2nd June 2020

Vibrio cholerae Issues

Background

At present, when undertaking analysis, we need to take into account that there are two different types of *V. cholerae* O1 and O139: toxic and non-toxic. Whilst the IMO D-2 Ballast Water Performance Standard recognizes this and talks about "toxicogenic Vibrio cholerae (O1 and O139)", the IMO BWMS Code (test validity criteria) does not differentiate between the toxic and non-toxic forms in its section "Sample analysis for determining efficacy in meeting the discharge standard" (see BWMS Code Annex, 4.7.4). Therefore, if a treatment is effective at killing *V. cholerae*, then we feel that this should be enough as an end-point for type approval testing. This means whenever *V. cholerae* was confirmed the toxicity is irrelevant for type approval testing.

However, from a Port State Control point of view, the toxicogenic evaluation may be necessary because it is included in the D-2 standard and it is a possible public health issue.

Further, and going beyond the toxicogenic issue, Global TestNet has in the past had discussions about replacing *V. cholerae* with another indicator microbe. This is because *V. cholerae* is not common in most waters (and toxicogenic forms are very rare indeed). This means that it's hard to verify whether a BWMS will kill it if it is not generally present in the water that we use for testing. The use of more cosmopolitan microbes might be a better indicator of efficacy.

As you may remember, Tim and Stephan volunteered to work on the two Cholera-related issues as listed in the Actions & Updates from the 11th Annual Meeting of Global TestNet. **Please can you complete the tables below for us to evaluate the situation within Global TestNet:**

Issue 1

Is every yellow colony analysed for the presence of toxicogenic O1 & O139 V. cholerae and if so how?

Table 1: Comparison table indicating whether or not each yellow suspect colony is analysed for the presence of toxicogenic V. cholerae

Test facility	Comment
GCDC	Not all yellow suspects are analysed with the TCBS method. In shipboard testing the indicative New Horizons method is used and samples are sent to a laboratory for confirmation.

PML Applications	Whole TCBS filters that have yellow colonies are analysed using the New Horizons DFA methods which only determines presence of O1 and O139 serotypes and NOT whether they are toxic or non-toxic forms.
MBRIJ	The TCBS filters with yellow suspect colonies are analysed for confirmation. The analysis is conducted based on the Ballast Water Management System Approved Biological Analysis Manual. https://www.classnk.or.jp/hp/pdf/activities/statutory/ballastwater/unyo/seibutubunseki_rev2_2010.pdf

Issue 2

The documentation of the presence of toxicogenic *Vibrio cholerae* is a cumbersome process, it also cannot be analysed without certain safety rules, which are difficult to implement, e.g., in shipboard sample analysis. Authorised labs may not (easily) be within reach to work on these samples. In addition, toxicogenic *V. cholerae* have not been found in the almost 20 years of BWMS testing. It was therefore discussed whether or not a better indicator microbe exists.

Three questions arose for you to please try to answer:

Table 2: Are you in favour to find an alternative microbe for V. cholerae?

Test facility	Comment
PML Applications	Yes
GCDC	Yes, possibly an indicator microbe detectable also during shipboard testing, i.e., be widely spread/present in the world.
MBRIJ	Yes

Table 3: What research is necessary to find an alternative microbe for V. cholerae?

Test facility	Comment
GCDC	Changing BWMC by amending the D-2 standard will not happen soon, but may become an option after the ongoing Experience Building Phase. We would need to check if there are other INDICATOR microbes in wide use for which validated documentation methods are available. Are there any candidate species in use for bathing or drinking water quality analysis or some other bacteria which are widely spread/present in the world?

PML Applications	We need to decide what the purpose is for any alternative indicator microbe to replace V. cholerae. We have two satisfactory indicator microbes already (E. coli & Enterococcus) so do we need another? If the purpose is to make sure that we kill V. cholerae then another vibrio species would seem appropriate and research might be directed that way or do we simply remove the specified serotypes and the word toxicogenic from the test requirements and only count yellow colonies?
MBRIJ	The alternative indicator microorganisms that will replace V. cholerae should be defined. If the aim is to kill the V. cholera, other candidate species such as related species of Vibrio or those with higher resistance should be considered.

Table 4: For all three performance tests (TA, Commissioning and CME [compliance monitoring and enforcement]) do we need to write a Global TestNet guidance document?

Test facility	**Comment**
GCDC	Guidance would be helpful to clarify matters. For TA the BWMS Code does not address the toxicity (see above). The IMO commissioning testing guidance will after the next MEPC likely exclude bacteria so that this issue would not need to be addressed. CME needs to show that D-2 is met so that toxicity tests are needed. However, PSC is outside the Global TestNet work as this is not done by test facilities. Any guidance document needs to address also the newly added bacteria replacing *V. cholera*.
PML Applications	Recommendations and guidance are always helpful.
MBRIJ	Guidance is important and necessary.