Exploring the Application of ATP Bioluminescence Method (Rapid Microbiological Method) to Microbial Enumeration Testing in Pharmaceutical Water Monitoring

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Introduction / Scope of Study

✓In microbial enumeration tests for water monitoring, the culture method is commonly used, which typically requires up to a week to obtain results. To enable prompt resumption of production in the event of system abnormalities and ensure timely water quality management, we explored the application of **ATP bioluminescence method (ATP method) with Rapica instrument** (HORIBA Advanced Techno, Co., Ltd.) as an alternative to the culture method, **offering results automatically within 2.5 hours.**

✓In this study, we **evaluated its equivalency compared to the culture method**. We also **propose examples of setting action level and alert level for ATP method** in pharmaceutical water.

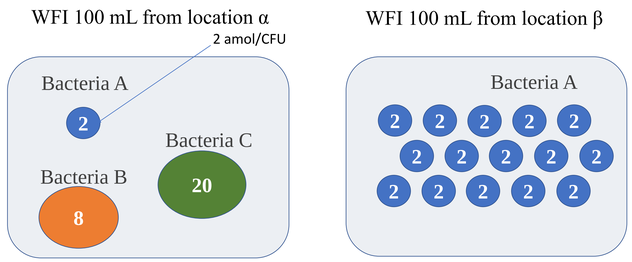
Background

Differences between culture method and ATP method

✓Culture method (units: CFU) and ATP method (units: amol) differ in their measurement units.

✓Because ATP varies among species and their physiological condition, **CFU counts may differ even at the same ATP level**.
(An example is shown below.)

✓Improperly setting the threshold values in ATP method may cause the risk of overlooking threshold exceedances in culture method.



How to apply ATP method to pharmaceutical water management?

To application of ATP method to microbial enumeration testing, our approaches are following:

1) We performed analytical validation of the ATP method.*
2) We proposed examples of setting action level and alert level for ATP method.

***Analytical method validation for ATP method has already been performed using an ATP standard solution according to** USP 1223 and ICH Q2(R1) (data not shown). Results of equivalency for ATP method and culture method are described in following sections.

The Evaluation of Equivalency

Materials and Methods

We evaluated Equivalency with reference to USP 1223 and PDA Technical report No.33.

Test condition	Acceptance Criteria
Test strains: See below. **Concentration:** 200, 100, 50, 25, 10, and 5 CFU/mL **Number of repetitions:** 6 Each sample was measured using both the culture method and the ATP method.	**Correlation:** Plot the measured values of the ATP method against of the culture method. The coefficient of determination (r^2) is ≥ 0.9025. **Precision:** The coefficient of variation (CV) for the ATP method at 100 CFU/mL and 50 CFU/mL should be less than 35% each.*

*If CV ≥ 35%, the upper limit of the 95% confidence interval for the ATP method ≤ the upper limit of the 95% confidence interval for the culture method +10%.

<Test strains and Culture conditions>

Test strain	Culture Medium	Culture condition	Notes
S.aureus, P.aeruginosa, E.coli, B.subtilis	PCA	30℃ – 35℃/48 hours to 72 hours	N/A
P.fluorescens, M.extorquens	R2A	20℃ – 25℃/4 days to 7 days	Starved
In-house isolate	R2A	30℃ – 35℃/4 days to 7 days	

Results

Test strains	Correlation ($r^2 \geq 0.9025$)	Precision (CV < 35%)						ATP amount per CFU (amol/CFU)*
		100 CFU			50 CFU			
	r^2	Mean (amol)	SD (amol)	CV (%)	Mean (amol)	SD (amol)	CV (%)	
S.aureus	**0.9750**	298.2	52.8	**18**	96.7	16.2	**17**	3.8
P.aeruginosa	**0.9662**	264.4	34.9	**13**	157.9	39.7	**25**	2.2
B.subtilis	**0.9279**	187.7	23.6	**13**	138.8	34.5	**25**	2.1
E.coli	**0.9661**	243.9	36.9	**15**	120.9	24.2	**20**	3.1
P.fluorescens	**0.9542**	68.1	12.6	**19**	32.5	4.8	**15**	1.0
M.extorquens	**0.9528**	82.5	4.6	**6**	25.6	6.9	**27**	0.9
In-house isolate	**0.9522**	80.3	9.4	**12**	40.2	4.4	**11**	0.6

* Calculated by using the measured value at 100 CFU/mL.

✓**All test strains met their acceptance criteria, which suggests that the ATP method is capable of obtaining results comparable to the culture method.**

✓**It has been confirmed that the ATP method also can detect starved microorganisms, which closely resembles the conditions in the actual pharmaceutical water system environment.**

✓**There is a possibility that starved strains have lower ATP level compared to non-starved strains.**

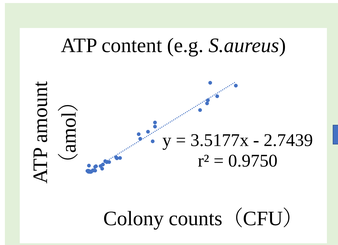
Proposal of examples for setting Alert/Action Levels

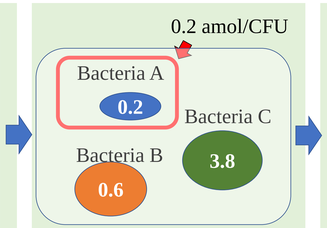
Example of setting flow for Alert/Action levels for ATP method

<Step1>
Measure the ATP amount per CFU for each isolated species in the pharmaceutical water facility.

<Step2>
Select the isolate with the lowest ATP content.
(In the case of the diagram below, 0.2 amol/CFU is selected.)

<Step3>
Convert the threshold of the culture method into ATP amount using the ATP content of the selected isolate.





Converted as 0.2 amol/CFU

	Action Levels
Purified water	100 CFU/mL → 20 amol/mL
Water for injection	10 CFU/100mL → 2.0 amol/100 mL

Proposal of examples for Alert/Action levels for ATP method

Water species	Alert levels*	Action levels
Purified water	**The lower of “ the 50% value of Action Levels (10 amol/mL) ” or “mean +2σ”**	**20 amol/mL**
Water for injection	**The lower of “ the 50% value of Action Levels (1.0 amol/100 mL)” or “mean +2σ”**	**2.0 amol/100 mL**

*We propose initially setting it using 50% of the intervention threshold value. Additionally, as data accumulates, we suggest calculating mean+2σ from the data and setting it to the lower of the two values.

✓ We should evaluate the validity of the proposed threshold over a period of one year, taking into account the seasonal variations of the microbial community.

Conclusion

✓**It has been suggested that the ATP method is capable of obtaining results comparable to the culture method.**

✓**We suggest examples of setting Action/Alert levels for the ATP method in pharmaceutical water.**