

## **Proficiency Testing with Microorganism Standards**

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### **The Current Situation in Microbiology Control**

We are living in a world with an increasing amount of regulation. However, in the field of microbiology, there is still some uncertainty with regard to quality control parameters. Microbial safety is a major issue for water, plants, and the food and beverage industry, but there is also a great deal of pressure to reduce costs while increasing productivity.

We currently have the following situation:

- diverse recommendations & regulations
- wide range of methods
- trend toward more standardization
- non regulated or new steps/situations/samples

Organizations such as ISO, AFNOR, UKAS, ASTM and ILAC propose guidelines and offer support regarding standardization in microbiology quality control.

The problems in a microbiology QC lab are broad, and compared to chemical analysis, there are more unanswered questions with regard to unknown and uncontrolled variables. It is sometimes difficult to decide if results obtained are correct or if they are the result of errors or a natural phenomenon.

Here are some examples of problems:

- large deviations in analysis
- discrepancies between labs
- difficult to compare (parameters, methods and results)
- confusion in determining the most suitable methods
- validations need a lot of time
- human error (handling, calculation, reporting)
- equipment and culture media failures

There is no question that we are moving in the right direction with the current trend toward standardization, especially in light of the fact that knowledge about microbiology quality control has increased over the last 20 years. The methods employed are more accurate and labs do a better job of self-regulation.

The following needs to occur to ensure more reliable results in microbiology quality control:

- trend to ISO, UKAS, EU Regulation 388/2012, FDA, other National Accreditation bodies → standardization of methods
- good and stable performance of test (qualitative and quantitative)
- **standards** (reference strains, certified reference material)
- proficiency testing

### **Microorganism Standards**

One basic but vital tool needed to improve the accuracy of testing is **standards**. In the case of a microbiology lab, that means the need for both microorganism standards (or a control strain), as well as standards for calibration of equipment, such as balances or incubators. Reference cultures are required for testing the performance of media and tests but also for validation of new methods, and to confirm the competency of the lab. It is also possible to use reference strains, which are derivatives of national or international reference cultures, as long as it can be proven that the relevant properties for the application still exist. According ISO 11133-1, it is possible to culture reference strains one time to produce reference stock cultures which are then controlled for purity and for biochemical tests. They should be stored in a freezer or in a freeze-dried form in small aliquots; however, defrosted cultures should not be refrozen. It is preferable for working strain cultures to be made out of stock cultures, and they should not be subcultured again.



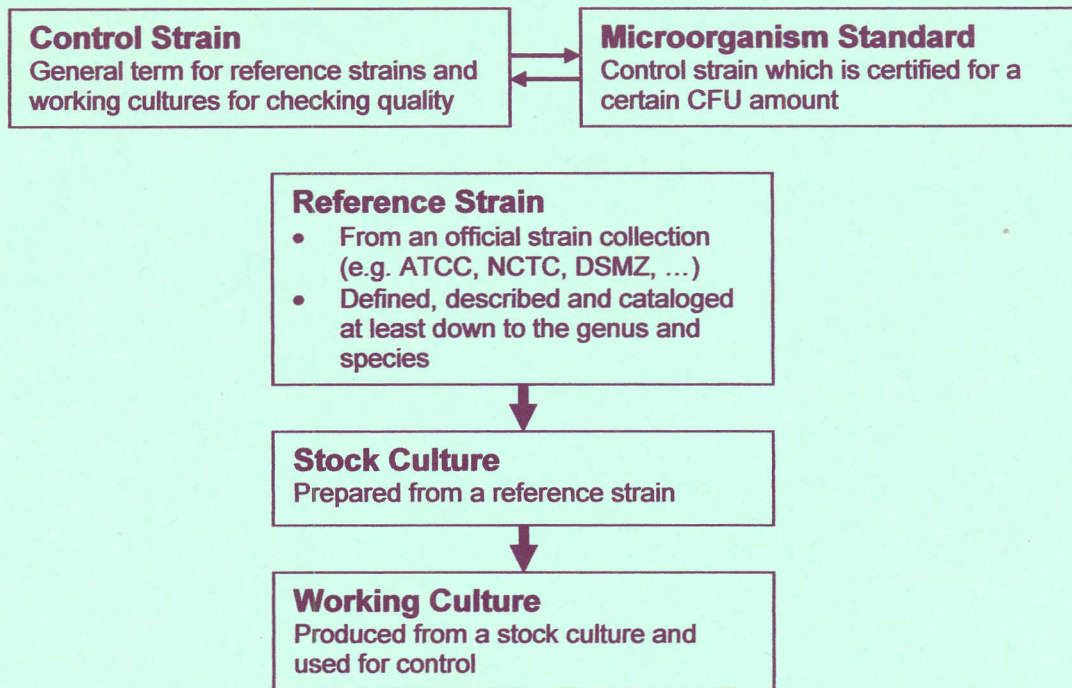


Figure 1: Control Strains

3.4 Where control media are used for comparative evaluation of performance, they should be prepared independently of the media under test and should be demonstrated to be suitable for control use, in that they are shown to provide consistency of appropriate performance. Conformance with ISO/IEC 17025:2005 necessitates control strains (i.e. reference materials) being traceable to certified materials, where possible. Using cultures obtained from a recognised national culture collection or from a reference materials producer accredited to ISO Guide 34:2000 (PD6532-5:2000) would provide a suitable level of assurance. In-house maintenance of control cultures must guard against contamination and deterioration. Guidance on the preservation and handling of control strains may be found in DD ENV ISO 11133-1:2009. If microbiological certified reference materials are used, they should comply with the definition for CRMs given in ISO Guide 30:1992 (PD 6532-1:1993) and need to contain an appropriate assigned number of organisms.

Figure 2: Official Guidelines from UKAS (Source: UKAS LAB 31 2nd Edition)

>>> What is important for a Microorganism Standard?

- specific defined organisms (reference strains from organizations like ATCC, NCTC, DSMZ, ...)
- Certified Reference Material produced under ISO guide 34 and certified acc. ISO 17025
- highly reproducible



## Vitroids™ - A possible Solution for Microorganism Standards

An easy to use form of microorganism standards are Vitroids™, which are certified reference materials. They are made out of reliable reference strains from ATCC and NCTC, are produced under ISO guide 34, and the CFU value is certified under ISO 17025. The organisms are placed in a disc and in this form, can be controlled in number and stability. The possible range is 30 to  $10^9$  CFU per disc and the reproducibility is 3% at levels of 100 CFU.

The discs are easy to use since they can be placed directly in water, diluent, broth, or even on agar plates. The Vitroids™ contain highly viable bacteria and when placed in contact with media, they dissolve rapidly and start to grow without a lag-phase. The viability of the CFU in a disc is stable for at least one year (for most organisms, more than two years) when kept under refrigeration ( $-20^{\circ}\text{C}$ ). It is also acceptable for the product to be briefly transported at ambient temperature. Each disc is packed in an individual tube with some desiccant and the tubes are then packed in mylar foil. Each package comes with a comprehensive certificate of analysis reporting the CFU and standard deviation.

All of the above mentioned features help microbiologist to have reliable results, save a lot of time (laboratory, documentation), and lower their costs.

### Overview feature and benefits:

- Standards in concentrations of 30- 50,000 -CFU per disc
- Produced acc. ISO Guide 34
- CFU certified acc. ISO 17025
- Delivered with detailed certificate of origin
- Reference strains from ATCC, NCTC, etc.
- Minimum 1 year shelf life at  $-20^{\circ}\text{C}$  (usually 2 years)
- No lag-phase
- Amazingly little standard deviation (e.g. 100 CFU  $\pm$  3%)
- No maintenance of stock and working cultures
- Recovery time not needed, organisms are ready to grow
- Pre-enrichment step not necessary
- More reliability (controlled by ISO certified process)
- Easy and ready to use
- Saves cost and time

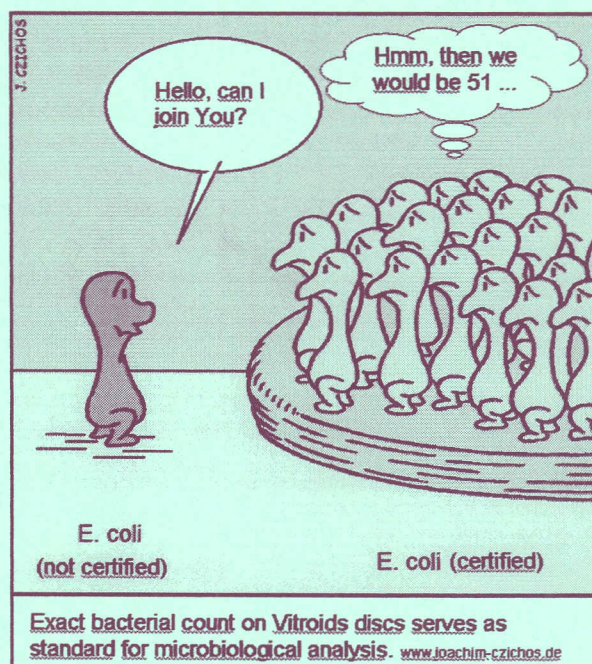


Figure 3: Vitroids™ Cartoon for Visualization

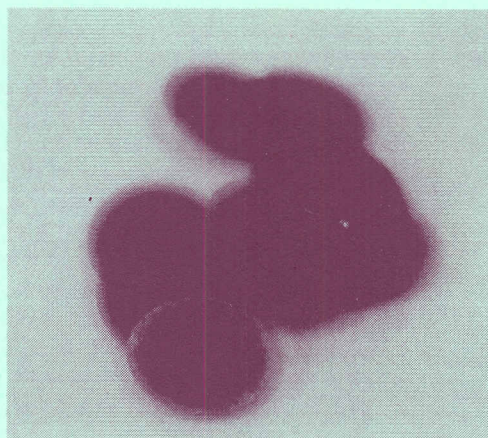


Figure 4: Vitroids™ Discs

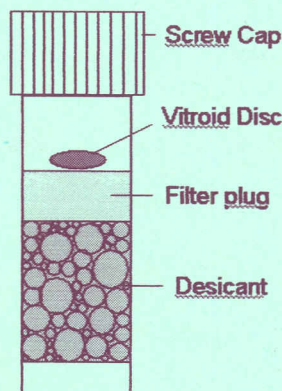


Figure 5: Packaging of the Vitroids™



**Preparation:**

Rehydrate the disc with a common phosphate buffer, or place the disc onto a solid or into a liquid medium. The rehydration process takes approximately 10 minutes. On solid media, the disc forms a droplet that can be spread with a loop. Liquid media may simply be shaken to dissolve the disc. The discs can be rehydrated in as little as 100uL of water or added into larger volumes, e.g. 100mL, for general water testing methods (MF, MTF, Quanti-Tray, etc). It is also possible to add the disc to the media for pour plate techniques.

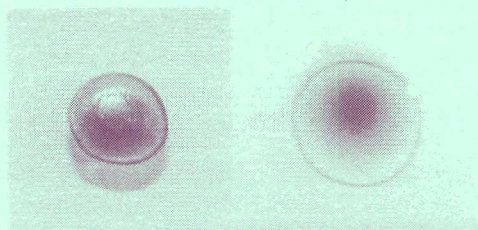


Figure 7: A single Vitroids™ disc on an agar plate. After about ten minutes on a plate it is rehydrated automatically and forms a droplet (no water addition is needed). The drop can be spread with a loop.

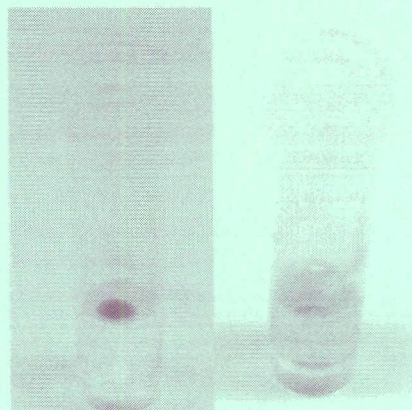


Figure 6: A Vitroid™ just put into buffer (left) and after 10 minutes, it is completely dissolved (right).

| Vitroids™ Test Strains  | Origin | Strain # | CFU   | Cat #    |
|---|--------|----------|-------|----------|
| <i>Aspergillus niger</i>  | ATCC   | 16404™   | 80    | RQC15003 |
| <i>Bacillus subtilis</i>  | ATCC   | 6633™    | 80    | RQC16003 |
| <i>Bacillus subtilis</i>  | ATCC   | 6633™    | 10000 | RQC02258 |
| <i>Candida albicans</i>   | ATCC   | 10231™   | 80    | RQC14003 |
| <i>Clostridium perfringens</i>  | NCTC   | 10240    | 30    | RQC02351 |
| <i>Clostridium perfringens</i>  | NCTC   | 10240    | 500   | RQC20106 |
| <i>Clostridium sporogenes</i>   | ATCC   | 19404™   | 80    | RQC19003 |
| <i>Enterobacter aerogenes</i>   | ATCC   | 13048™   | 50    | RQC01652 |
| <i>Enterobacter aerogenes</i>   | ATCC   | 13048™   | 200   | RQC01655 |
| <i>Enterobacter aerogenes</i>   | ATCC   | 13048™   | 1000  | RQC01657 |
| <i>Enterococcus cloacae</i>   | ATCC   | 35030™   | 50    | RQC21102 |
| <i>Enterococcus faecalis</i>  | ATCC   | 19433™   | 50    | RQC01772 |
| <i>Enterococcus faecalis</i>  | ATCC   | 19433™   | 200   | RQC01774 |
| <i>Enterococcus faecalis</i>  | ATCC   | 19433™   | 500   | RQC01775 |
| <i>Enterococcus faecalis</i>  | ATCC   | 19433™   | 1000  | RQC01777 |
| <i>Escherichia coli</i>   | ATCC   | 11775™   | 50    | RQC01702 |
| <i>Escherichia coli</i>   | ATCC   | 11775™   | 200   | RQC01705 |
| <i>Escherichia coli</i>   | ATCC   | 11775™   | 1000  | RQC01707 |
| <i>Escherichia coli</i>   | ATCC   | 8739™    | 80    | RQC11003 |
| Heterotrophic Organisms   |        |          | 100   | RQC02504 |
| <i>Legionella bozemanii</i>   | NCTC   | 11368    | 50000 | RQC02908 |
| <i>Legionella pneumophila</i>   | NCTC   | 12821    | 50000 | RQC02008 |
| <i>Listeria monocytogenes</i>   | ATCC   | 19115™   | 30    | RQC01901 |
| <i>Pseudomonas aeruginosa</i>   | ATCC   | 9027™    | 30    | RQC02202 |
| <i>Pseudomonas aeruginosa</i>   | ATCC   | 9027™    | 50    | RQC12002 |
| <i>Pseudomonas aeruginosa</i>   | ATCC   | 9027™    | 100   | RQC02204 |
| <i>Pseudomonas aeruginosa</i>   | ATCC   | 9027™    | 200   | RQC12005 |
| <i>Pseudomonas aeruginosa</i>   | ATCC   | 9027™    | 1000  | RQC12007 |
| <i>Salmonella enterica</i> subsp. <i>Enterica</i> serovar Abony       | NCTC   | 6017     | 80    | RQC18003 |
| <i>Salmonella enterica</i> subsp. <i>Enterica</i> serovar Typhimurium | ATCC   | 14028™   | 50    | RQC17002 |
| <i>Salmonella goldcoast</i>   | NCTC   | 13175    | 30    | RQC02301 |
| <i>Staphylococcus aureus</i> susp. <i>Aureus</i>                      | ATCC   | 6538™    | 50    | RQC13002 |
| Vitroids™ Blank   | -      | -        | 0     | RQC0001  |

Table 1: Available Range of Vitroids™



## Proficiency Testing (PT) and ISO 17025

PT is an effective tool in helping laboratories to assure themselves that they report correct results.

**Principle:** Samples of known, but undisclosed content go through the routine procedures

**Result:** Independent external assessment of performance assure the results of the testing laboratory.

**Philosophy of ISO/IEC 17025:** The same sample at different times, from different analysis, and from many different laboratories should reflect an agreeable result

### ILAC – International Laboratory Accreditation Cooperation

*"Proficiency testing is one of the important tools used by laboratories and Accreditation Bodies for monitoring test and calibration results and for verifying the effectiveness of the accreditation process. As such, it is an important element in establishing confidence in the competence of Signatories and their accredited laboratories covered by this Arrangement."*

### ILAC-P1 ILAC Mutual Recognition Arrangement: Requirements for the Evaluation of Accreditation Bodies

P1 further mandates Accreditation Bodies (AB) "... to demonstrate the technical competence of its accredited laboratories by their satisfactory participation in Proficiency Testing Activity."

In the flow chart below, the process of a PT cycle is shown. It starts as a program and the PT organization (e.g. Sigma-Aldrich) assumes the coordination for the participating laboratories. The kits, which contain different Vitroids with water as sample matrix, are sent out to the labs. The labs complete their testing and submit the results to the PT organization. The PT organization collects all results and performs a statistical analysis. A report is then generated and sent back to the participating laboratories.

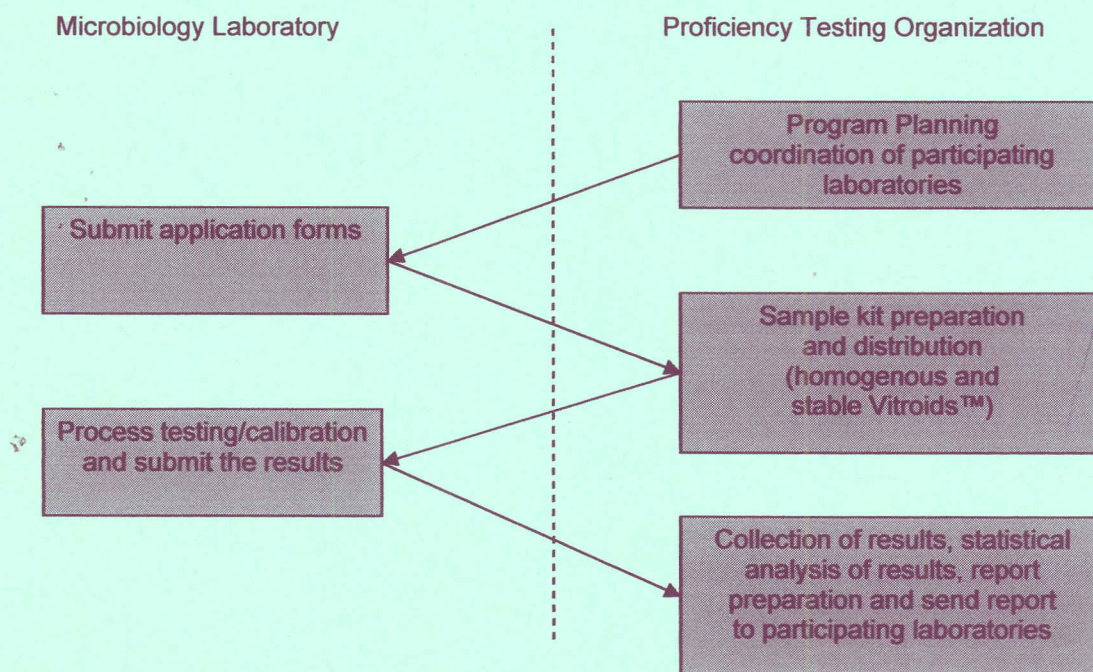


Figure 8: PT Flow Chart

### What can I do when I get incorrect results?

- Check for all possible sources of error
- Repeat the process with standards, e.g. Vitroids™, or use a quality control kit which is similar to the PT kit (may also do before the PT)
- Implement corrective actions to avoid future errors
- Repeat PT (as confirmation)