White Paper

THORNTON
Leading Pure Water Analytics

Don't Miss a Bug
with Real-Time Microbial Monitoring

Measuring the bioburden in Purified Water and Water for Injection has almost exclusively depended on time-consuming and error-prone culture-based lab measurements. Recent developments in spectroscopic technology now offer accurate, continuous, on-line determination of microbial and inert particle contamination in pharmaceutical water systems.

Introduction

Increasing competition, expiring patents and changes in regulations are placing ever-growing pressures on pharmaceutical companies. These stresses are resulting in an increasing focus, from regulators to drug manufacturers, on how modern production methodologies can be applied in the pharmaceutical industry that will enable greater efficiencies alongside increased product safety.

The FDA's Process Analytical Technology (PAT) initiative, EMA Guidance on Real Time Release, ICH Guidelines Q 8 – 10, etc. all offer opportunities to increase production efficiency for even the most conservative drug companies. It is not surprising then, that there has been growing adoption of continuous analytical technologies that can rapidly identify out-of-specification conditions and lessen the workload burden on laboratories.

The requirement for real-time microbial analysis

Maintaining the quality of Purified Water and Water for Injection is vital in the pharmaceutical industry. Here, on-line analytics plays a major role in real-time monitoring of water conductivity and total organic carbon (TOC). However, due to a lack of such instrumentation for the measurement of microbial contamination, this vital measurement has been dominated by laboratory culture-based methods. This situation causes great frustration as on-line conductivity and TOC sensors allow real-time release of pharmaceutical waters, yet any bioburden excursions may not be identified for many days.

Further, manual sampling of a Pharmaceutical water distribution loop or multiple points-of-use to conduct traditional bacterial culture tests can result in a high percentage of false-positive results.

METTLER TOLEDO

The investigation of false-positives is time consuming and expensive, with some industry estimates putting the cost per event between USD 5,000 to 18,000.

What is more, due to the high number of POUs in a production facility, a single point may only be tested a few times in a month. This can make identification and remediation of local microbial issues very challenging, and is compounded by the fact that when a sample is collected for testing, it represents only a small volume of the water system or point-of-use at that specific time.

The pharmaceutical industry welcomes the advances in technology that have led in recent years to the development of microbial analyzers that significantly reduce the need for culture-based methods. Measurement techniques such as polymerase chain reaction (PCR) amplification are significantly faster than traditional lab methods; however, they require the use of dyes and reagents to treat water samples and cannot be used on-line.

Industry workgroup supports on-line analyzers

In 2011, the FDA published their "Advancing Regulatory Science at FDA" document in which they support the need to "Develop sensitive, rapid, high-throughput methods to detect, identify, and enumerate microbial contaminants and validate their utility in assessing product sterility." [1]

In recognition of this and the pharmaceutical industry's requirement for on-line microbial measurement instruments; a group of seven leading pharmaceutical companies formed the Online Water Bioburden Analyzer (OWBA) workgroup in 2013. Their objective is to provide guidance to instrument manufacturers regarding the development of new microbial measurement systems in order that such equipment satisfies the needs of industry and regulators.

"The development and implementation of an on-line water bioburden analyzer (OWBA) offers the potential to improve pharmaceutical water system operations, reduce costs, and ensure water quality." [2]

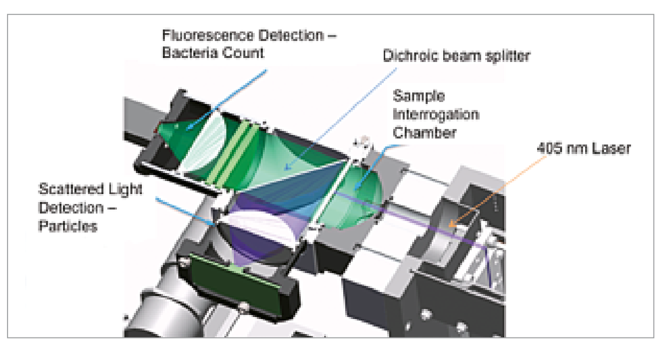
The OWBA believes that pharmaceutical companies can benefit from an on-line real time microbial detection system in a number of ways, including:

1. Lower costs through a reduction in labor due to reduced sampling, and reduced conventional testing and materials.
2. Fewer investigations of, and improved responsiveness to, microbial excursions.
3. Greater process understanding and product safety through real-time monitoring.
4. Real-time release of ingredient water, product intermediates, and process buffers / solutions.
5. Less frequent heat sanitization cycles through verification of system capability.

The workgroup has also issued technical system requirements, which include specifications for instrument sensitivity, and a limit of detection that equals that for culture-based methods (10 CFU / 100 mL, the limit for Water for Injection and 100 CFU / mL for Purified Water).

Cutting-edge spectroscopic technology

Laser-induced fluorescence (LIF) is a bioburden measurement technique that can meet industry needs. All microorganisms use metabolites (e.g., NADH, riboflavin) to regulate their growth and development. These metabolites produce intrinsic fluorescence emissions when exposed to light of certain wavelengths. LIF is a highly sensitive technique that exploits this phenomenon to detect microbes. Air analyzers using LIF have been available for some years, and advances in the technology means that it is now possible to use LIF to measure microbial levels in water.



Analysis of fluorescence and scattered light distinguishes microorganisms from inert material.

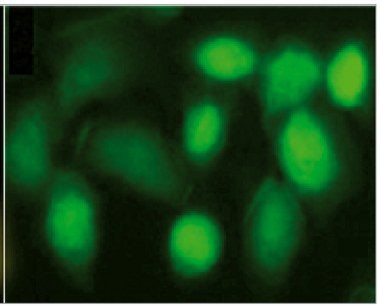
Real-time bacterial detection analyzer

METTLER TOLEDO Thornton's 7000RMS is an on-line analyzer for real-time, continuous measurement of bioburden in pharmaceutical waters. It uses LIF to instantly measure microbial contamination without any requirement for consumables such as stains or reagents. Further, the 7000RMS also measures inert particles that can come from sources such as diaphragms, filters and O-Rings.

A sample stream from the water supply is connected to the analyzer's flow cell. A 405 nm laser is directed through the sample and induces fluorescence in the metabolites present in the microbes. Any emitted fluorescence is detected by a photomultiplier tube. The quantity of particles in the water is determined by another sensor via Mie scattering. Data from the two detectors is processed using advanced, proprietary algorithms.

Most inert materials do not fluoresce, but some including certain polymers do, therefore there is a risk that such particles can be counted as biological. By analyzing both the emitted fluorescence and the scattered light, the algorithms in the 7000RMS are able to accurately distinguish microbes from non-microbial particles.

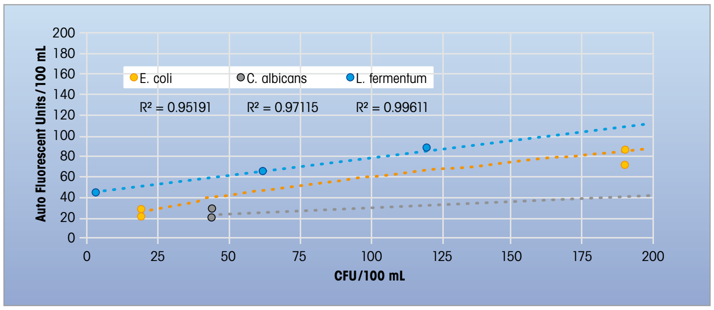
Inert



Biological

The analyzer's touchscreen interface displays the readings for microbes in bio-counts (one bio-count approximates one Colony Forming Unit (CFU)) and the number of inert particles. Alarms can be set for alert, action and specification limits. The 7000RMS measures particles between 0.52 µm and 50 µm and accepts sample temperatures of up to 90 °C. It offers SCADA connectivity with ModBus TCP, multiple analog outputs and is Wi-Fi capable.

Accuracy of the analyzer is very high. Testing the 7000RMS against a traditional plate count method shows a correlation of $R_2 > 0.9$, as shown in the graph below.



Correlation graph of plate count (CFUs) and 7000RMS (Auto Fluorescent Units)

Lower risk, greater process control

"USP < 1231 > Water for Pharmaceutical Purposes" recommends that pharmaceutical water systems should be monitored at a frequency that ensures the system is in control and continues to produce water of acceptable quality. The general information chapter endorses operating monitoring instruments continuously in order that historical in-process data can be recorded for examination. Over time, trend analysis can be used as a basis for conducting loop maintenance.

Real-time and historical data from the 7000RMS enables rapid identification of deteriorating or improving microbiological control. Use of 7000RMS analyzers at various POUs or distribution sampling points will help pinpoint the source of any problems and provide rapid assurance of successful remediation.

The 7000RMS enables risk reduction and greater process control, and offers significant costs savings from the combined decrease in laboratory testing and false-positive results. A facility operating eight water systems and ten 7000RMS analyzers can more than recoup the investment cost in less than a year.

Conclusions

Initiatives such as PAT and QBD, and the pharmaceutical industry's recognition of a need for increased, real-time monitoring of pure pharmaceutical waters has led to instrumentation that allows life science companies to rely less on time-consuming, culture-based lab measurements of bacteria.

Rapid microbiological methods hold the potential to accelerate and even improve microbe quality control of Pharmaceutical Water Systems. The improved testing and speed of response will allow pharmaceutical products to reach the market faster and improve the understanding of the water process. Rapid microbiology detection will permit timely and effective investigations of any microbial event and enable corrective actions to be taken swiftly.

The advanced LIF technology employed in METTLER TOLEDO Thornton's 7000RMS analyzer provides continuous and accurate data on bioburden and inert particle contamination throughout water systems. Assurance of in-specification microbial levels and real-time identification of excursions leads to improved product quality, greater process understanding, risk reduction and lower operating costs.

1. "Advancing Regulatory Science at FDA – A Strategic Plan: August 2011" www.fda.gov/regulatoryscience

2. "Novel Concept for Online Water Bioburden Analysis: Key Considerations, Applications, and Business Benefits for Microbiological Risk Reduction" American Pharmaceutical Review, May/June 2013.

► **www.mt.com/7000RMS**

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