Development of guidance on how to analyze a ballast water sample

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EMSA Workshop on Ballast Water Sampling for Enforcement – Presentation of the Research Results

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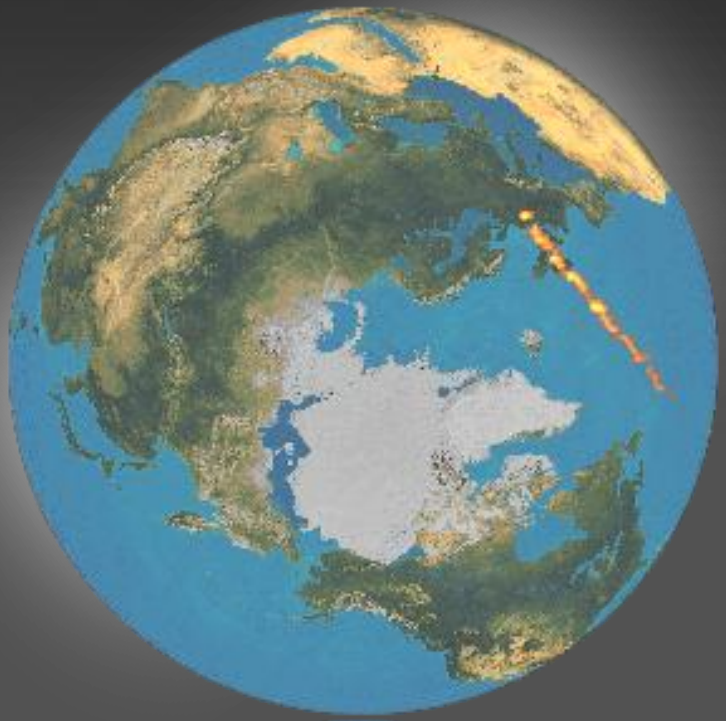
The Shortest Possible Description of DHI

- Spin Off from the Technical University of Denmark
- Research based (100 man years own R&D)
- 1,000 staff (850 MSc/PhD, 80 tech., 70 support)
- Turnover 100 mio. € (75% international)
- R&D core funding from Ministry of Science (5%)
- Offices in 25 countries (65 pct of staff)
- Representation in further 40 countries
- Private, no owners, not-for-profit

MISSION

Leading Technologies and Solutions



- Water in River Basins
- Water in Cities and Industry
- Water in Marine Areas
- Health, Environment and Climate

BWMS Test facilities in Denmark and Singapore

Aim of the project

The aim of this project was to develop guidance on how to analyze and process the results from a representative ballast water sampling protocol.

Is discharged ballast water in compliance with the D2 requirements?

Organism category	Standard
Organisms ≥ 50 µm	< 10 viable organisms/m^3
Organism size: ≥10 µm - < 50 µm	< 10 viable organisms /mL
Toxicogenic *Vibrio cholerae*	< 1 cfu/100 mL
Eschericha coli	< 250 cfu/100 mL
Intestinal Enterococci	< 100 cfu/100 mL

The answer depends on

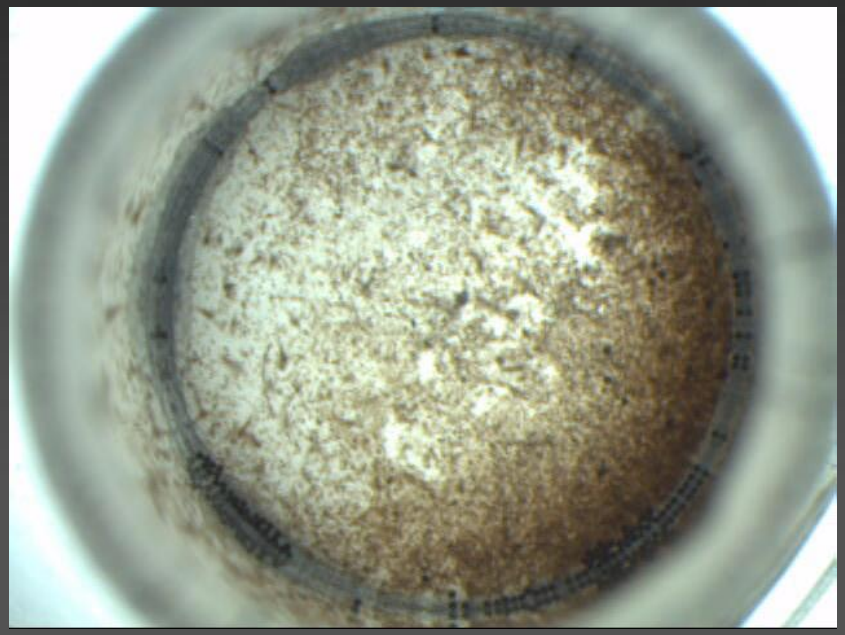
- The eye that sees
- Who sees

The eye that sees:

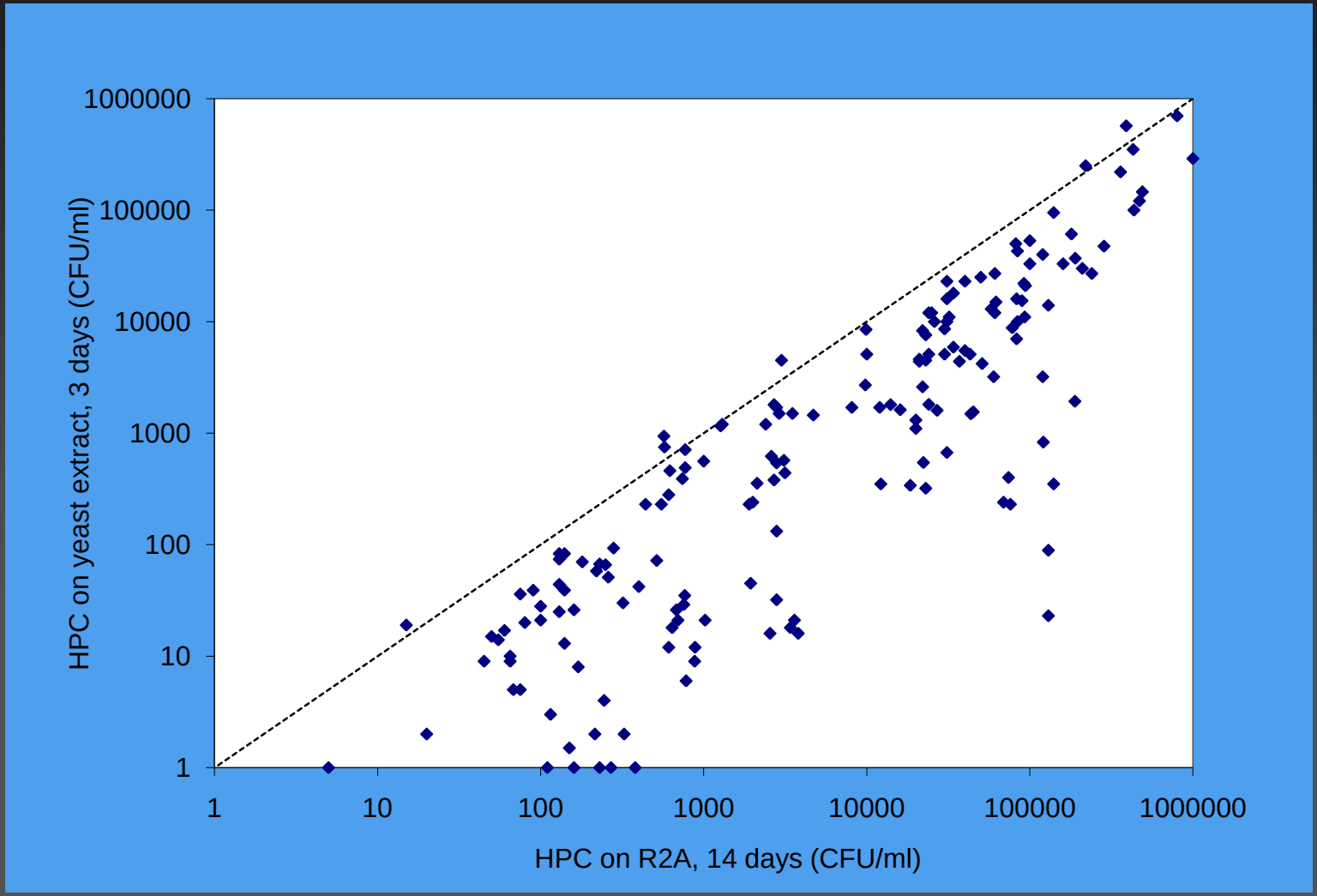
- Detection principle
- Death criterion

- Movement
- Cell membrane integrity
- Enzyme activity
- Ability to grow

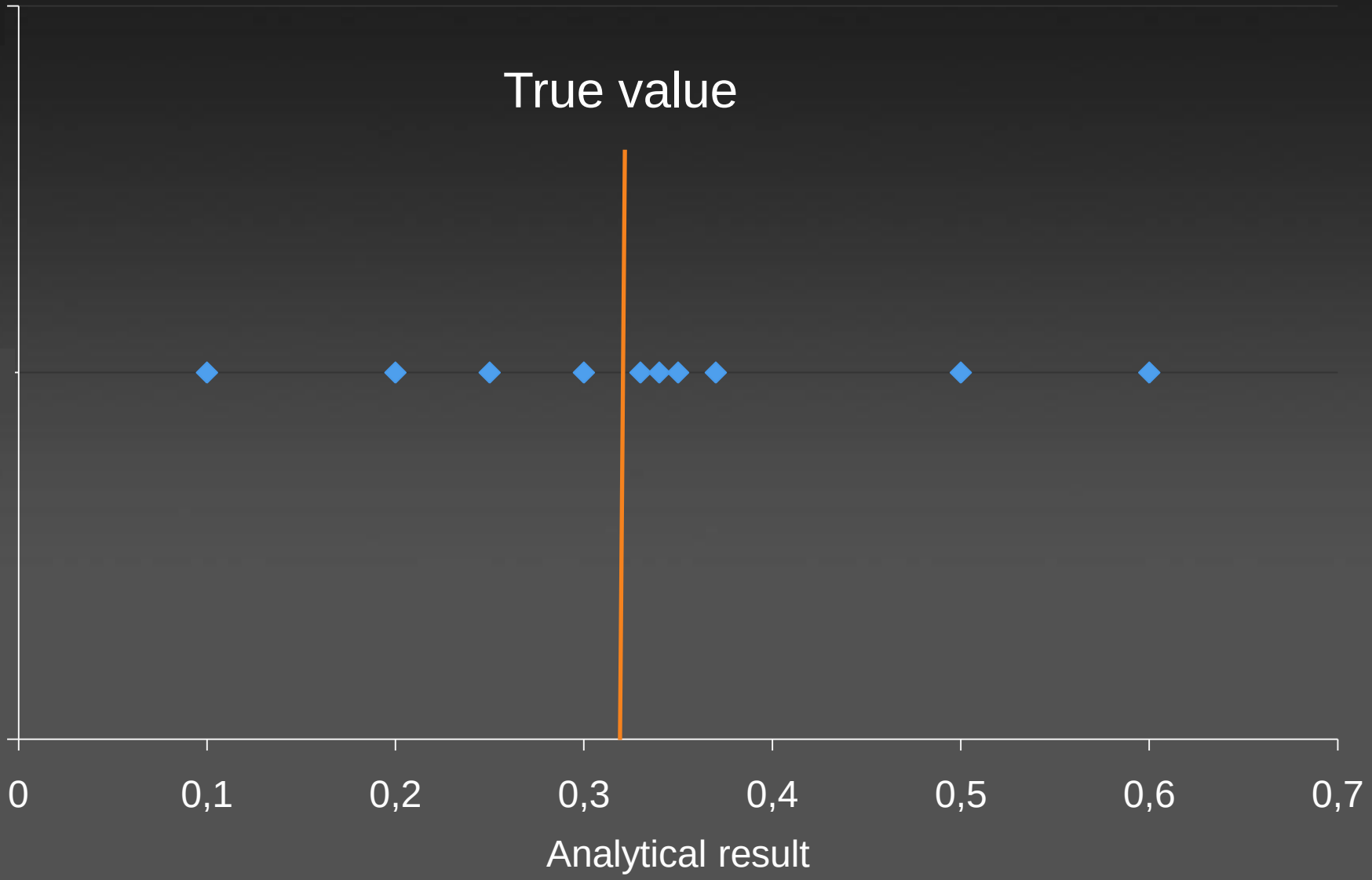
- Bias is likely to occur



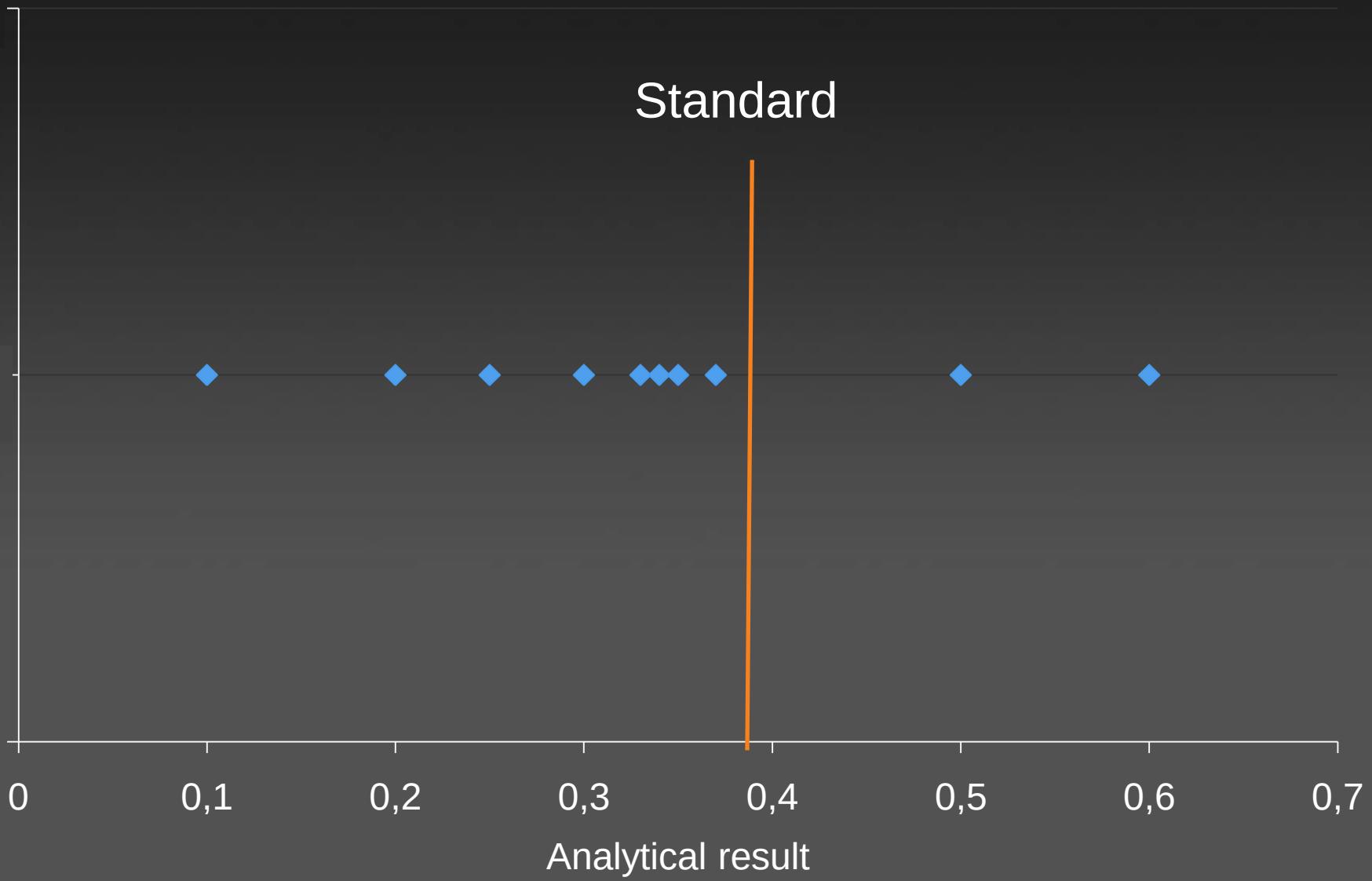
Example of analytic bias: Bacteria



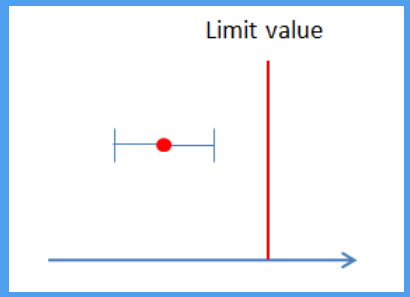
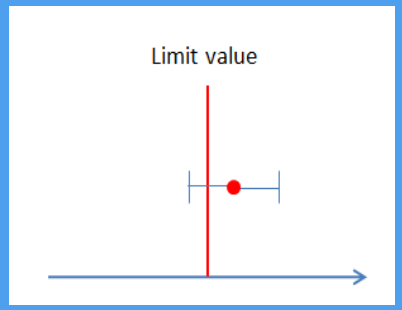
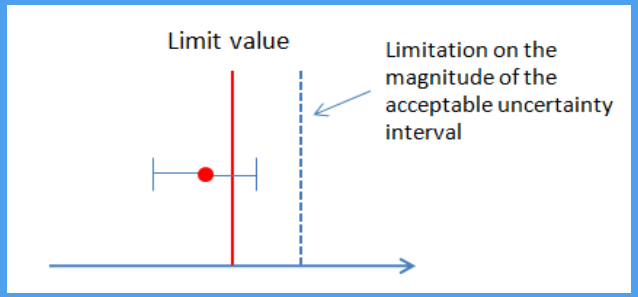
Analytical variability



Analytical variability



Who sees

No	Relating test results to an upper limit value	Evaluation rule	Comments
1		The estimated average value plus the uncertainty must be below the limit value	A high degree of protection is achieved as the entire uncertainty range must be below the limit value. In some cases, this may lead to too strict criteria depending on the consequences of making a wrong decision
2		The estimated average value minus the uncertainty must not exceed the limit value	There is no incentive to reduce the uncertainty, quite the contrary - a high uncertainty will increase the chances of compliance. Such an evaluation rule should always be accompanied by a specification of the magnitude of the acceptable uncertainty interval
3		The estimated average value must be below the limit value and the average value plus the uncertainty must not exceed a specified value	It is most likely that the true value is below the limit value. However, with this evaluation rule it is accepted that some of the individual test results exceed the limit value up to a specified value set based upon restriction on the magnitude of the uncertainty interval

An example from the bathing water directive

For coastal waters and transitional waters

	A	B	C	D	E
	Parameter	Excellent quality	Good quality	Sufficient	Reference methods of analysis
1	Intestinal enterococci (cfu/100 ml)	100 (*)	200 (*)	185 (**)	ISO 7899-1 or ISO 7899-2
2	Escherichia coli (cfu/100 ml)	250 (*)	500 (*)	500 (**)	ISO 9308-3 or ISO 9308-1

(*) Based upon a 95-percentile evaluation. See Annex II.
(**) Based upon a 90-percentile evaluation. See Annex II.

Identifying the relevant methods

- Searched the scientific literature
- Received information from the test facilities

Selecting the best analytical methods

1	Does the analysis have a sufficient limit of detection (LoD) and limit of quantification (LoQ)?
2	Interference
a	Does the analysis distinguish between size of the organisms?
b	Does the method distinguish correctly between viable and non-viable, i.e. is the death criterion acceptable when testing ballast water?
c	Are there other interferences?
3	Is the accuracy acceptable?
a	Does sample pre-treatment give rise to bias?
b	Does the analyst give rise to bias?
c	Precision?
d	Information on reproducibility and repeatability
4	Is the analysis robust?
a	Geographical variations
i	Temperature
ii	Salinity
b	Type of organisms: autotrophic, mixotrophic, heterotrophic
c	Range (high/low number of organisms)
d	TSS/DOC/POC levels
e	Transport/handling
5	Practicality
a	Complex/simple (many/few operations)
b	Availability
c	Cost (high or low)
d	Time to result
e	Does the analysis require highly skilled analysts

Recommendation: Organisms > 50 µm

It is recommended to use simple microscopic examination of the organisms ≥ 50 µm for ballast water compliance monitoring. The microscopic examination of organisms is a robust, simple and cheap method, and laboratories for this analysis are available world-wide. Viability is determined by observing movement. Movement is induced by poking the individual animals and by tapping the counting chamber. In addition, it is recommended to validate the methods using vital staining and video recording for analysis of viable organism and to reduce the possibly bias for motility during transport and storing of samples.

Recommendation: Organisms < 50 µm, > 10 µm

Based on the evaluation made in the project, it is recommended to use FDA/CMFDA in combination with epi-fluorescence microscopy for determining the number of viable organisms in ballast water. However, as high degrees of skill and experience are needed and as the many subjective judgments increase the uncertainty, it is recommended to initiate development of a robust and more objective method(s) for compliance monitoring.

Recommendation: Bacteria

For the indicator bacteria (*E. coli* and Enterococci), it is recommended to use the national analyses used for bathing water analysis.

Analysis of *V. cholera* is recommended to be performed by traditional culturing methods such as ISO/TS 21872-1

Recommendation for all

Aanalysis should be carried out by accredited laboratories.

Average vs instantaneous approach

The “average” approach

The “average” approach is here defined as taking and analyzing more than two samples and evaluating compliance by comparing average and variation of the results to the standard.

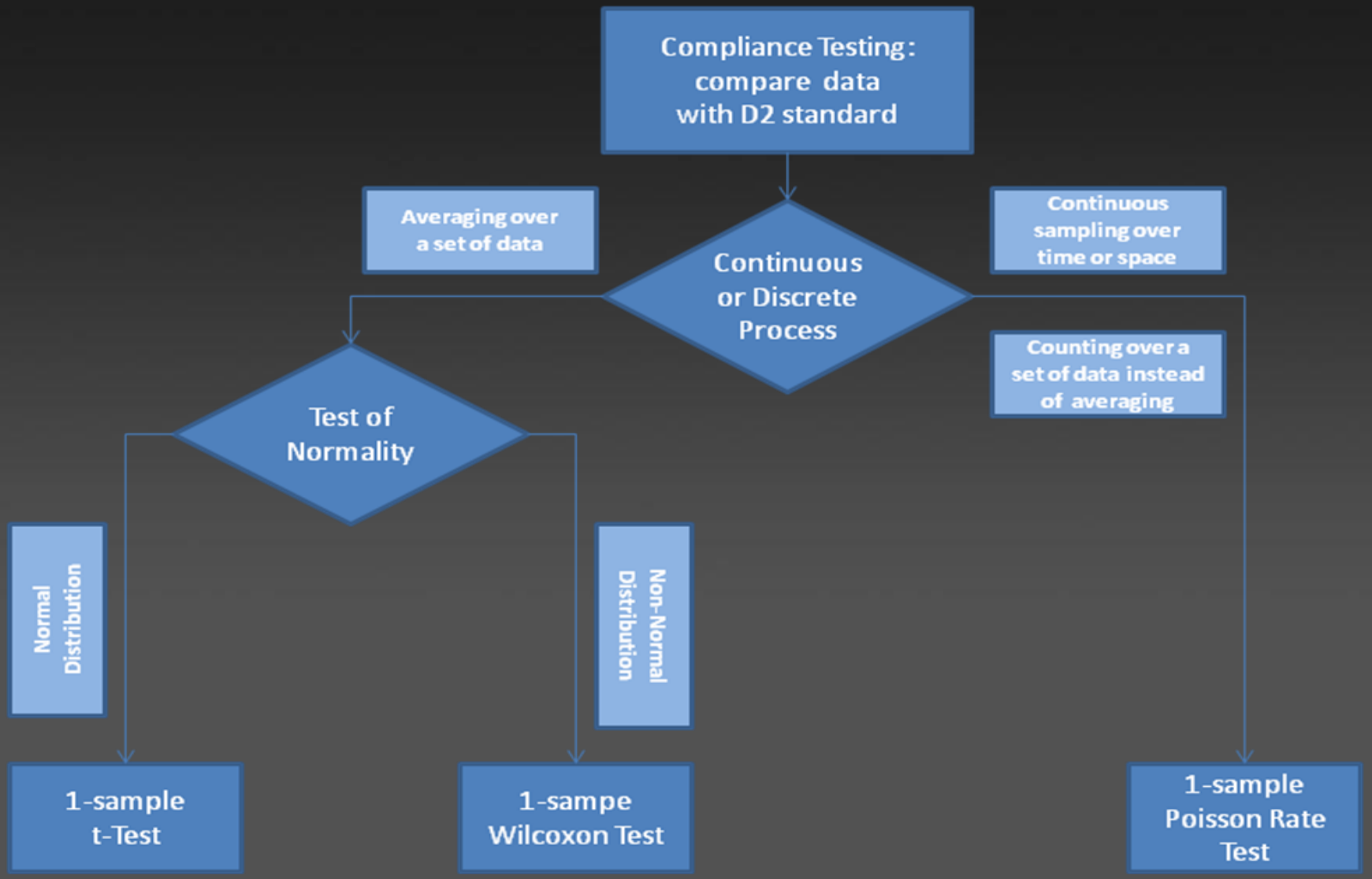
The “instantaneous” approach

The “instantaneous” approach is here defined as taking and analyzing one or more samples and comparing them with the standard on an individual basis.

A statistical evaluation based on an evaluation rule is recommended

After obtaining the analytical results, the compliance evaluation should be performed according to an evaluation rule. The evaluation rule does not change or add to the D2-standard but it describes the principle of how to assess compliance. The evaluation rule could be either of the rules presented by application of an appropriate statistical test and selection of a level of decision certainty.

Statistical decision tree



Recommendations

It is recommended to apply an evaluation rule that considers uncertainty.

It is also recommended to take either a flow-integrated sample or discrete samples of sufficient size and assess compliance by use of statistics based on the Poisson distribution. The flow-integrated sampling has the advantage of higher representativeness and, for long ballast water discharge events, the disadvantage of possible introduction of a negative analytical bias. Discrete sampling has the advantage of reducing the risk of negative bias and the disadvantage of a possible lower representativeness due to the risk of missing high or low peaks of organisms.