Royal Biotech GmbH

Vial LAB

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Royal Biotech GmbH – VIAL LAB

BASIC SYSTEM

USER MANUAL

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1.1 Introduction

Dear User, thank you for purchasing **RB – 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 color change in the suspension formed in the analysis vial used when the test sample is added: the suspension changes color (turns) if there are microorganisms, the greater the amount of microorganisms, the more rapid is the change of color.
The main features of the **RB – VIAL LAB** are:

- **Speed**: analysis time, from preparation to the achievement of results, from 2 to 5 times less than traditional methods;
- **Ease of use**: Anyone, anywhere can do the analysis without the need for other reagents or special equipment;
- **Sensitivity**: you can detect even a single microorganism present in the sample;
- **Selectivity**: it can detect different species of microbial organisms to the experimental limit of 99.999%;
- **Cost**: The cost of each analysis turns out to be 2 to 4 times cheaper than traditional methods.

The 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 (Totals ed *E. coli*) – CO-A02;
3. *Enterobacteriaceae* – EB-A03;
4. *Staphylococcus aureus* – SP-A04;
5. *Pseudomonas aeruginosa* – PAO-A05;
6. *Salmonella* spp. – SL-A06;
7. *Listeria* spp. – LY-A07;
8. *Enterococcus faecalis* – EF-A09
9. Yeasts *(Saccharomyces spp.*) – SC-A11.

1.2 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).

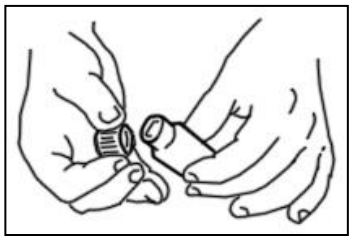
To perform the analysis you must also have a bacteriology thermostatic incubator programmable to 30°, 37° or 44 °C.

Before handling the vials and proceeding with the analysis a thorough hand washing is recommended.

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 sample**, **liquid sample**, or **surface analysis**.

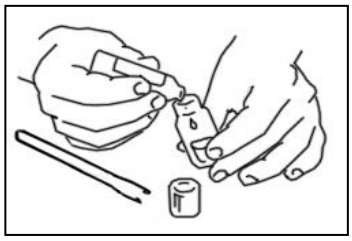
1.2.1 Solid sample

Step 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.

Step 2. Start analysis



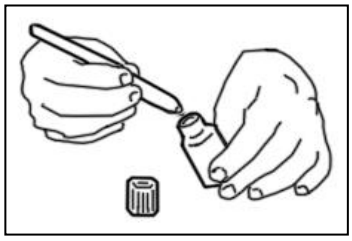
- Open the vial of water supplied with the reaction vial, and insert the entire contents of the vial itself.
- Mix by inverting the vial several times to completely dissolve the reagent (20 seconds).

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: *Using the device RB-RVLM, for the determination of Total Viable Count (CBT), wait for 10 minutes at least from the dissolution of the reagent, before inserting the sample to analyze in to the vial.*

Step 3. Inserting the 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).



- If necessary, fill in the Quality Control Sheet.
- Place the vial in the incubator thermostat.

Note: the size or the exact weight of the sample to be examined is not 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.*

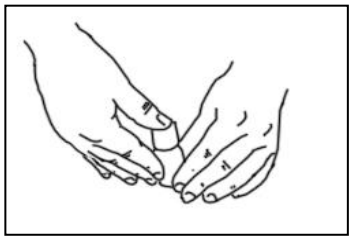
Step 4. Control of the analysis results

- You only need to check the color of the vial once, after a set number of hours depending on the type of analysis. This number of hours 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 Sheets).
- Compare the color of the vial with the correlation table set out in paragraph 1.3 or in Quality Control Sheet. The analysis result is positive if, and only if, occurs a **complete color change** of the vial content.
- If you have started the compilation of the Quality Control Sheet, fill it in with the result of the analysis.
- In the case of analysis for detection of *E. coli* and **only if the result is positive** (bright yellow vial), you can perform a test to confirm the presence of *E. coli*, adding 3-5 drops of Kovacs reagent to the vial at the end of the analysis, **before the sterilization**. If there is *E. coli*, within a few seconds you will see the development of a small surface layer of purple-red liquid.

Note: to make the test for confirmation of E. coli, open the vial carefully*, avoiding contamination with the liquid contained in it, because if the color is tacked, the vial will*

contain a significant number of bacteria and it is therefore preferable to use disposable gloves, even non-sterile, for the operation of opening the vial. If you come in contact with the liquid, wash the affected area immediately and thoroughly with antibacterial soap or normal soap and then with a disinfectant.

Step 5. Post-analysis sterilization



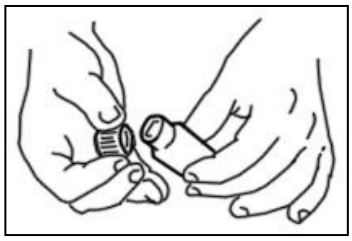
- Without opening the vial, firmly press the top of the cap and shake for about 10 seconds. The addition of the sterilizing agent can cause a color change. After 5-10 minutes the contents of the vial are completely sterilized.

Note: *when the sterilization of the microorganisms are destroyed, the reagents are rendered inert and the vial can be safely disposed of as a "not hazardous sanitary waste". Such waste, after sterilization, can be disposed of in the same way as drugs which have expired.*

1.2.2 Liquid sample

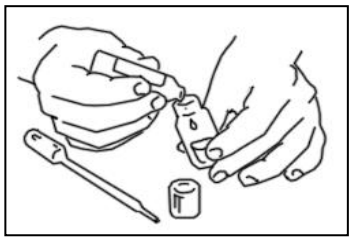
Step 1. Opening

- 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.



Step 2. Start analysis

- Open the vial of water supplied with the reaction vial, and insert the entire contents of the vial itself.
- Mix by inverting the vial several times to completely dissolve the reagent (20 seconds).

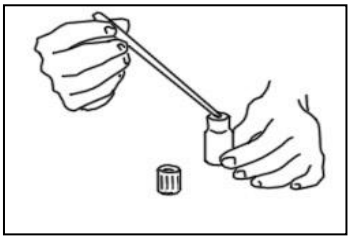


Note: *the vial grow in 2 liquid phases: the upper one consisting of white oil transparent (does not change during the analysis), formed at the bottom of the vial from the water with which the reagent is dissolved. The color of the second phase will develop fully within 15-20 minutes from the dissolution of the reagent and that is what must be observed for the evaluation of the results of the analysis.*

Note: *Using the device RB-RVLM, for the determination of Total Viable Count (CBT), wait for 10 minutes at least from the dissolution of the reagent, before inserting the sample to analyze in to the vial.*

Step 3. Inserting the sample

- Using a pasteur dropper, sterile, disposable (supplied on request) draw a quantity of fluid to examine and introduce about 1 ml of that liquid into the vial.
- If necessary, fill in the Quality Control Sheet.
- Place the vial in the incubator thermostat.



Step 4. Control of the analysis result

- You only need to check the color of the vial once, after a set number of hours depending on the type of analysis. This number of hours 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 Sheets).
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- If you have started the compilation of the Quality Control Sheet, fill it in with the result of the analysis.
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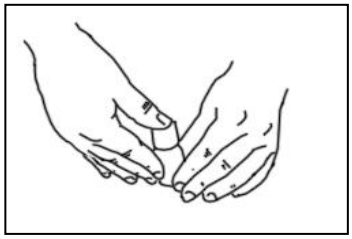
***Note: to make the test for confirmation of E. coli, open the vial carefully**, avoiding contamination with the liquid contained in it, because if the color is tacked, the vial will contain a significant number of bacteria and it is therefore preferable to use disposable gloves, even non-sterile, for the operation of opening the vial. If you come in contact with the liquid, wash the affected area immediately and thoroughly with antibacterial soap or normal soap and then with a disinfectant.*

Step 5. Post-analysis sterilization

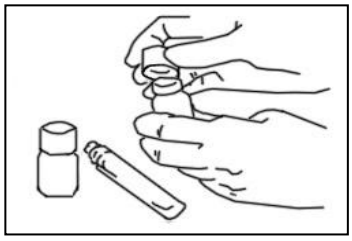
- Without opening the vial, firmly press the top of the cap and shake for about 10 seconds. The addition of the sterilizing agent can cause a color change. After 5-10 minutes the contents of the vial are completely sterilized.



Note: *when the sterilization of the microorganisms are destroyed, the reagents are rendered inert and the vial can be safely disposed of as a "not hazardous sanitary waste". Such waste, after sterilization, can be disposed of in the same way as drugs which have expired.*

1.2.3 Surface analysis

Step 1. Preparation of the <u>reaction vial</u>



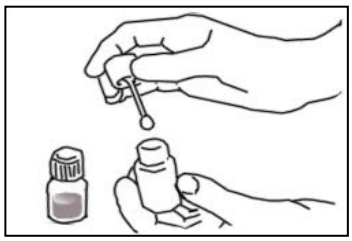
- Open the vial of water supplied with the vial for analysis, and insert the entire contents of the reaction vial itself.
- Mix by inverting the vial several times to completely dissolve the reagent (20 seconds).

Note: *after dissolution of the reagent, it is advisable to unscrew the reaction vial cap of analysis to facilitate subsequent operations.*

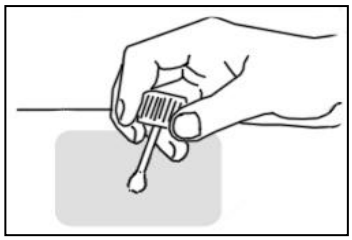
Note: *the vial will grow in 2 liquid phases: the upper one consisting of white transparent oil (does not change during the analysis), formed at the bottom of the vial from the water with which the reagent is dissolved. The color of the second phase will develop fully within 15-20 minutes from the dissolution of the reagent and that is what must be observed for the evaluation of the results of the analysis.*

Note: *<u>Using the device RB-RVLM</u>, for the determination of Total Viable Count (CBT), wait for 10 minutes at least from the dissolution of the reagent, before inserting the sample to analyze in to the vial.*

Step 2. Sampling

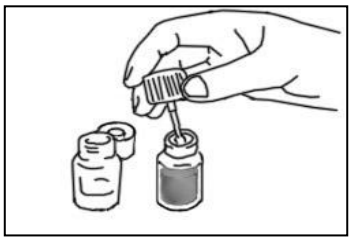


- Open the <u>cotton swab vial</u> (TM-A14) containing the neutralizing solution.
- Rub the swab on the surface trying to cover a square area of about 10 cm for side.



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

Step 3. Start analysis



- Open the previously prepared reaction vial.
- Insert the swab into reaction vial.
- If necessary, fill in the Quality Control Sheet.
- Place the vial in the incubator thermostat.

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

Step 4. Control of the analysis result

- You only need to check the color of the vial only once, after a set number of hours depending on the type of analysis. This number of hours 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).
- Compare the color of the vial with the correlation table set out in paragraph 1.3 or in Quality Control Sheet. The analysis result is positive if, and only if, occurs a **complete color change** of the vial content.
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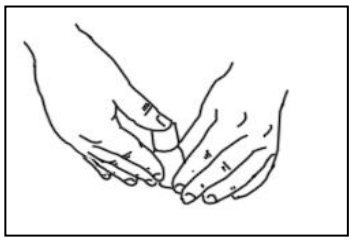
Note: to make the test for confirmation of E. coli, open the vial carefully*, avoiding contamination with the liquid contained in it, because if the color is tacked, the vial will contain a significant number of bacteria and it is therefore preferable to use disposable gloves, even non-sterile, for the operation of opening the vial. If you come in contact*

with the liquid, wash the affected area immediately and thoroughly with antibacterial soap or normal soap and then with a disinfectant.

Step 5. Post-analysis sterilization



- Without opening the vial, firmly press the top of the cap and shake for about 10 seconds. The addition of the sterilizing agent can cause a color change. After 5-10 minutes the contents of the vial is completely sterilized.

Note: *with the sterilization of the microorganisms are destroyed, the reagents are rendered inert and the vial can be safely disposed of as a "not hazardous sanitary waste". Such waste, after sterilization, can be disposed of in the same way as drugs which have expired.*

1.2.4 Notes on 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 (peptone water, distilled water, phisiological solution ...) and to analyze 1ml of the solution. Note that it is not necessary to adhere exactly to the amount indicated, since the variability of microbiological statistical analysis is about 60-70%. 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/DIS 7954).

- Example 1

  Food: Salad

  Type of analysis: *Escherichia coli* (CO-A02)

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

  Result analysis: 1.436E02 CFU/g

  Real contamination: **1.436E03 CFU/g**, i.e. 1.436E02 x **10** (dilution factor).

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: 2.583E04 CFU/g

  Real contamination: **2.583E05 CFU/g**, i.e. 2.584E04 x **10** (dilution factor).