

Introduction:

« The costs engendered by a microbial contagion in the petroleum industry have caused and continue to cause millions of Euros in damage and operational costs.

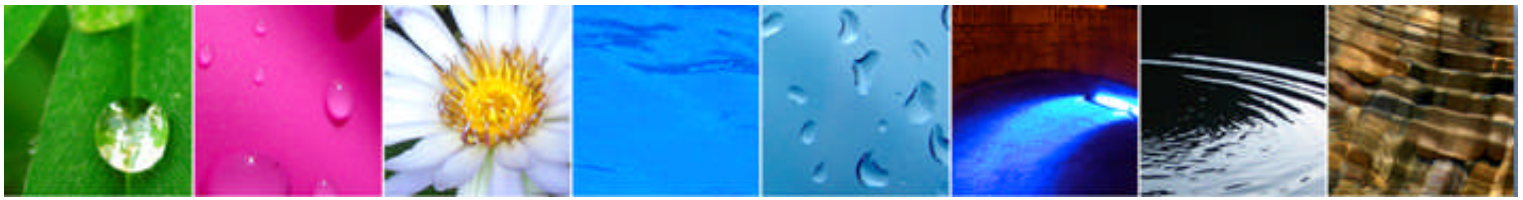
The proliferation of microorganism in the oil has for consequence problems like corrosion (pipeline...), fouling (line of fuel, filters, injectors) throughout the production of the crude oil up to finished products (diesel, lubricant).

Nowadays, the Aqua-tools ATP-metry kit, allows within 10 minutes, an immediately measurement of the microbial contagion using the ATP parameter.

With an immediate result, the industrial can react quickly in front of an operational drift by setting up preventive treatments. He can also decrease continuous treatments (biocide, bio-dispersing treatments) by real quantification of the microbial contamination in order to economize chemical products and money.

The ATP-metry, a non specific technique, allows decreasing of SRB culture test frequency because:

- If the ATP is low that means that the installation is secure regarding SBR contamination**
- If the ATP is high (see table of thresholds p.15), prepare SBR dilution bottles for specific tests and implement immediate corrective actions to solve the microbial problem. »**



Follow-up the quality of petroleum from the extraction until the distribution by a real-time microbiological control

The Proliferation of bacteria is a major concern in the **three segments** of petroleum sector:



In « **Upstream** » sector, we find the activities of exploration and extraction of the crude oil.

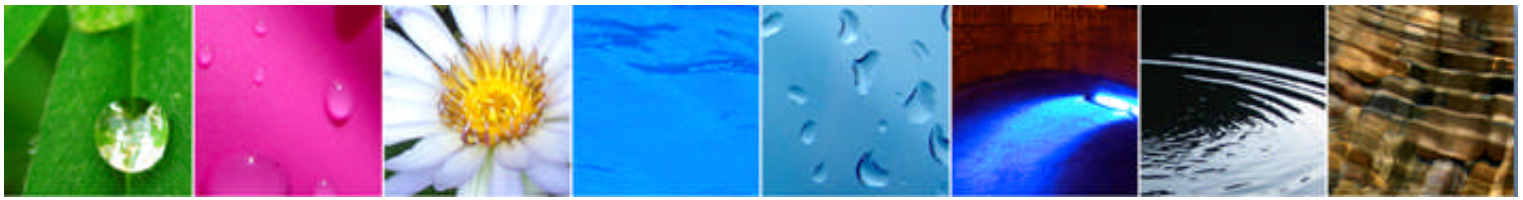


In « **Downstream** » sector, the crude oil pursues its road via tankers or pipelines towards refineries to obtain fuels, lubricants, oil or products for the chemical industry (polymers...)



In « **Midstream** », we find the part of distribution via pipelines and storage of the finished product in stores, petrol stations, airports...





Microorganisms can contaminate a wide range of **petroleum fuels** from heavy residual fuel oils to gasolines and cause very costly operational problems for:

✓ **Suppliers**

✓ **Distributors**

✓ **Airlines**

✓ **Ship**

✓ **Small boat operator**

✓ **Haulage companies**

✓ **Bus and train operators**

✓ **Power generators**

✓ **Car companies**

✓ **Petrol stations**

ORIGIN OF PETROLEUM CONTAMINATION

Although the microbes need water to grow, very fee is needed and they are able to proliferate within pipelines and closed recipients.

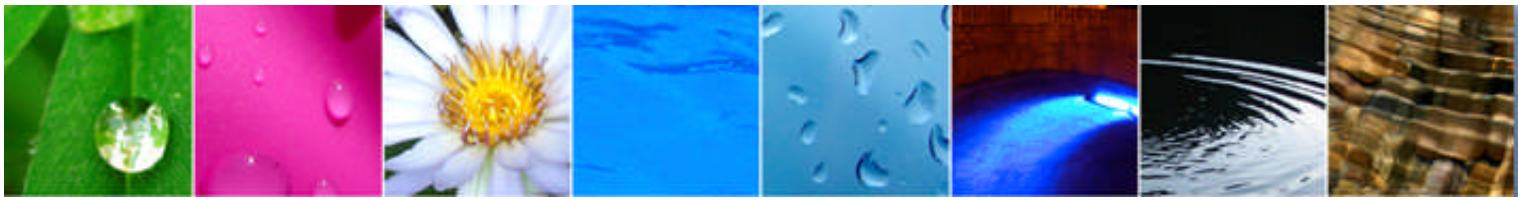
In practice microbial contamination in petroleum can have different origins:

- ❖ **Entry of air -Tank** at the stage of balancing the air pressure because the atmosphere contains a multitude of water droplets transporting microorganisms (“Upstream”)
- ❖ **Transport** - there is often sufficient water in the bottom of large storage tanks and even in the fuel tanks of road vehicles such as trucks and buses for some microbial growth to occur (“Midstream”)
- ❖ **Water in the network** – water may contain a very strong concentration of microorganisms (pipe line...)
- ❖ **Water injection** via the wells which contain a strong water contamination and bacteria.

CONSEQUENCES OF A BIOLOGICAL DETERIORATION

The consequences of a biological deterioration in oil industry can be distinguished in 4 groups:

- Microorganisms attack hydrocarbon and non-hydrocarbon molecules involving a **change of the characteristics and chemical properties of the crude oil**
- Microorganisms grow up at the interface of oil and water or in the biofilm and **produce biosulfates, detergent molecules** and H₂S molecules.
- Weak molecular weight secretions from microorganisms react with crude oil and accelerate the formation of particles what **make fuel more corrosive**.



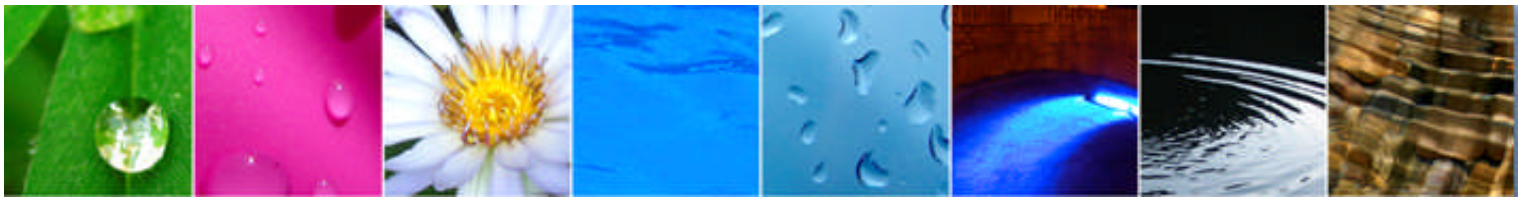
DISORDER STATES CAUSED BY MICROBIAL CONTAMINATION OF THE EQUIPMENTS

The principal disorders caused by a bacterial proliferation result in:

- ✓ **Fouling:** by slimes produced by bacteria, yeasts and/or moulds can cause **severe filter plugging, blocking of fuel lines and injectors** and consequently then cause excessive **wear and failure of engines and system components**. Fuel fouled by microbial slimes **can fail particulate specifications**.
- ✓ **Corrosion:** **microbial growth in fuel tanks and fuel systems can** result in rapid and **severe corrosion**. For example **occasional problems occur in aircraft wing tanks** due to the growth of moulds and also yeasts and bacteria which **produce organic acids and also stimulate corrosion** by creating oxygen gradients which enhance electrochemical corrosion cells.
 - In steel tanks, growth of **Sulphate Reducing Bacteria (SRB)** in water and sludge in the bottoms of tanks can **cause pitting corrosion**.
 - Growth of **Sulphate Reducing Bacteria (SRB) in fuel tanks** can also cause sulphide **spoilage of fuels**. The fuel becomes **corrosive** and will **fail sulphide limit specifications**.

Although early microbiologists thought that sulfate reducing bacteria (SRB) were the only microbes involved in microbial influenced corrosion (MIC), we now know that, while SRB play a role in MIC, they are not unique. Non-SRB (such as acid-producing, iron-reducing, and slime-forming bacteria) contribute to MIC in a variety of ways. The two most common are the production of biofilm (causing chemical and electro-potential gradients between surfaces that are covered with biofilm and those that aren't), and production of weak organic acids which react with chloride, sulfate, nitrate and nitrite ions to form organic salts and strong inorganic acids (hydrochloric, sulfuric, nitric and nitrous acids) within biofilms; particularly within MIC pits.

- ✓ **Microbial surfactants** can stimulate the suspension of water in fuel causing it to become **hazy** and **causing failure of fuel water separators**
- ✓ When the tank contents are disturbed, for example when the tank is refilled, the **microbes become suspended** in the bulk fuel where they cause **fouling** and may be passed on down the distribution chain to **contaminate facilities downstream (motors...)**.



**Diesel-Bacteria
growing on the
bottom of tanks**



The microbes tend to be most **active at the interface between water and fuel** and also on internal tank and system surfaces. Microorganisms, such as bacteria, virus, fungi and algae, degrade oil products. Greyish, slimy compounds are formed in the oil, and these are gradually forming a slimy layer of mud covering tanks and piping, **clogging pipes and filters that are harmful for engines**. The costs to disinfect equipments are very high and can be estimated to several **millions of Euros**, for example:

- ✓ In 2000, the entire Australian private aviation industry was closed down with some estimating the costs at US\$180 million (€125million) because of a microbial contamination
- ✓ The most recent case where fuel contamination contributed to a major disaster was in the floods caused by Hurricane Katrina in the US in 2005. Several emergency generators systems failed due to contamination problems.

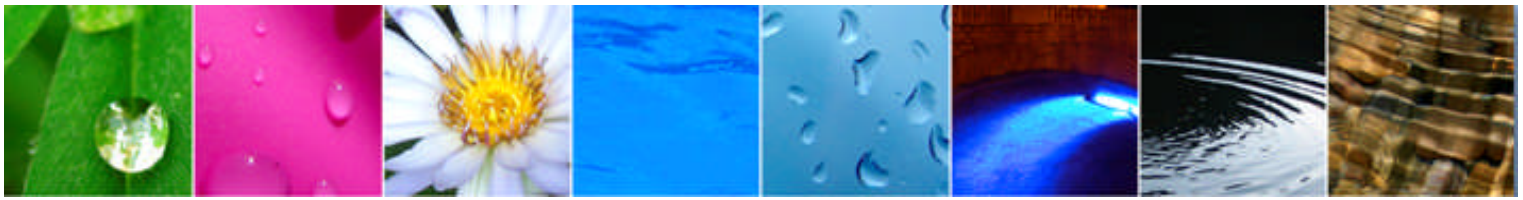
The difficulty of microbial quantification in the oil industry: fuel tank, pipe line, filters, injectors..., resides in the fact that they are very **charged in organic compounds**, which is an obstacle in the follow-up of the biological activity. **No powerful microbiological tool** was n available for **following the activity and controlling the proliferation of microorganisms**.

The **best solution** for **an oil industrialist would** be thus:

- **Fast and anticipated detection of microbiological drifts**
- **Proactive** rather than **corrective** actions and treatments

Today, a fast detection is possible via the ATP-metry QGO-M™ kit, developed by the company **LuminUltra™** (Canada) and marketed by **Aqua-tools** in Europe.

The QGO-M™ kit is intended to any type of **organic fluids**, including conventional **gasoline, diesel and jet fuel, plus ethanol/gasoline blends, biodiesel, lubrication oils, hydraulic fluids, pipeline streams, and metalworking fluids...**



I. ATP-metry technology

1. Short introduction to APT-metry

The 2nd generation of ATP-metry kits developed by LuminUltra™ (Canada), allow measuring the concentration of living biomass through the quantification of intracellular Adenosine TriPhosphate. The **intracellular ATP** is the energy stored in any living cell, which is degraded in few minutes, when it is released in the environment. By the measurement of the intracellular ATP, contained in the samples, **we can know, in less than 10 minutes, the total load of living microorganism**, called **active biomass**. Today, this technique is the best **indicator of total viable flora, allowing without interference the fast measurement of active biomass in the oil products.**

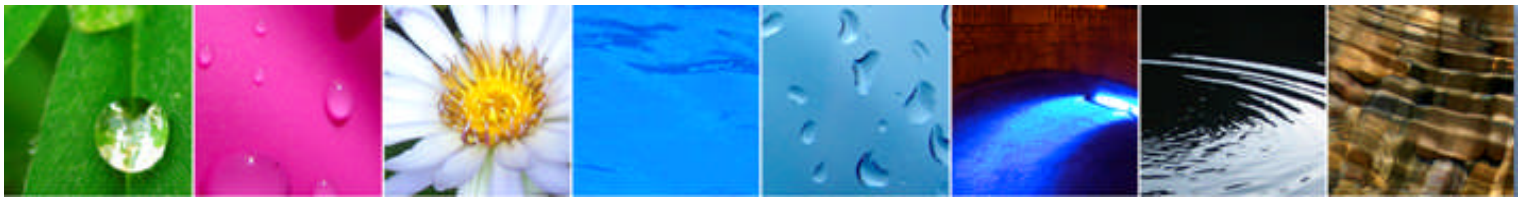
2. Principle of the technique of ATP-metry

The technique of ATP-metry allows a fast measure of the concentration of **active biomass in water**. This method is based on the quantification of Adenosine Triphosphate (ATP), which is an essential molecule in the microbial life.

ATP molecule can be found in all living organisms and is indicative of their metabolic activity and the viability of the biomass present in environmental samples. The ATP is a carrier of energy situated inside the living cells. ATP manages all the biological functions, such as nutrition, maintenance and reproduction.

The principle of ATP measurement is based on a bioluminescence technique, in which one photon of light is produced in the enzymatic reaction between Luciferase enzyme and an ATP molecule. Intensity of light is measured with **a luminometer**. The quantity of produced light is directly proportional to the active biomass present in the sample. ATP measurement is directly proportional to living microbial flora: it allows quantifying rapidly the concentration, health and activity of all microorganisms contained in water or sludge sample, used for the follow up of the functioning of the biological waste water treatment plants (WWTP).





II. Presentation of QGO-M™ kit

1. Content of the kit

Quench-Gone Organic-Modified™ (QGO-M)™ is based on the measure of ATP and is intended for the samples containing organic compounds like the fluids in oil, metallurgical or chemical industry.

Included in QGO-M Test Kits
Luminase (Luciferase Enzyme Reagent)
UltraCheck 1 (1 ng/mL ATP Standard)
LumiClean (Filter Wash Reagent)
UltraLyse 7 (ATP Extraction Reagent)
UltraLute (ATP Dilution Buffer)

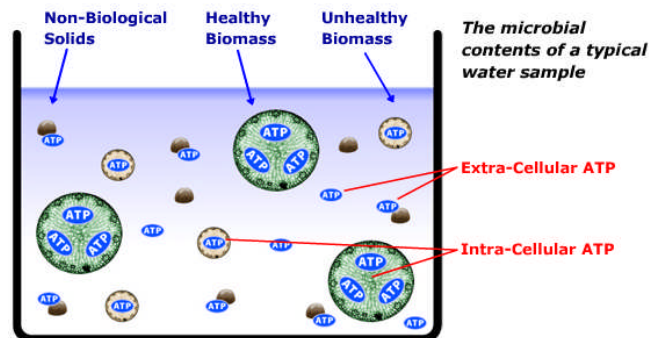
QGO-M™ measures the total quantity of microbiological activity through a single analysis within minutes. Compared to other methods measuring total microbial load (also known as total revivable flora), QGO-M provides immediate feedback, is not prone to interferences, and measures the total living population. Use the real time knowledge gained through QGO-M analyses to assess microbiological contamination and prevent process deterioration, optimize maintenance, and troubleshoot disinfection programs. **QGO-M™ is optimized to detect between 4 and 2×10^6 pg.**

The kit Quench-Gone Organic-Modified™ (QGO-M™) contains all the reagents necessary to carry out 100 analysis.

2. Measured parameters

Within a sample of water containing microorganisms, the kit QGO-M™ gives an instantaneous measurement of the concentration of living biomass which is represented by the following parameter:

- **Intracellular ATP (cATP)** that is the ATP contained in the living biological cells. cATP is representative of the concentration of living microorganisms in the sample.

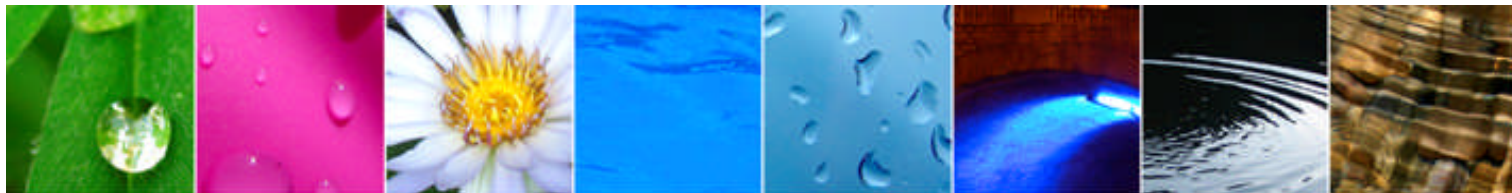


Intracellular ATP (cATP) = Measurement of living biomass in any sample

In order to make available the cATP to the bioluminescence reaction, the sample of water is subjected to bacterial lyses, which makes it possible, by destroying the cellular membranes and releasing the cATP

In the range of the kits QGO-M™, the measurement gives a **direct and only** access to the value of **cATP™**, thanks to a preliminary filtration of the sample.

The monitoring of the Intracellular ATP makes possible to quantify in real time living active biomass and to follow its growth under optimal conditions or to detect early microbial proliferation drifts, responsible for corrosion and biofouling.



III. Case studies with the Quench Gone Organic-Modified™ kit

To realize the effectiveness of these follow-ups by ATP-métrie, we present here three [studies realized in oil industry](#) by user of our ATP-metry technique for the monitoring of various sectors (upstream, downstream and midstream).

1. Use of QGO-M™ kit for follow-up of samples in the Upstream's field



This first study was realised at the stage of crude oil production. Microbes indigenous to underground wells can build up and corrode pipes and equipment. Waters used for common maintenance activities such as well fracturing can introduce microbes into upstream infrastructure.

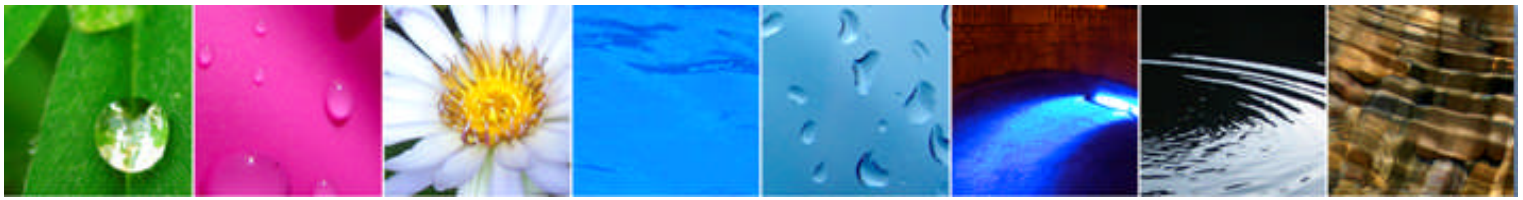
The QGO-M™ measurement shows good reproducibility when measured on bacteria in Upstream field. Table 1 summarizes duplicate results for 4 samples and presents the calculation

of the Coefficient of Variation (CV), which is a measure of the variability between replicate measurements. Low CV represents superior reproducibility.

Table 1 Samples of oil bacteria in water on Upstream

Sample	[ATP] Rep 1 (pg ATP/mL)	[ATP] Rep 2 (pg ATP/mL)	Average [ATP] (pg ATP/mL)	[ATP] Standard Deviation	[ATP] Coefficient of variation
#1	14673	16066	15370	985	6%
#2	3165	3100	3133	46	1%
#3	524	473	498	36	7%
#4	146	140	143	4	3%

In general, the Coefficient of Variation is considered to be satisfying in the range of 10 to 25%. For all samples, reproducibility was within this range, **indicating good performance in the Upstream applications.**

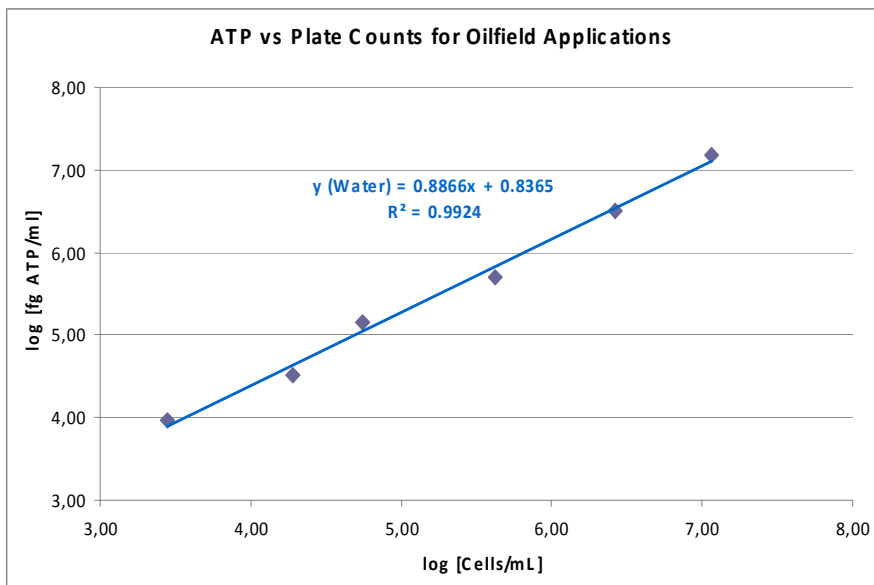


The standard for measurement of microorganisms in petroleum industry is the culture test. The disadvantage of this method is that it quantifies only viable and cultivable bacteria contrary to the **QGO-M™** method **which quantifies all the bacteria: cultivable or not cultivable**. Moreover, conventional microbial tests require organisms to be cultured and later counted, which takes days (2 or 3 days) and are longer when temperatures are colder or bacteria grow slowly (i.e. tests for sulfate reducing bacteria can require up to 21 days). ATP-métrie provides an immediate answer in a few minutes.

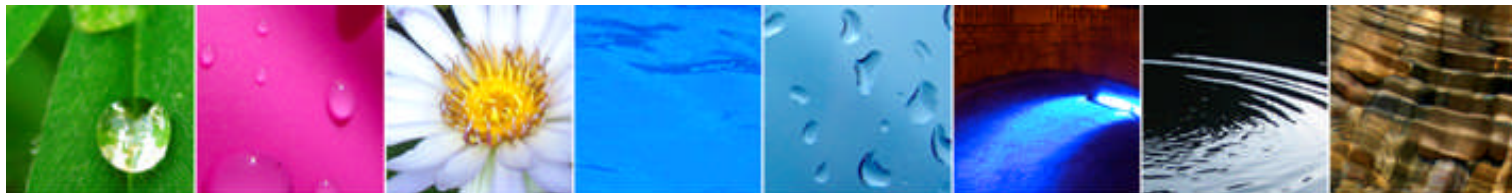
Table 2 summarizes the results of three different microbial monitoring systems for the same samples as above. QGO-M is reported as concentration of pg ATP/mL and converted in equivalent microorganism/mL (**1pg/mL is equivalent to 1000 bacteria**). Culture tests are reported as CFU/mL. All numbers are reported as the average of duplicate analyses

Table 2: Comparison of Microbial Measurement Methods in Upstream

Sample	Kit QGO-M™ (pg/mL)	Kit QGO-M™ (Equivalent microorganismes/mL)	3M Petrifilm™ Méthode de culture (CFU/L)
#1	15370	1.5 x10 ⁷	1.16 x10 ⁷
#2	3133	3.1 x10 ⁶	2.64 x10 ⁶
#3	498	4.9 x10 ⁵	4.2 x10 ⁵
#4	143	1.4 x10 ⁵	5.6 x10 ⁴



QGO-M™ is a suitable method for a real quantification of the viable cultivable or not cultivable microorganisms. According to the results, a good relationship between QGO-M™ and the culture test exists. In this following figure, these results are presented using a linear fit on a log-log plot.



2. Use of QGO-M™ Kit for the follow-up of samples in the Midstream field



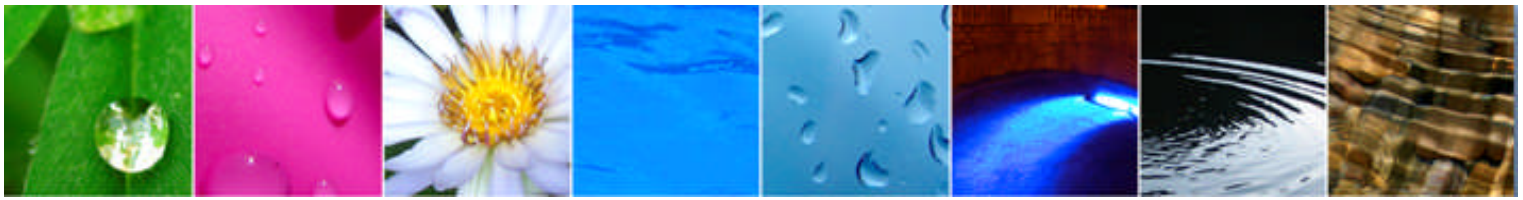
This second study was carried out by the same company during a regular follow-up on the second sector of his activity, this time on the **midstream application**. Measurements are carried out in the **pipes line bringing back the crude oil towards the refineries**.

The measurements taken with the QGO-M™ kit show an excellent reproducibility on the oil samples in the pipes line. **Table 3** summarizes duplicate results for 4 samples and presents the calculation of the Coefficient of Variation (CV), which is a measure of the variability between replicate measurements.

Table 3: Samples of bacteria in the pipes line in midstream field

Sample	[ATP] Rep 1 (pg ATP/mL)	[ATP] Rep 2 (pg ATP/mL)	Average [ATP] (pg ATP/mL)	[ATP] Standard Deviation	[ATP] Coefficient of variation
#1	24765	22697	23731	1462	6%
#2	10457	8837	9647	1146	12%
#3	2290	1875	2083	294	14%
#4	732	853	793	85	11%

Once more the coefficient of variation is between 10 and 25%, this is an **excellent performance** for a biological method, knowing that is a **different branch of the oil activity**.

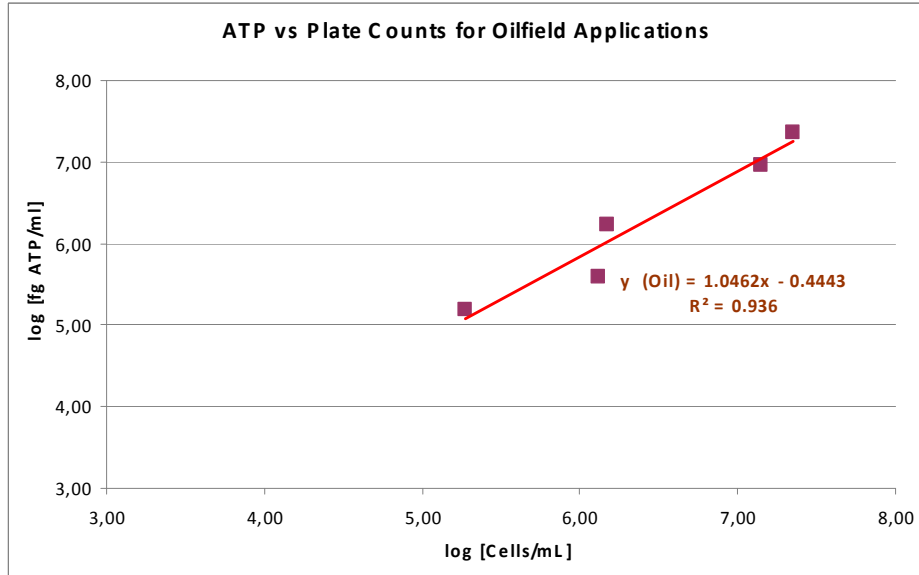


The standard for measurement of microorganisms in petroleum industry, even in the Midstream activities, is the culture test. As we known on the last study, this **method quantify only the viable and cultivable bacteria** contrary to the **QGO-M™** method **which quantifies all the bacteria: cultivable and not cultivable**.

Table 4 summarizes the results of 4 different microbial monitoring systems for the same samples above. QGO-M is reported as concentration of pg ATP/mL and converted in Equivalent Microorganisms/mL (**1pg/mL is equivalent to 1000 bacteria**). Culture test results are reported as CFU/mL. All numbers are reported as the average of duplicate analyses.

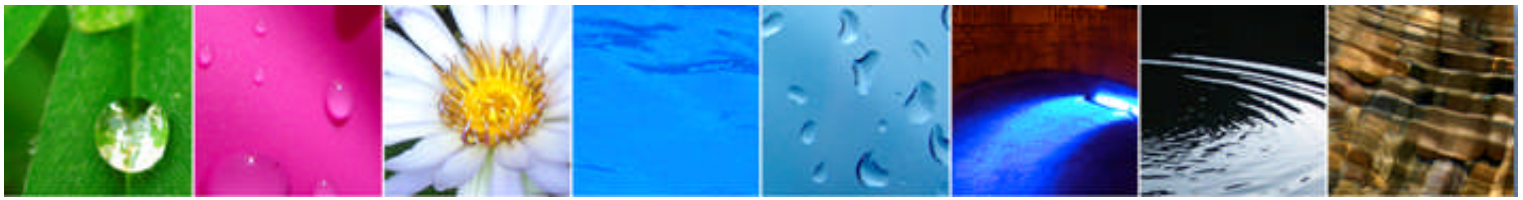
Table 4: Comparison of Microbial Measurement Methods in Midstream

Sample	Kit QGO-M™ (pg/mL)	Kit QGO-M™ (Equivalent microorganisms/mL)	3M Petrifilm™ Plate count (CFU/L)
#1	23731	2.3 x10 ⁷	2.25 x10 ⁷
#2	9647	9.6 x10 ⁶	1.39 x10 ⁷
#3	2083	2.1 x10 ⁴	1.47 x10 ⁶
#4	793	7.9 x10 ⁵	1.30 x10 ⁶



We can observe a **good correlation** between results obtained with the kit **QGO-M™** and the **culture method**. There appears to be a good relationship between QGO-M™ and the culture test when we are in a log-log plot. According to these results, QGO-M™ Kit seems to be one **suitable method for a real quantification of microorganisms concentration** also in a Midstream branch, towards the refineries.

more especially in the pipe lines bringing the crude oil



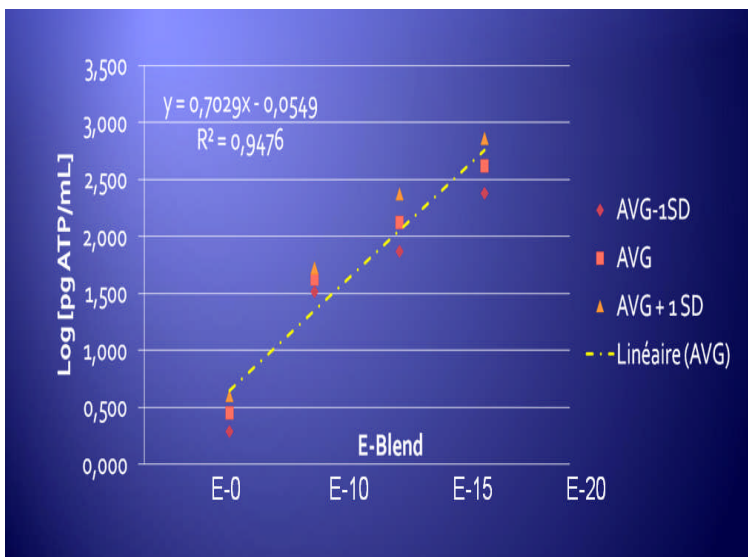
3. Use of Kit QGO-M™ for the follow-up of samples in Downstream field



This last study was carried out by our customer to validate the **repeatability and the reproducibility of ATP-metry measurements** via QGO-M™ kit to follow the **microbial contamination in finished products like fuel** containing a layer of water. Proliferations of micro-organisms in the finished products like diesel or fuel **can contaminate the equipment on downstream** (cars and planes engine...) and cause their **breakdowns**. The analyses are carried out with various factors of dilution.

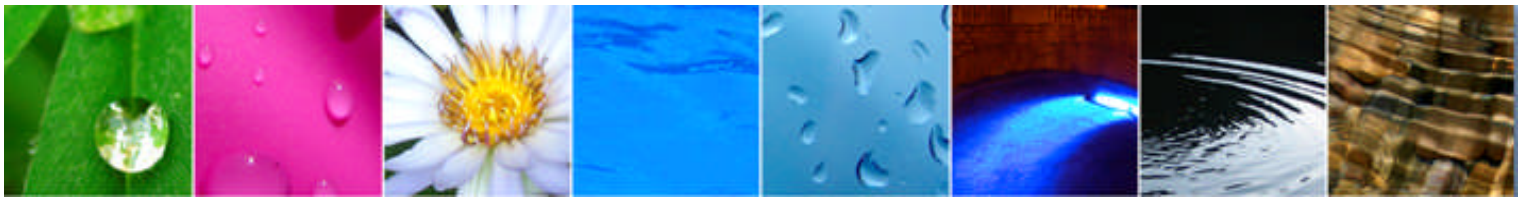
Table 5 Samples of bacteria in the fuel in Downstream applications

Sample and dilution factor	[ATP] Rep 1 (pg ATP/mL)	[ATP] Rep 2 (pg ATP/mL)	[ATP] Rep 3 (pg ATP/mL)	Average [ATP] (pg ATP/mL)	[ATP] Standard deviation	[ATP] Coefficient of variation
E-0	0.620	0.319	0.398	0.445	0.156	35.0%
E-10	1.699	1.661	1.501	1.620	0.105	6.5%
E-15	2.352	1.850	2.164	2.122	0.254	11.9%
E-20	2.598	2.875	2.391	2.621	0.243	9.3%



The QGO-M™ measurement shows **excellent reproducibility** when measured on diluted fuel samples. Table 5 summarizes duplicate results for 4 samples and presents the calculation of the Coefficient of Variation (CV), which is a measure of the variability between replicate measurements. A lower CV represents superior reproducibility. In general, the Coefficient of Variation is considered to be satisfactory in the range of 10 to 25%. For all but 1 sample, **reproducibility was within or below this range**. The only outlier was for a sample

with very low microbial loading. In spite of this value apart from the range, we find linearity between the averages of ATP values and the various factors of dilution.



Recommended thresholds

Upon examination of the QGO-M™ ATP and culture test results, it is apparent that there are some ranges of concentration for each parameter which equate to one another.

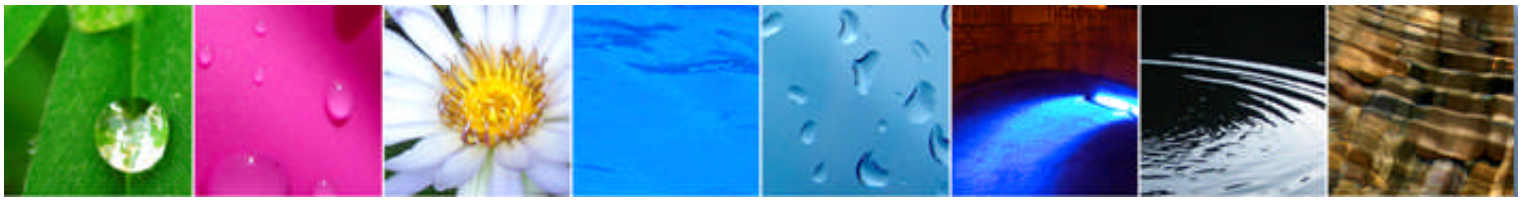
QGO-M™ ATP	3M Petrifilm™ Plate count Magnitude Range
0 à 50 pg ATP/ mL	0 à 10 ³ CFU/mL
50 à 250 pg ATP/ mL	10 ⁴ à 10 ⁵ CFU/mL
250 pg ATP/ mL et plus	10 ⁶ CFU/mL et plus

With a regular update of our customer's data, we can draw up a table of **recommended values of cATP** to follow up the **quality** of the oil in the each part of the petroleum industry (“**Upstream, Midstream and Downstream**”):

Process	Parameter	Good Control	Prevention Action Required	Corrective Action Required
Metalworking emulsions, oil tank, bottom water, Fire-retardant, Lubrication Fluids	cATP(pg/mL)	<1000	1000 à 20000	> 20000
Upstream and Downstream Applications	cATP(pg/mL)	<5	5 à 50	> 100
Conventional Lubrication Fluids, gasoil, jet A1... (« Midstream)	cATP(pg/mL)	<1	1 à 20	> 20

The general process that our customers follow is as follows:

1. Test your sample for ATP.
2. If ATP is low, the installation is secure.
3. If ATP is high, prepare SBR dilution bottles PLUS take corrective action immediately to solve the microbial problem.



IV. The advantage of QGO-M™ kit

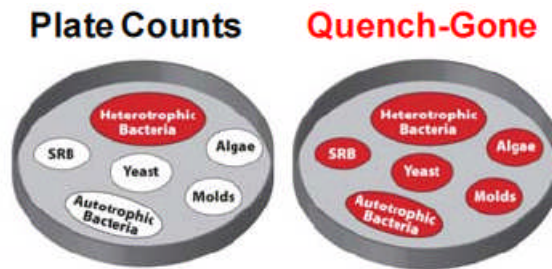
Aqua-tools's 2nd Generation ATP monitoring test kits can be used in all oil production operations where culture techniques have traditionally been used. Furthermore, obtaining fast, accurate feedback helps to **guide biocide dosing programs and ensures** that problems are known as quickly as possible. This ultimately eliminates delays in addressing bacteria issues and thus prevents from equipment deterioration and chemical consumption in all the segments of oil industry:

- ✓ **Upstream:** extraction, exploration of the crude oil, pipe line, contamination of water...
- ✓ **Downstream :** pipe line towards the refineries, production of gasoil, lubricants, kerosene, hydraulic liquids ...
- ✓ **Midstream:** pipe line, reserve, tank of storage, gasoline distributors...

QGO-M™ kit has the advantage to be more sensitive and in the same time providing an answer in few minutes whereas several days are necessary for the culture test. QGO-M™ kit gives multiple possibilities of uses for the petroleum industry:

- **Prevent** from microbial Corrosion
- **Prevent** from material **breakdowns**
- **Measure in real time** the concentration of microorganisms in the samples and give an immediate indication on contamination levels.
- **Carry out in real time quality controls, in the Fuel, in the lines of Lubricant, in the reserves and in the distribution network...**
- **Control the microbial growth** in water, in biofilm and deposits
- **Control the microbial contamination from the raw material (crude oil) to the finished product (fuel...)**
- **Early detect** the degradation of oil storage and transport facilities. Localize critical zones for bacteria proliferation in production, distribution and storage systems.
- **Validate the effectiveness of the treatments** on cultivable and no cultivable microorganisms
- **Optimize** the concentration, the frequency, and injection points of biocides, **and follow-up treatment effectiveness in water and oil phase**
- **Identify the origins of microbial contamination** - raw materials (crude oil, fuel...) ; distribution and production equipments (extraction and distribution pipes)
- **Optimize controls:** reduce culture test analysis, with long time to result. Check relevance of cleaning action on the biofilm (2 to 3 times per year)

- **Control effectiveness of treatments** implemented on offshore platform or in distribution networks
- **Correct and validate** cleaning and disinfection actions related to the microbial contamination
- **Quantify** all the living organisms (heterotrophic and autotrophic bacteria, algae, sulfato-reducing bacteria, yeasts, moulds), whereas with regulatory method by culture, only a small percentage (2%) of bacteria able to form colonies are detected (heterotrophic bacteria).



Measurement	Traditional Total Counts	LuminUltra Quench-Gone
Speed	Days	Minutes
Portability	Not portable	Portable
Accuracy	Counts only microbial 'particles' regardless of size	Counts all cells individually
Specificity	Detects only organisms that can grow on media	Detects all organisms


Microbiological threats are best addressed in their early stages of growth. The real-time feedback and full portability of test kits allows operators to:

- **Identify and locate the critical points** on the ground by biological mapping in order to address and validate threats during the same shift
- **Validate the effectiveness of the treatments.**

The kit provides you with an affordable complement to your existing microbiological measurement programs that helps you save **time & money and reduce risk.**

The new generation of ATP-metry kits commercialized by Aqua-tools is based on bioluminescence and allows measurements in less than 10 minutes on the field for a higher reactivity for implementing preventive and corrective action.

The kits are implemented in the following types of installations:

- Cooling towers
- Sanitary water
- Production & distribution of potable water
- Process water
- Biofilm
- Pure and ultra-pure water
- Metalworking fluids
- Oil & Gaz
- Cataphoresis baths
- Surface treatment
- Activated sludge in water treatment bioreactors 
- Reuse water
- Other...