Technical Whitepaper

An Overview of LuminUltra's qPCR Assays

LuminUltra's GeneCount qPCR products and services can be used to rapidly quantify the presence of detrimental or unwanted organisms in your system or process. In the past decade, simplified, yet powerful qPCR technology has become commercially available to users around the world. qPCR is short for quantitative Polymerase Chain Reaction, which is a microbiology technique used to detect and quantify DNA from targeted organisms. LuminUltra offers a line of field and lab-ready instruments and test kits which allows users of all experience levels to quantify the organisms which are relevant to them. We're dedicated to expanding our offering of assays which are important to our clients and their industries. This whitepaper will highlight the currently available assays and offers some technical information about them and the organisms they target.

Total Prokaryote:

Prokaryote is a term used to describe single-celled organisms that do not contain a distinct nucleus or intracellular organelles, and includes bacteria and archaea. When thinking of microorganisms, bacteria often come to mind first, but archaea are common in natural and engineered environments as well. While we generally think of 'bugs' as bacteria, many industrially important organisms, including methanogens, are archaea. Bacteria and archaea are not closely related, however they are grouped together as prokaryotes for convenience, and can be selectively targeted by qPCR due to their 16S rRNA gene.

This qPCR assay will give quantitative data on all the bacterial and archaeal organisms present in a sample and represents the total microbial level. This information can be used to collect information about contamination events, compare different locations or systems, and gives the ability to take mitigation action before a problem gets out of control. LuminUltra uses this assay to convert 16S rRNA NGS data from relative abundance (%) to approximate cell counts (ie. cell/mL, cell/g, cell/cm^2).

Sulfate-Reducing Prokaryotes:

LuminUltra's Sulfate-Reducing Prokaryotes (SRP) assay offers coverage for both bacteria and archaea that are responsible for the reduction of sulfur substrates, primarily sulfate, to sulfide through their respiration pathways. SRP are one of (if not) the main contributors to microbially influenced corrosion (MIC) through the chemical product of sulfur reduction, hydrogen sulfide (H_2S) which has been shown to corrode iron and other metals. SRP are present primarily in anoxic sulfur-rich environments like oil & gas pipelines, marine sediments, and bogs & marshes.

SRP contribute to corrosion by CMIC (chemical microbially influenced corrosion) and EMIC (electrical microbially influenced corrosion). The chemical method of corrosion is the most common and refers to the corrosive chemical products that are generated by sulfur reduction, mainly H_2S which reacts with iron to produce hydrogen gas and iron sulfides (characteristic products of SRP corrosion). EMIC is less common and only demonstrated in certain species of sulfate-reducers, while CMIC is present in all species.

The majority of SRP are anaerobic bacteria, however there are some bacteria that operate in aerobic environments. Additionally, multiple genera of sulfate-reducing archaea are known as well, which can contribute to corrosion in systems too hot for bacteria to proliferate. SRP can also be found in wastewater treatment plants and collection systems where they will produce hydrogen sulfide once dissolved oxygen and nitrates have been removed. Additionally, H_2S gas results in foul odors.

Methanogens:

Methanogens are a group of microorganisms defined by their ability to produce methane through their metabolic pathway. Methanogens include a diverse range of organisms, with all currently known methanogens belonging to the archaea domain. Most commonly, they produce methane by the reduction of CO_2 in the presence of H_2, however methanogens can also use simple organic molecules such as acetate, formate or methylamine to produce methane. Methanogens are very sensitive to oxygen and operate exclusively in anoxic or anaerobic environments. There are both mesophilic and thermophilic methanogens, and therefore they exist in environments ranging from room temperature to upwards of 100 °C.

These organisms can be found naturally in many different environments including marine sediments, wetlands, and the digestive tracts of animals (including humans). In addition, methanogens can be present in engineered environments, like wastewater treatment plants, biogas plants, and oil & gas pipelines with either beneficial or detrimental effects.

Biogas is considered as a potential alternative energy source for the replacement of more conventional energy resources. Biogas is a term used to describe the mixture of gases that are created by the breakdown of organic matter, such as wastewater sludge, by microorganisms in an anaerobic environment. Methane is the main component of biogas, and therefore organisms with the ability to produce methane from CO_2 or other simple organic molecules have potential for exploitation as a renewable energy source. Methane can be used directly as a fuel source or processed to make industrial products.

Further, the anaerobic environment of oil & gas pipelines provides a breeding ground for methanogens, which have been shown to contribute to corrosion of carbon-steel surfaces via their methane-producing metabolic pathways. Methanogens are able to oxidize elemental iron within steel infrastructure converting it to dissolved ferrous iron. The role of methanogens in MIC is often understated relative to SRP, however methanogens have been shown to cause catastrophic damage. The reason they are often understated may be due to a lack of culture-based growth method. The addition of qPCR addresses this issue.

Iron-Reducing Bacteria (*Shewanella*):

MIC is caused by a coalition of organisms that act synergistically to degrade metal infrastructure and lead to corrosion. Many operators and managers of pipeline systems are aware of sulfate-reducing and acid-producing bacteria and archaea which are the face of pipeline corrosion, but they may not be aware of Iron-Reducing Bacteria (IRB) like *Shewanella*. LuminUltra's Iron Reducing Bacteria qPCR assay is designed to measure the range of *Shewanella* species which are the most common IRB.

Through the metabolic pathways of iron-reducing bacteria, Fe^{3+} is reduced to Fe^{2+} which leads to the formation of iron oxides and iron hydroxides which cause local corrosion of the metal surface. In addition, the ability of IRBs to corrode has been shown to be improved in the presence of SRP, making these two groups a deadly duo for pipelines.

Further, certain species of *Shewanella* are known to produce biofilm. The formation of biofilm on the inside of a pipeline is usually the single most important factor to allowing corrosion to take place. Once biofilm has been established, it can harbor a plethora of unwanted organisms, including SRP, methanogens and sulfur-oxidizing bacteria, among others. These organisms can then act to degrade the pipeline, while the biofilm provides protection from chemical treatments and undesirable environmental conditions.

Sulfur-Oxidizing Bacteria:

The grouping of organisms known as sulfur-oxidizing bacteria (SOB) oxidize mainly hydrogen sulfide, but also elemental sulfur to sulfate, generating sulfuric acid as an end product. Sulfuric acid has a corrosive effect on both metal and concrete structures. SOB are naturally found in marine sediments and hydrothermal systems where they play important roles in the global sulfur cycle. These organisms can live in either aerobic or anaerobic conditions but are most commonly found in anaerobic conditions such as oil & gas pipelines and wastewater treatment plants.

A specific type of corrosion known as biogenic sulfide corrosion is that caused by sulfuric acid created from the conversion of sulfate as described above. This is a bacterially mediated process and is especially relevant in wastewater treatment plants where the anaerobic sections contribute to hydrogen sulfide creation, which is then converted in the presence of oxygen to sulfuric acid. SOB are also used strategically in wastewater treatment plants to remove excess hydrogen sulfide when dealing with high-sulfur influent streams.

SOB can partner with SRP to produce stronger corrosion than either of the two groupings or organisms could alone. They work together in oil & gas pipelines to degrade metal as the SRP produce the hydrogen sulfide that SOB metabolize to survive.

Total *E. coli*:

E. coli is a gram-negative bacteria that is naturally present in the intestinal tracts and feces of animals, including birds and mammals, but it is not typically found in other places in the natural environment. For this reason, *E. coli* is often used as an indicator organism – meaning that if *E. coli* is found in water or food, it is an indicator of fecal contamination from animals. Most *E. coli* are harmless, however there are certain strains that are pathogenic to humans if consumed through contaminated food or water.

Exposure to pathogenic *E. coli* can result in diarrhea, cramps, and vomiting. Healthy persons will generally recover within one week from the infection. For the young, the elderly, or the immunocompromised, *E. coli* infection can be more serious and in extreme cases can lead to hemolytic uremic syndrome, which precedes kidney failure.

When an *E. coli* outbreak is reported in the news, it is usually caused by a Shiga-Toxin *E. coli* (STEC) pathotype bacteria, of which serotype *E. coli* O157 is the most common. LuminUltra's Total *E. coli* qPCR assay offers broad spectrum coverage for both pathogenic and non-pathogenic *E. coli* organisms. This is consistent with current regulations and standard methods like the IDEXX Colilert, which detects coliforms and *E. coli* simultaneously. LuminUltra's Total *E. coli* qPCR assay will detect the same *E. coli* portion of organisms as Colilert. If specificity is required, an assay validated by the AOAC for *E. coli* O157 is also available.

Total Fungi:

The term fungi is used to describe a physically and genetically diverse kingdom of eukaryotic organisms with chitin present in their cell walls. This large kingdom includes the familiar yeasts, molds, and mushrooms, along with other groupings that are less well known. All fungi are heterotrophs (consume organic material) and are the main decomposers of organic material in ecological systems. Fungi are ubiquitous in the environment and mostly microscopic; they become observable when producing fruiting bodies (mushrooms) or filamentous hyphae networks.

Yeasts may be the oldest human-domesticated microorganisms and have been used for thousands of years to produce bread and alcoholic beverages. They are still used today in many fermentation processes to produce CO_2, alcohols, soy sauce, as well as medicine (penicillin). The single-celled yeast *Saccharomyces cerevisiae* is one of the most well-studied organisms in the world and is used in research and production as its genome can be manipulated easily. Many fungi live as symbionts with other organisms, while other genera are pathogenic to animals and plants. Fungi that infect plants are known as rusts due to the rust-colored spots they create on leaves.

Fungi are important to consider for industrial systems such as cooling tower circuits, where they can form biofilms that harbor other microorganisms, giving protection from biocides. If wood is present, molds can lead to white or brown rot, while yeasts can produce the slime that forms biofilms. Fungi can also grow in fuel tanks when water is present, either at the fuel-water interface or in fungal biofilms. For industries that use metalworking fluids, different types of fungi can grow in these fluids, potentially causing fluid degradation and therefore lowered heat transfer efficiency, financial losses, and illness to system operators. Agricultural products such as fruits and vegetables are also degraded by fungi and their quantification can lead to better food preservation procedures.