Executive Summary: Primary Validation of AQU@Sense MB

Approved by

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1. Introduction

The AQU@Sense MB is a measuring device for the detection of intact cells in pharmaceutical water, using an alternative method to the traditional testing of waterborne viable particles, such as pour-plate agar or membrane filtration method typically used in the pharmaceutical industry. The traditional membrane filtration method used as a comparison, referred to as HPC in this document, is described in general for the use in non-sterile products in Ph. Eur. monograph 01/2021:20612 [2] and specifically for Purified Water (PW) and Water for Injection (WFI) in 04/2018:0008 and 04/2017:0169, respectively. The sample volume for WFI using HPC is defined as at least 200 mL, whereas for PW, a sample volume is not defined in the Ph. Eur. [3] [1]. The AQU@Sense MB analyses a fixed volume of 90 µL per replicate and the traditional and alternative methods are compared using the same sample volume of 90 µL. As defined in the Ph. Eur. 5.1.6, it is the supplier's responsibility to provide the primary validation and the description of the technique of the alternative method [4].

This document briefly summarizes the results of the execution of the primary validation. The protocols and argumentations used, as well as the description of the technique and the risk–benefit analysis can be found in the Validation Master Plan (VMP). The detailed results of the experiments and the corresponding statistical analysis can be found in the Validation Summary Report (VSR).

Assuming the end user's appropriate PQ, the AQU@Sense MB can be used in addition or as a substitute of traditional methods to control water systems and enumerate viable particles in pharmaceutical-grade water. The customer's PQ scope depends on the intended use and a corresponding risk assessment. As part of the PQ, the results described in this document can either be confirmed or referenced. In any case, it must be considered that the AQU@Sense MB does enumerate and report cells as Intact Cell Counts (ICC) and not as the traditional unit Colony-Forming Units (CFU), referred to in the guiding documents (see Chapter 9.1 Guiding Documents and References in the VMP). Because the method used is non-growth based, it allows for detecting cells that do not grow on agar media used for the traditional method. Even though the traditional method does not detect these bacteria, scientists have found that they are still viable and therefore named them Viable But Non-Culturable (VBNC) [5]. These bacteria can still proliferate, given enough time under appropriate conditions, and they may still be infectious.

2. Summary of Results

The results are listed in the table below per parameter tested. Each test-setup is shortly described in the column "Summary of Test Procedure".

Table 2-1: Test procedure and results for each parameter tested during the primary validation.

Parameter	Summary of Test Procedure	Pass / Fail	Comment
ACCURACY	- 3 strains - 6 concentrations - 5 replicates	PASS	- AQU@Sense MB non-inferior to HPC - dependent on strain (0 -34.1 cells/90µL)
PRECISION	- 3 strains - 2 concentrations - 3 days	PASS	- PRECISION = intermediate PRECISION and REPEATABILIY - both methods RSD < 30%
SPECIFICITY A1	- 10 strains - 1 concentrations (low)	PASS	- all strains detected - similar or more sensitive to HPC
SPECIFICITY A2	- 3 strains in PW/WFI - 1 concentrations (low)	PASS	- all strains detected by AQU@Sense MB (not by HPC) - AQU@Sense MB on average 1.15 more counts than HPC
SPECIFICITY B	- live / dead - particles - sterile WFI	PASS	- no interference by polystyrol and FeOH
LINEARITY	- data from ACCURACY experiment used	PASS	- strictly linear - non-inferior to HPC - R^2 = 94.9-98.2
RANGE	- data from ACCURACY experiment used	PASS	- LOQ similar (lower end of range) - not assessed against criteria
LOD	- data from ACCURACY experiment used	PASS	- LOQ ≤ 3 cells / 90µL for AQU@Sense MB - dependent on strain
LOQ	- data from ACCURACY experiment used	PASS	- LOQ ≤ 5 cells / 90µL for both methods - dependent on strain
ROBUSTNESS	- environmental and sample temp. varied	PASS	- non-inferior to reference setting
RUGGEDNESS	- 3 instruments - 3 batches	PASS	- non-inferior for all setting

3. References

[1] European Pharmacopoeia Commission, “WATER PURIFIED,” in *European Pharmacopoeia 10.0*, 04/2018:0008.

[2] European Pharmacopoeia Commission, “2.6.12. MICROBIOLOGICAL EXAMINATION OF NON-STERILE PRODUCTS: MICROBIAL ENUMERATION TESTS,” in *European Pharmacopoeia 10.0* , 01/2021:20612.

[3] European Pharmacopoeia Commission , “WATER FOR INJECTIONS,” in *European Pharmacopoeia 10.0*, 01/2017:0169.

[4] European Pharmacopoeia Commission , “5.1.6. ALTERNATIVE METHODS FOR CONTROL OF MICROBIOLOGICAL QUALITY,” in *European Pharmacopoeia 10.0*, 07/2017:50106.

[5] A. G. G. P. P. S. S. Thandavarayan Ramamurthy, “Current perspectives on viable but non-culturable (VBNC) pathogenic bacteria,” 31 06 2014. [Online]. Available: https://www.frontiersin.org/articles/10.3389/fpubh.2014.00103/full. [Accessed 11 05 2021].

4. Revision history

Revision	Modification	Date	Initials
0	First issue	05.07.2022	FTH
1	Second revision	01.10.2023	JOAN