system.

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Nelson has received and accorded several accolades including the 2015 Winner of the Asia Packaging Award, Top 10 Most Inspiring Entrepreneur 2015, Winner of the Global Star, Asia Star and Singapore Star Packaging Awards 2014, 2015, 2016, 2017, 2014, Top Entrepreneur Award-Singapore Small Medium Business Association, Asia Excellence Award 2014 and Top 20 Innovation Award 2013 from Small Medium Business Association.

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He has been a member of NACE for 26 years. He is the current Technical Director of Magna Group of Companies.

Professor Ernesto Beltrán-Partida

Professor Ernesto Beltran-Partida obtained his bachelor's degree in Biological and Pharmaceutical Chemistry and his Ph.D. in Biomaterials Sciences both with Honors from the Autonomous University of Baja California.

During his Ph.D., Dr. Beltrán was a visiting student at the National Institute of Rehabilitation in Mexico City, the School of Stomatology and Medicine of the Autonomous University of San Luis Potosi and at the School of Medicine of the University of California San Diego, USA.

He is a professor of biomaterials science, tissue engineering, microbiology, and molecular biology at the institute of engineering of Autonomous University of Baja California Mexico.

He has authored different peer-reviewed articles and a book chapter. Moreover, Dr. Beltrán has directed several researchers, granted from different government institutions. He has also served as a reviewer of different high impact journals such as Materials Science and Engineering C, Nanomedicine: Nanotechnology, Biology and Medicine, and Biotechnology and Biotechnological Equipment. His research interests are focused on Biomaterials, Tissue Engineering, Cellular and Molecular Biology, and Corrosion of Materials.

Dr. Ernesto Alonso Valdez Salas

Dr. Ernesto Alonso Valdez Salas is a passionate and renounced physician based in Mexicali Baja California. He has a medical degree and a master's degree in surgery with a specialization in gastroenterology at the Universidad Autonoma de Guadalajara, he received his of Doctor of Sciences from Universidad Autonoma de Baja California.

His research activities include the generation of functionalized and non-functionalized natural compounds with mineral nanoparticles for medical purposes.

He has participated and published many articles on NanoMedicine in International Scientific Journals, in addition to collaborating with the engineering institute of the Autonomous University of Baja California in projects involving areas of health.

He is the founder and director of Ixchel Medical Center and is the Medical Adviser of the Magna Think Tank Group.