Royal Biotech
Vial LAB

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Royal Biotech – VIAL LAB

BASIC SYSTEM

USER MANUAL

Royal Biotech

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1 INTRODUCTION

Dear User, thank you for purchasing our **VIAL LAB**, an innovative rapid colorimetric system to perform microbiological tests on food, water and surfaces.
The method of analysis is based on the observation of the change of color in the suspension formed in the analysis vial used when the test sample is added: the suspension changes color if there are microorganisms, the greater the amount of microorganisms, the more rapid the change of color.
The MBS method has been validated according to ISO 16140:2003 "Microbiology of food and animal feeding stuffs - Protocol for the validation of alternative methods".

Available reagents for the selective search of the following microorganisms:

1. Total Viable Count – **CBT-A01**
2. Coliforms – **CO-A02**
3. *Escherichia coli* **– EC-A22**
4. *Enterobacteriaceae* – **EB-A03**
5. *Staphylococcus aureus* – **SP-A04**
6. *Pseudomonas aeruginosa* – **PAO-A05**
7. *Salmonella* spp. – **SL-A06**
8. *Listeria* spp. – **LY-A07**
9. *Enterococcus faecalis* – **EF-A09**
10. *Saccharomyces* spp. – **SC-A11**.

Note: Before use, it is recommended to download the Material Safety Data Sheet from our website.

2 DISCLAIMER

VIAL LAB must be used only for the purpose for which it was intended; any other use is considered improper and dangerous.
The users must, under their own responsibility, observe the current laws concerning health and safety.
Royal Biotech, therefore, decline any responsibility for damage to persons, animals or objects that may, directly or indirectly, follow the use of the product.

3 HOW TO USE

The kit comes in a pack containing all the material for the analysis: the reaction vial (vial) and a vial of distilled water (vial of water).
To perform the analysis you must also have a bacteriology thermostatic incubator programmable to 30°, 37° or 44°C.

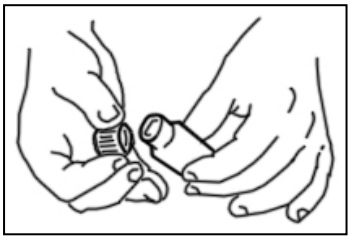
Before handling the vials and proceeding with the analysis a thorough hand washing is recommended.
Furthermore, **you should follow the current regulations for the sampling procedures and the instructions given in the following paragraphs.**

The performance analysis can be summarized into 5 phases: opening, insertion of the sample, early analysis, order analysis and sterilization. It also differs by type of sample to be analyzed: **solid**, **liquid** or **swab for surface's analysis**.

Note: for Salmonella spp. analysis on 25 g of sample, using the selective enrichment broth ESL-A32, follow the operating procedures of Appendix I.

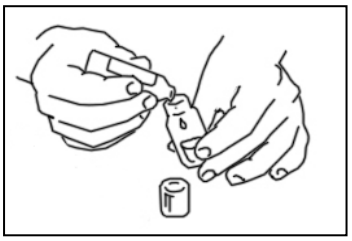
3.1 Opening

Open the vial, taking care to flip the cap so that the inner surface does not come into contact with the surface to avoid contamination.



3.2 Start analysis

Open the vial of water supplied with the reaction vial, and insert the entire contents of the vial itself.
Mix by shake the vial until the reagent is completely dissolved and no solid powder is present (20 seconds using a vortex).

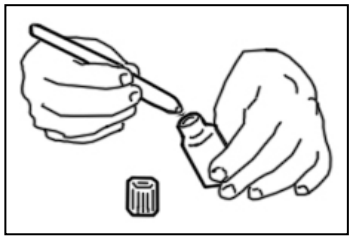


Note: the vial grows 2 liquid phases: the upper one consisting of white transparent oil (does not change during the analysis), formed at the bottom of the vial from the water with which the reagent is dissolved. The color of the second phase will develop fully within 15-20 minutes from the dissolution of the reagent and that is what must be observed for the evaluation of the results of the analysis.
Note: when using the MBS-MR/RVLM device to determine the Total Viable Count (CBT), wait at least 10 minutes from the dissolution of the reagent, before inserting into the vial the sample to analyze.

3.3 Inserting the sample

3.3.1 Solid sample

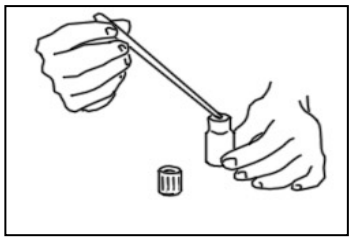
Take a small aliquot of the food (about the volume of a grain of corn, approximately corresponding to 1 g) with a tool used during the processing of the food itself and insert it into the vial (alternatively you can use sterile tweezers).
Accurately mix the sample with the solution contained into the vial by inverting the vial several times.
Place the vial in the incubator thermostat.



Note: the size or the exact weight of the sample to be examined is not so important. However, the sample must be reduced to a very small parts (maximum size 2-3 mm). Infact, the statistical variability microbiological examination of food samples is such that no method of analysis (including the reference method) has a variability of less than ± 60-70%. Therefore, the analysis has the same intrinsic value if instead of 1 g into the vial are inserted 2 g or 0.5 g. This variability is due both to the method of analysis, and to the sample itself since these microorganisms often grow in grouped colonies and therefore are not dispersed evenly in the middle.

Note: for inserting the sample into the vial, we recommend using a tool used during the processing of the food itself, since by so doing, you will be able to detect any contamination of the food due to extrinsic causes.

3.3.2 Liquid sample

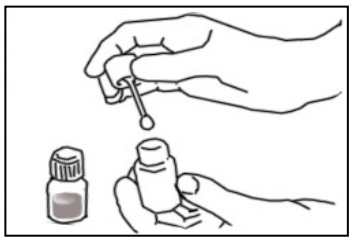


Using a pasteur dropper, sterile, disposable (supplied on request) draw about 1 ml of liquid to examine and introduce it into the vial.

Accurately mix the sample with the solution contained into the vial by inverting the vial several times.

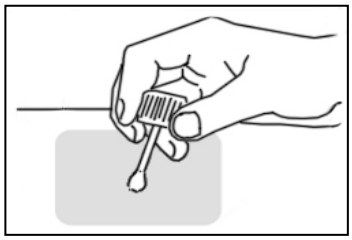
Place the vial in the incubator thermostat.

3.3.3 Swab for surface's analysis



Open the cotton swab vial (TM-A14) containing the neutralizing solution.

Rub the swab on the surface trying to cover a square area of about 10 cm per side. Insert the swab into analysis vial (previously prepared as described in 2.2)

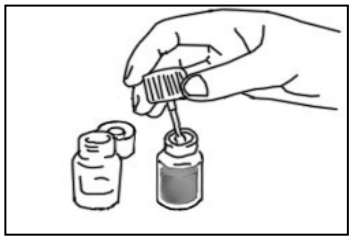


Accurately shake the vial by inverting several times.

Place the vial in the incubator thermostat.

Note: do not re-insert the swab into the vial containing the neutralizing solution.

Note: you can reseal the vial containing the neutralizing solution with the cap of reaction vial before disposal.



3.4 Check of the analysis result

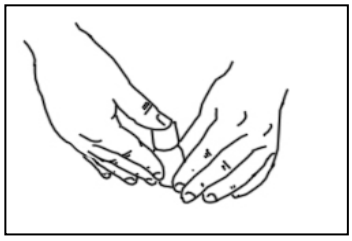
If you are not using the MBS-MR/RVLM device, you need to check the color of the vial only once, after a set number of hours. This time does not depend only on the type of analysis, but includes the operational limit of acceptable bacterial load in the sample (the generally accepted limits for the type of sample taken by law, are shown, **by way of illustration**, on the Quality Control Sheet).

The analysis result is positive if, and only if, occurs a **complete color change** of the vial content.

3.5 Post-analysis sterilization

After analysis, without opening the vial, firmly press the top of the cap and shake for about 10 seconds.

After 5-10 minutes the contents of the vial is completely sterilized.



Note: *The addition of the sterilizing agent can cause a color change*.

Note: After sterilization, the vial may be disposed of waste with EWC code n. 18 01 07 [Wastes from human or animal health care and/or related research (except kitchen and restaurant wastes not arising from immediate health care) – wastes from natal care, diagnosis, treatment or prevention of disease in humans – chemicals other than those mentioned in 18 01 06] and hazard properties HP4.

4 NOTES ABOUT MICROBIOLOGICAL ANALYSIS

Foods with particular physical-chemical characteristics (eg: low specific weight, strong acidity / alkalinity, high viscosity, marked coloration ...) may interfere with the analysis in a case-specific way.

In these circumstances, it is recommend to dilute 1g or 1ml of sample in 10ml of suitable sterile solvent (buffered peptone water, distilled water, physiological solution ...) and to analyze 1ml of the solution. *Note: if, after dilution, the sample is not completely dissolved or forms "flakes", it is necessary to take 1g and proceed as described above.*

After analysis, it is possible to identify possible initial contamination taking into account the dilution used, as required by reference methods (ISO 7218:2007).

- **Example 1**

  Food: Salad

  Type of analysis: Escherichia coli (EC-A22)

  Dilution factor: 10; diluting about 1g of food in 10ml of solvent

  Result analysis: 1.43E02 CFU/g

  Real contamination: **1.43E03 CFU/g** = 1.43E02 x **10** (dilution factor).

Similarly, products containing food additives, like preservatives, acidity regulators, antioxidants, emulsifiers, etc.., could influence the course of the analysis. Therefore, the most appropriate solution for each individual case must be sought, or sample dilution must be carried out, as described above.

- **Example 2**

  Food: Mayonnaise

  Type of Analysis: Total Viable Count at 30 °C (CBT-A01)

  Dilution factor: 10; diluting about 1g of food in 10ml of solvent

  Result analysis: 2.58E04 CFU/g

  Real contamination: **2.58E05 CFU/g** = 2.58E04 x **10** (dilution factor).

5 APPENDIX I

The following operating procedure is applicable to the combined use of the SL-A06 – Salmonella spp. reaction vials and the ESL-A32 – *Single dose preparation of Salmonella selective enrichment broth.*

The enrichment step of a food sample requires the following:

- an ESL-A32 single-dose vial;
- a highly resistant sterile plastic bag (e.g. Stomacher bags), not provided in the kit;
- sterile saline solution, not provided in the kit.

Dissolve the ESL-A32 vial content in 225 ml of sterile saline solution.

Weigh 25 g of the food sample to be analyzed in a sterile plastic bag and add the reconstituted enrichment broth.

Mix or homogenize for about 2 minutes; incubate at 37°C ± 1°C for 18 h ± 2 h.

Inoculate 1 ml of the obtained culture in the SL-A06 reaction vials, and proceed with the analysis following the standard operating procedures, previously described.

After maximum 48 hours the analysis ends with only two possible outcomes:

- the reaction vials turn yellow, indicating the **presence** of Salmonella spp.;
- the reaction vials do not change color, indicating the **absence** of Salmonella spp.

Note: in the event of results indicating the presence of Salmonella spp., it is recommended to perform confirmatory tests, in accordance with ISO 6579:2002.