CURRENT STATE OF ENUMERATION METHODS FOR *Escherichia coli* IN REGULATORY BATHING WATER QUALITY MONITORING IN EUROPE

1. Background
The European Bathing Water Directive (BWD) references methods to use in microbiological quality monitoring. During meetings in 2016, the EMEG subgroup of experts, in representation of their respective Member States (MS), debated these reference methods, the acceptance procedure for alternative methods and the new EN ISO 9308-1:2014 method, including its applicability for bathing waters. No simple answer was found for the concerns that were identified. The purpose of this document is to summarize the main aspects related to applicability of the different methods in use for bathing water *E. coli* monitoring in Europe. However, the outline is not complete since formal data on the situation for each MS were not available during the preparation of this document.

2. Reference methods in BWD for *E. coli* enumeration
The Annex I of the Bathing Water Directive 2006/7/EC defines the analytical methods accepted for the microbiological monitoring. For *E. coli* monitoring, the reference methods are ISO 9308-3 (miniaturised MPN method, liquid medium) and ISO 9308-1 (membrane filtration method, solid medium). As directives are revised, so are the standard methods, in order to follow progress in science. This means that the reference methods referenced in a directive may change also. According to the ISO rules and laboratory accreditation requirements (of EN ISO 17025), the latest edition of the standard method (including any amendments) should be used by accredited laboratories. The reference methods in the BWD are undated, and therefore it is mandatory to use the latest editions of the reference methods for compliance monitoring.

3. Considerations about the applicability of ISO methods for *E. coli* monitoring under the BWD
This method is an old version of EN ISO 9308-1 and was withdrawn in 2014 when the revised version of the EN ISO 9308-1 standard was published. The old version published in 2000/2001 was a reference method for the BWD for nine years: from 2006 to 2014. The 2001 version of the standard was a membrane filtration method using a Lactose TTC Tergitol (LTTC) medium. The scope of the method was corrected in 2007 to stress the high sensitivity of the LTTC medium. The standard method was applicable only for monitoring of very clean and/or disinfected (drinking) water. In addition to the standard method, EN ISO 9308-1:2001 also included a rapid test using TSA/TBA media. Some Member States successfully used the rapid TSA/TBA test for *E. coli* enumeration under the scope of the BWD. However, as mentioned above, this 2001 version of the standard has now been withdrawn. If a laboratory wishes to use this old method in the future, the method should be referenced as an internal method, not as a standard method, implying that a new audit for the accreditation is required.

Since 2014, the new version of the EN ISO 9308-1:2014 method has been in force. This revised standard method is drastically different from the previous standard version from 2001. Among other
changes, other kinds of water than drinking water (such as bathing water) are not included at all in the scope of the EN ISO 9308-1. The main technical difference in the new method is that the method is based on the chromogenic coliform agar and that the rapid test (using TSA/TBA) is no longer included in the standard. The title and the scope of the new ISO 9308-1 published in 2014 states that the method is not suitable for waters with high bacterial background flora. It must be noted that bathing water is generally considered as water with high background flora. Therefore, there is an urgent need to change the Annex I of the BWD, since the reference method ISO 9308-1 is no longer valid for the bathing water monitoring. Further information from the MS is needed to indicate if this method is in use for monitoring under BWD in some countries. In such cases, further clarification from these countries is needed. For example, if these countries have made successful modifications to the standard protocol or if the ambient bathing waters in these countries could be considered as having only low background flora.

The MPN method ISO 9308-2 was not available in its current form at the time when the BWD was published in 2006. This method is thus not listed as a reference method in the current BWD. However, the scope of this new method includes bathing water. Furthermore, the method has been validated for bathing water monitoring in European marine and freshwater bathing sites and is currently in use. The ISO 9308-2 is based on Colilert-18 method (IDEXX trademark), and has been accepted in some countries to be used as alternative method for E. coli monitoring under BWD. According to the BWD, the acceptance of the alternative methods should follow the method comparison protocol described in the standard EN ISO 17994. Currently, if a MS wish to verify the use of ISO 9308-2 as alternative method in their country, the earlier comparison data from other MS can be used as a reference to minimize the work load needed for the comparison. However, in order to avoid unnecessary work load from multiple comparison trials in several countries, there is a need to consider the inclusion of EN ISO 9308-2 into the BWD. This inclusion is justified based on the fact that bathing water is included in the scope of ISO 9308-2. The change could be done as a revision of the Annex I or later on in conjunction of the BWD revision.

EN ISO 9308-3:1998 “Water quality - Detection and enumeration of Escherichia coli and coliform bacteria — Part 3: miniaturized method (most probable number) for the detection and enumeration of E. coli in surface and waste water”
This miniaturized MPN method has been developed in the frame of an EU project ("Performance of reference methods and validation of commonly used methods in the E.U. for the enumeration of E. coli and intestinal enterococci in fresh- and sea- bathing waters") and was established as EN ISO method in 1998. The method is included as a reference method in the current version of the BWD. The EN ISO 9308-3 method is successfully used for bathing water monitoring in Europe, but not in all countries. This method is robust and reliable, and the scope of the method is surface and waste waters. The analyzed sample volume in this method is always less than 10 ml, which is considered too small a volume for clean bathing waters to detect target bacteria in some countries. With other standard methods outlined herein, at least a ten-fold sample volume (100 ml) can be analyzed. Low numbers of microorganisms per sample volume have a high variability due to the characteristics of the Poisson distribution. Therefore, E. coli numbers nearby the detection limit of the method usually produce a large variation in the data, potentially leading to misclassification of the water quality as based on the percentile calculation. In the Epibathe study, the ISO 9308-3 method produced some false positive findings when clean marine (Mediterranian) waters were
monitored (Epibathe, EU FP6 Project Reference 022618). For successful use, the selectivity criteria included in ISO 9308-3 (Annex E) has to be followed.

4. A need for better *E. coli* methods to use in the European Union’s Member States

It is European consensus to include EN-ISO standard methods as reference methods in specific directives, such as the Bathing Water Directive. **Therefore, it is essential that the European Commission and its Member States ensure the nomination of relevant experts with sufficient (laboratory) resources to implement the method standardization.** Microbiological EN ISO standard methods for water quality monitoring purposes are being prepared by the ISO Technical Committee 147 - Subcommittee 4 (TC147/SC4), which holds secretariat at the German Institute for Standardization (DIN). According to its recommendations, TC147/SC4 welcomes proposals from the national member bodies as “preliminary work item proposals” or “new work item proposals”. The need for new methods proposals has been noticed especially in the **ISO 9308 series (Water quality – Detection and enumeration of Escherichia coli and coliform bacteria), which is currently lacking a membrane filtration method suitable for bathing water *E. coli* monitoring.** The national member bodies of the International Organization for Standardization (ISO) preparing proposals for new standard methods should be aware that, prior to publication, the performance characteristics of each new standard method must be validated. TC147/SC4 is currently in process to revise the validation guidance entitled: “ISO/DIS 13843 Water quality — Requirements for establishing performance characteristics of quantitative microbiological methods”. The revised standard ISO 13843 or at least its final draft should become available through the national standardization bodies in 2017.

5. Steps forward

EMEG prepared this document for briefing the discussions in the next Bathing Water Expert Group meeting which will take place at 7th October 2016. The outcome of the Expert Group meeting is awaited to define the further progress and the need for additional advice (if any) from EMEG.