The dilemma in Microbial test for Water

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Purified Water system and Water-for-injection requires periodic monitoring as assurance to the water quality that is being used by the processes.

While other parameters are not big issues, the parameter on microbial test creates quite a stir and uneasiness amongst the local manufacturers (in Malaysia)

The 'uneasy' scenario was created due to the approved test method for microbial test on grab samples of water taken from the water system.
While there is no problem in conducting membrane filtration method on 100ml samples for Water–For-Injection, the same is not true for Purified Water samples.

What can be so difficult in complying to National Pharmaceutical Control Bureau (NPCB)'s directives that water samples be tested via membrane filtration using 100ml sample?
NPCB simply requires compliance to BP for testing of water samples. See extracts of BP 2004 below.

(Ph Eur monograph 0008)

H_2O 18.02 *7732-18-5*

Ph Eur

DEFINITION

Water for the preparation of medicines other than those that are required to be both sterile and apyrogenic, unless otherwise justified and authorized.

PURIFIED WATER IN BULK

PRODUCTION

Purified water in bulk is prepared by distillation, by ion exchange, by reverse osmosis or by any other suitable method from water that complies with the regulations on water intended for human consumption laid down by the competent authority.

During production and subsequent storage, appropriate measures are taken to ensure that the total viable aerobic count is adequately controlled and monitored. Appropriate alert and action limits are set so as to detect adverse trends. Under normal conditions, an appropriate action limit is a total viable aerobic count *(2.6.12)* of 100 micro-organisms per milliliter, **determined by membrane filtration**, using agar medium S and incubating at 30-35 °C for 5 days. **The size of the sample** is to be chosen in relation to the expected result.

While BP stated that this is the requirement, it is interesting to note that USP allows pour plate method for microbial test on Purified water USP.

See extracts of USP on the next page.

USP 28, <1231>, Pg. 2762

Methodologies that can be recommended as generally satisfactory form monitoring pharmaceutical water system are as follows:

Drinking Water	POUR PLATE METHOD Minimum sample – 1.0ml Plate count agar 42 to 72 hours incubation at 30^0C to 35^0C
Purified Water	POUR PLATE METHOD Minimum sample – 1.0ml Plate count agar 48 to 72 hours incubation at 30^0C to 35^0C
Water for Injection	MEMBRANE FILTRATION METHOD Minimum sample – 100ml Plate count agar 48 to 72 hours incubation at 30^0C to 35^0C

Inquiries made to NPCB states that BP method is the choice for microbial testing of water and the sample size must indeed be 100ml. Justification was based on the fact that 1ml sample as used in the Pour plate method is not representative of the whole water system where thousands of liters are produced daily.

Reply from an officer in NPCB *(in Malay)*

BIRO telah menyaran pengujian mengguna kaedah membrane filtration on at least 100 ml sample in order to gain better assurance that the resulting colony count is more statistically representative (only 1.0 ml sample is taken using pour plate method). Pihak syarikat can set the limit according to BP or more stringent. Jika expected count 10 cfu/ml - pihak syarikat masih boleh jalankan ujian ke atas 100 ml sampel by splitting sample into portions (eg 4 x 25 ml or 5 x 20 ml or 10 x 10 ml) - filter each portion onto a separate membrane. Expected count on each membrane would be 250 or 200 or 100 cfu (which is still possible to count). Cfu/100 ml will be the sum of cfu from all membranes & calculate cfu/ml.

- extracted reply

Translating the above reply in Malay, where the expected results are about 10cfu/ml, NPCB suggested that the 100ml samples be sub-divided to 4 or 5 sub-samples. This will allow readings be made and the total cfu on all the sub-samples will be used for the final result that represents a 100ml sample.

However, BP clearly states that it do not specifically requires use of 100ml sample during the test – "**The size of the sample** is to be chosen in relation to the expected result". Thus, a reasonable volume should be selected (utilized) in the test. The volume used should meet the expected results of the water samples to be tested.

Let's say you expected your water sample to have readings of < 10cfu/ml. The ideal volume to be used shall be 25ml.
NPCB argue that you should used 25ml x 4 to make up to 100ml.
However, you may conduct a study and will definitely reach a conclusion that all 4 samples of 25ml will have a reading almost similar to each other. Maybe I'm wrong, but I believe the distribution of microbial population should be uniform in the 100ml sampled.

So, what is the purpose of conducting 4 tests when one should suffices.
Manufacturers may be happy with my comment. Their microbiologist will surely be reduced with the high burden of testing 4 samples.

To further amplify this argument, if your expected result is 50cfu/ml, a suitable sample size will be 5ml. Thus, do we need to test 20 times to get all 100ml sample size? Isn't this ridiculous? I wonder how many of the manufacturers have 20 sets of membrane filtration units?

To make matters worse, most manufacturers have more than one sample to be tested at any one time. Some may need to test up to a number of 20 samples.! During validation, samples from every points-of-use must be tested. So, you can imagine the enormous work to be carried out by a microbiologist in the testing of a Purified water system .

If one sample requires 4 membrane filtration tests (as required by NPCB), 10 samples will require 40 membrane filtration test..!!! Who has this much membrane filtration set…??

In conduct of water monitoring exercise, I believe that the spirit of quality should be taken into consideration. Using a "suitable" volume for microbial test should be deemed as good as using sub-divided volumes multiply by the number of tests to make up o 100ml. Although the latter will be accurate, the former has no less value in determining the quality of the water samples. By all means, this is a monitoring exercise. Conducting test via membrane filtration with volume more than 1.0ml is already a better alternative to the pour plate method allowed by USP. So, why need to complicate matters….?

Comments from readers will be much appreciated. Please forward any comments to azmanaj@acissb.com
I will put up in my web-sites for other to view. Please state if you wish to stay anonymous.

Regards.