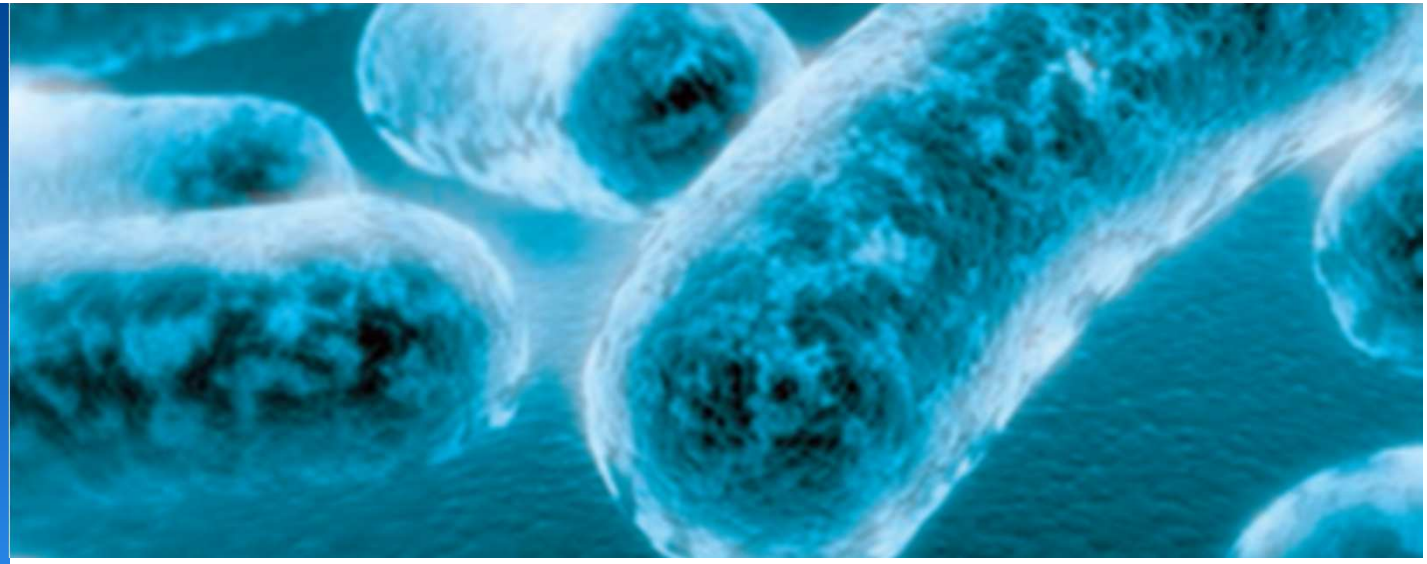
Melbourne
July 2016/R. Strebel



Rapid Microbial Measurement

The 2016 GMP & Validation Forum

METTLER TOLEDO

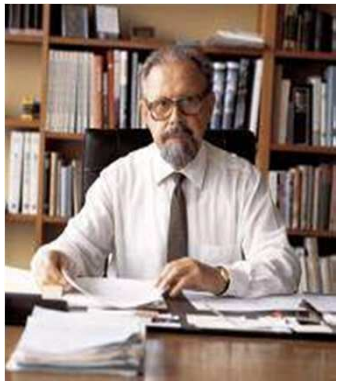
Agenda

1. Introduction
2. Short History of Water Analysis
3. Discussion on Alternative Methods
4. Correlation between plate count and alternative methods
5. Conclusion

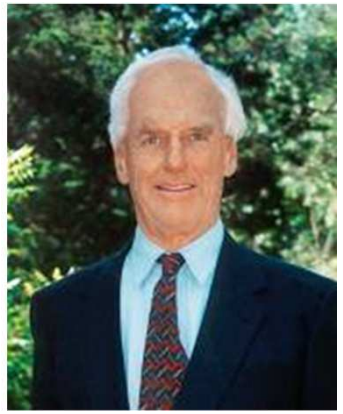
Company Background

60+ years of Process Analytical Expertise

- Ingold founded in 1948, acquired by Mettler Toledo in 1986
- Thornton founded in 1964, acquired by Mettler Toledo in 2001
- Sensor/Analyzer manufacturer for pH/ORP, Resistivity/Conductivity, TOC, DO and water quality with focus on:
  - Pharmaceutical and Biotech
  - Chemical
  - Microelectronics
  - Power
- Extensive research
  - Chemical and physical properties
  - New technologies
  - Product handling and asset management
- Active participation in ASTM, ISPE, PDA, Semi, EPRI, USP



Dr. Werner Ingold



Dr. Richard Thornton

Abstract

WHO Technical Report Series No. 970, 2012 states

- *"Water is the most widely used substance, raw material or starting material in the production, processing and formulation of pharmaceutical products. It has unique chemical properties due to its polarity and hydrogen bonds."*
- *"Control of the quality of water throughout the production, storage and distribution processes, including microbiological and chemical quality, is a major concern. Unlike other product and process ingredients, water is usually drawn from a system on demand, and is not subject to testing and batch or lot release before use. Assurance of quality to meet the on-demand expectation is, therefore, essential. Additionally, certain microbiological tests may require periods of incubation and, therefore, the results are likely to lag behind the water use."*

Consequently:

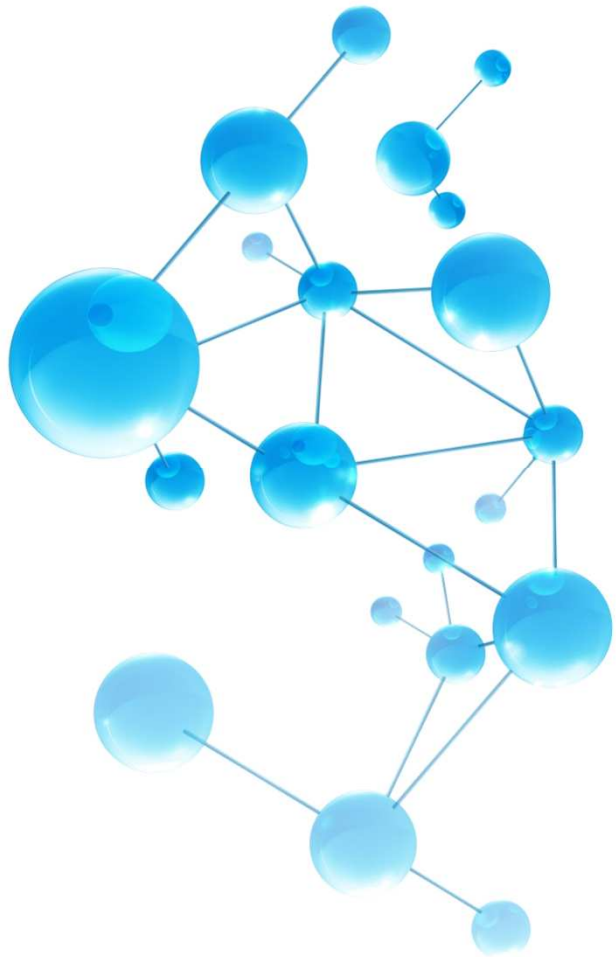
- Real-time measurement is replacing time consuming grab sample testing. As process control becomes more important and available technologies more sophisticated, Pharmacopoeias around the world have accepted real-time measurement to replace traditional, well established wet chemistry testing. Today, **Conductivity** and **TOC** are the two primary analysis methods to detect chemical and organic impurities in high purity pharmaceutical waters.

Agenda

Importance of Water

Water is multi-functional, it is used as:

- Raw material
- Solvent
- Ingredient
- Reagent
- Cleaning agent (hot water or steam)
- Sterile waters
  - Sterile water for irrigation
  - Sterile water for inhalation
  - Sterile purified water
  - Bacteriostatic water for injection
  - Water for hemodialysis
- Packaged waters
  - Purified water for small volume use



Water for Pharmaceutical purposes

<1231> reads:

- *"The chemical attributes of **Purified Water** and **Water for Injection** in effect prior to **USP 23** were specified by a series of chemistry tests for various specific and nonspecific attributes with the intent of detecting chemical species indicative of incomplete or inadequate purification. While these methods could have been considered barely adequate to control the quality of these waters, they nevertheless stood the test of time. This was partly because the operation of water systems was, and still is, based on on-line conductivity measurements and specifications generally thought to preclude failure of these archaic chemistry attribute tests."*
- *"USP moved away from these chemical attribute tests to contemporary analytical technologies for the bulk waters **Purified Water** and **Water for Injection**. The intent was to upgrade the analytical technologies without tightening the quality requirements. The two contemporary analytical technologies employed were TOC and conductivity."*

- Fact:
  Water purity can not be tested directly; the purity of water can only be concluded by proving the absence of impurities, such as organic or inorganic contamination, which included a variety of tests .

Water Quality Attributes

WFI Attribute[1]	USP 38	EP 8.3	JP XVI
Production Method	Distillation or suitable process	Distillation	Distillation or RO with UF from PW
Source Water	US, EU, Japan, WHO drinking water	Human consumption	JP water specification
Total Aerobic (cfu/100 mL)[2]	10	10	10
Conductivity (µS/cm at 25° C)[3]	1.3 (3 stage)	Same as USP	Same as USP
TOC (mg/L)	0.5	0.5	0.3 on- / 0.4 off-line
Bacterial Endotoxins (EU/mL)	0.25	0.25	0.25
Nitrates (ppm)		0.2	
Heavy Metals (ppm)			
Acidity/Alkalinity			
Chloride			
Sulfate			
Nitrite			
Ammonium (mg/mL)			
Residue on Evaporation (mg)			
…etc.[4]			

Note 1: All tests are maximum, unless otherwise stated.

Note 2: Microbiological testing is considered to be harmonized, with the exception noted that the EP test is written into the Production section, and the USP test is contained in a non-compendial general information chapter <1231>

Note 3: Limits are temperature dependent

Note 4: above list of attributes is not complete

Agenda

1. Introduction
2. Short History of Water Analysis
3. Discussion on Alternative Methods
4. Correlation between plate count and alternative methods
5. Conclusion

Characteristics of Impurities in Water

USP, FDA, WHO are accepting/promoting real-time measurement

Types of Impurities	Characteristics	Types of Tests
Inorganic	ionic	conductivity
<USP 645>, since 1995, test of total ionic contamination; has replaced antiquated wet chemistry tests plus pH		
Organic (non-living)	non-ionic	TOC
<USP643>, since 1996, test of total organic contamination; has replaced test for oxidizable substances. TOC does not replace the need to microbial testing		
Microbiological	living, organic, non-ionic	Sterility
<USP1231> and <USP 1223> discuss the benefits of real-time bioburden measurement vs traditional plate count methods		
Microbiological	dead, organic, non-ionic	Bacterial Endotoxin Test
<USP 85>, lab test, off-line		

Regulated quality attributes by USP

USP 39 – new Chapter

The latest issue of USP supports real-time measurement

- USP <**1231**> Water for Pharmaceutical Purposes recommendation*:*
  - *"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
- The new USP <**1223**> promotes and encourages the validation and development of alternative technologies

Drivers for Alternative Methods

Industry experts and regulatory bodies acknowledge the benefits

OWBA Workgroup

- Online Water Bioburden Analysis Workgroup
  - Established as a workgroup within the industry to promote development and implementation of online bioburden analyzers
  - Members from Merck, Novartis, Amgen, Fresinius, Baxter, P&G, Roche, Sanofi and Pfizer

FDA

- FDA PAT (Process Analytical Technologies) Initiative
- FDA Aseptic Processing Guidance - cGMPs
- FDA Strategic Plan for Regulatory Science
- FDA Microbiologists Support RMMs

Traditional Microbial Detection

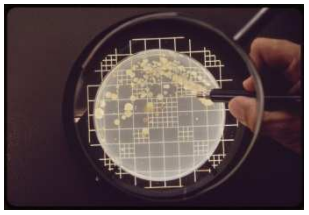
Microbial testing today essentially is still the same as in 1870's

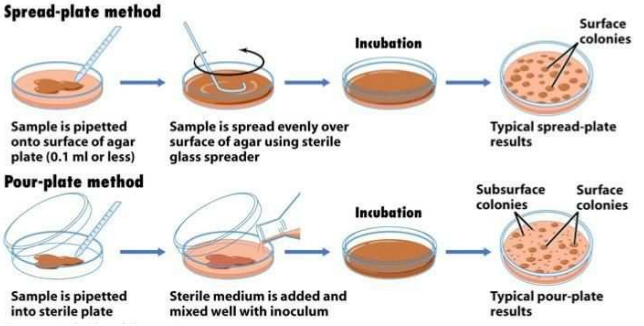
- A **colony-forming unit** (**CFU**) is a unit used to estimate the number of viable **bacteria** or **fungal** cells.
- Counting with CFU's requires culturing the microbes and counts only viable cells.
- Duration and temperature of incubation are also critical aspects of a microbiological test method.
- Classical methodologies, using high-nutrient media, are typically incubated at 30–35℃ for 48–72 hours.
- Because of the flora in certain water systems, incubation at lower temperatures (e.g., 20–25℃) for longer periods (e. g. 5–7 days) can recover higher microbial counts when compared to classical methods.
- Low-nutrient media are designed for these lower temperature and longer incubation conditions (sometimes as long as 14 and up to 21 days) to maximize recovery of very slow-growing oligotrophs or sanitizer-injured microorganisms.

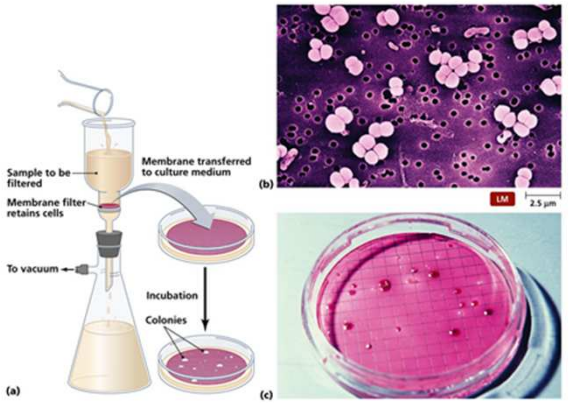
Manual work and visual inspection are potential sources of error

- The visual appearance of a colony in a cell culture requires significant growth. When counting colonies, it is uncertain if the colony arose from one cell or a group of cells.
- Plating and culturing bacteria can be done by a number of methods:
  - **The Pour Plate** method wherein the sample is suspended in a petri dish using molten agar cooled to approximately 40-45 ℃
  - **The Spread Plate** method wherein the sample (in a small volume) is spread across the surface of a nutrient agar plate and allowed to dry before incubation for counting.
  - **Membrane Filtration** wherein the sample is filtered through a membrane filter, then the filter placed on the surface of a nutrient agar

  All agar based methods request for an incubation period of a minimum of 5 to 7 days.



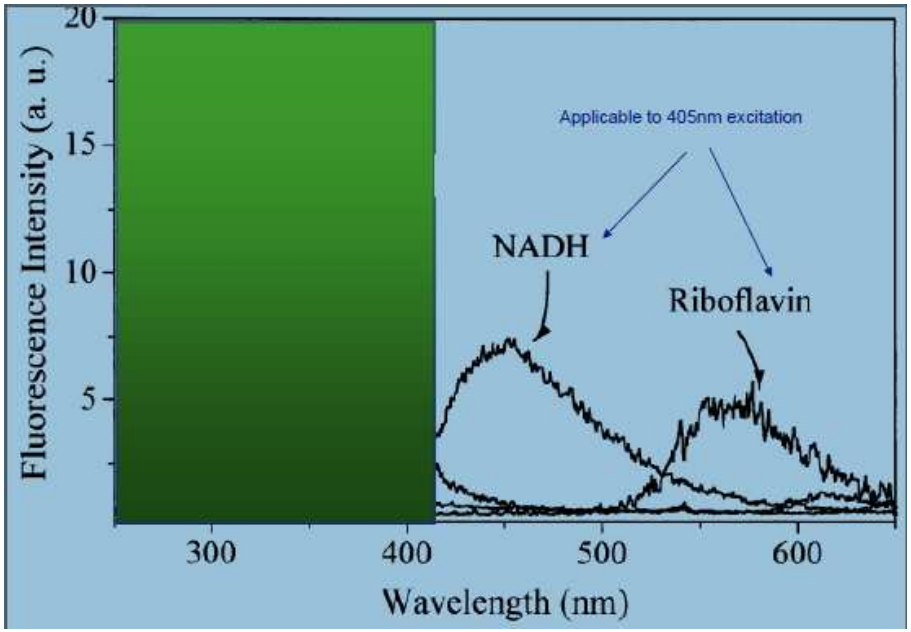




Alternative Method – Fluorescence

Light to detect microorganisms

- Microorganisms (bacteria, fungi, parasites, spores) use Metabolites (NADH, Riboflavin, & other proteins) to regulate their growth and development.
- These metabolites produce intrinsic fluorescence emissions when exposed to light of certain wavelengths.
- LIF (Laser-induced fluorescence) is a highly sensitive technique that exploits this phenomenon to detect microbes.

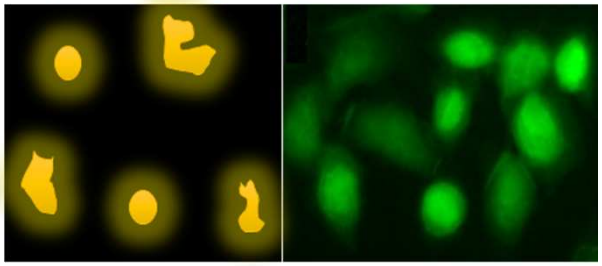


(Hill et al, Field Ana. Chem. & Tech, 3(4-5), 221, 1999)

Basic Principle of Operation

Combined Mie scattering and intrinsic fluorescence to detect bacteria

- Draw a sample through a flow cell into the interrogation zone
- UV laser light source is directed through the sample
- Scattered light is captured and collimated within a parabolic mirror
  - the scattering of light determines the size of the particle
- The intrinsic fluorescence is also captured
  - i.e. the amount of light emitted at a longer (than 405 nm) wavelength by a microorganism
- The two types of light (scattered/fluorescent) are separated and the data computed
- Software uses the combined data to differentiate and enumerate inert particles and biological cells
- When a particle is detected at PD and PMT at the same time (within x µsec), then this is a BIOCOUNT!



Inert Biological

Agenda

1. Introduction
2. Short History of Water Analysis
3. Discussion on Alternative Methods
4. Correlation between plate count and alternative methods
5. Conclusion

Plate Count vs. Rapid Microbial Systems 1/2

Why do I have higher counts online versus my plate counts?

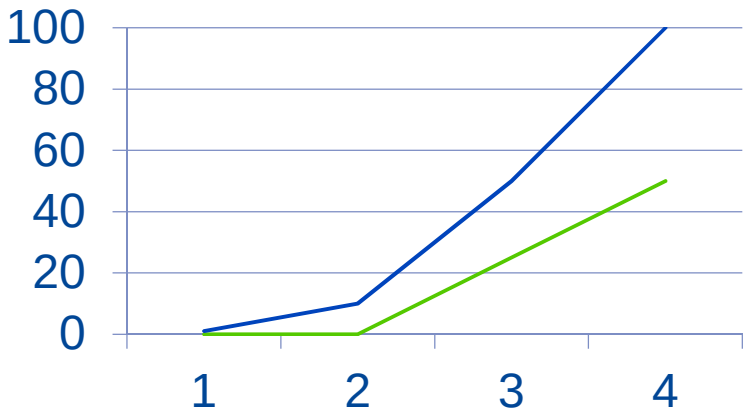
- USP <1223> "*It is important to understand that the cfu has always been an estimation of microorganisms present, rather than an actual count.*"
- "*Studies on the recovery of microorganisms have demonstrated that traditional plate-count methods reporting cell count estimates as colony-forming units (cfu) may recover 0.1%–1% of the actual microbial cells present in a sample.*"
- *"Most of the rapid microbiological methods are, to some extent, direct cell count methods. They, therefore, may provide a higher cell count estimate than the cfu method for a given sample."*
- *"Observations of cell counts that differ from cfu results are not a concern if the different methods and their different signals of cell presence are equivalent to or are non-inferior to referee methods in terms of assessing the microbiological safety of an article."*
- *"Higher cell counts must not be considered as necessarily indicative of greater risk given the inherent variability of standard growth methods."*

Plate Count vs. Rapid Microbial Systems 2/2

Why do I have higher counts online versus my plate counts?

- The CFU (Colony Formation Unit) has always been an estimation of microorganisms present, rather than an actual count – colony may be from 1 cell or 1,000 cells (plate count).
- Rapid microbiological methods are direct cell count methods, therefore a higher count is expected.
- The difference between the CFU from plate count and AFU direct cell count is smaller when the number of bacteria cells in the water sample is low. However, the difference is proportionally much higher when the number of bacteria cells in the sample water is high.

Comparison test per USP <1223>		
Bacteria cells/sample	Plate count (CFU)	Rapid Testing Biocount
1	0	1
10	0	10
50	25	50
100	50	100



Agenda

1. Introduction
2. Short History of Water Analysis
3. Discussion on Alternative Methods
4. Correlation between plate count and alternative methods
5. Conclusion

Summary and Q&A

Possible adaption of USP to the new technology

1890 - 2015

- Bacteria tests are:
  - Plate count
  - Qualitative
  - Subject to bias
  - Off-line
  - Affected by human error
  - Time consuming

2015 - 2016

- Current discussion concerning plate count vs on-line real-time instruments.
- USP<1223> now encourages validation of alternative microbial methods.
- PhEur. 5.1.6. "Alternative methods of control of microbiological quality".

2017?

- Accepted by USP as a compendial test.
- On-line real-time instruments are used to monitor the performance of water systems.

2018?

- Accepted by other Pharmacopoeias.