MBS-HACCP&WATER EASY TEST

BASIC SYSTEM

USER MANUAL

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1 INTRODUCTION

Dear User, thanks for purchasing the **MBS-HACCP&WATER Easy Test**, a colorimetric system for microbiological testing of food, water and surfaces, developed in collaboration with Roma Tre University, Rome (Italy).

Microbiological testing according to the Micro Biological Survey (MBS) method is performed in ready-to-use disposable reaction vials that contain original reagents in which samples can be analyzed with no preliminary treatment. In the presence of bacteria in the sample, the suspension within the vial changes color; the observation of this color change is indicative of the presence and of the concentration of bacteria: the greater the amount of microorganisms, the faster the color change.

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".

The available original reagents are listed below:

1. Total Viable Count – **CBT-L01**
2. Coliforms – **CO-L02**
3. *Escherichia coli* **– EC-L22**
4. Enterobacteriaceae – **EB-L03**
5. *Staphylococcus aureus* – **SP-L04**
6. *Pseudomonas aeruginosa* – **PAO-L05**
7. *Salmonella* spp. – **SL-L06**
8. *Listeria* spp. – **LY-L07**
9. *Enterococcus* spp. – **EF-L09**
10. Yeast – **SC-L11**.

Note: Before use, it is recommended to download the Material Safety Data Sheet from link: http://www.emmebiesse.net/schede-sicurezza/?lang=en.

2 DISCLAIMER

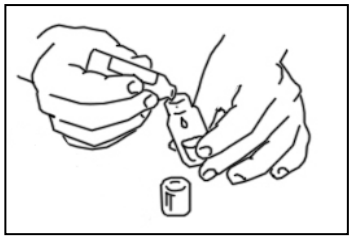
MBS-HACCP&WATER Easy test must be used only for the purpose for which it was intended; any other use is considered improper and dangerous. Users must, under their own responsibility, observe the current laws concerning health and safety.
MBS Srl, therefore, declines any responsibility for damage to persons, animals or objects that may, directly or indirectly, follow the use of the product.

3 INSTRUCTIONS FOR USE

The **MBS-HACCP&WATER Easy test** kit includes: blistered reaction vials (vial), blistered colored caps (cap), and vials containing distilled sterile water and paraffin oil (rehydration vial).
A microbiological incubator (incubation temperature range from +30° to +44°C) is required to perform analyses.
A proper hand hygiene is highly recommended before use.
Observing standard regulations for sampling procedures and complying with the instructions given in the following paragraphs maximizes the reliability of analyses.

The analytical procedure can be divided into 4 phases: vial preparation, sample inoculation, analysis and sterilization.
Specific protocols apply to different sample types: **solid samples, liquid samples** or **swabs for surface analysis.**
Note: for the detection of Salmonella spp. in 25 g of sample, follow the operating procedures of Annex I.

3.1 Vial preparation

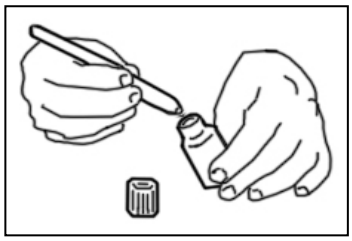


Remove vials from the blister. Open the vial and rehydrate with the rehydration vials provided in the kit. Recap using the rubber cap and shake until complete dissolution (around 20 seconds using a vortex). Remove and discard the rubber cup from the vial and insert the sample according to following sections.

Note: after rehydration, the vial content consists of 2 liquid phases: paraffin oil (upper phase) and reagent solution (lower phase). The starting color of the reagent solution will fully develop within 15-20 minutes from rehydration. Only the lower phase will change color during the analysis in the presence of bacteria.

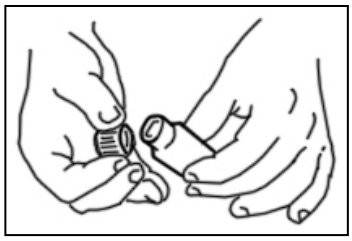
3.2 Sample inoculation

3.2.1 Solid samples



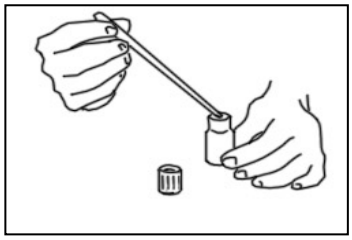
Insert 1 g of the sample in the vial using disposable or sterilizable tweezers. If not available it is recommended to use a tool employed during sample processing.

Close the reaction vial using the reaction plastic cap provided in the kit. Be sure to pick up the cap by its upper side avoiding any kind of contamination. Thoroughly shake the vial by inverting it several times.

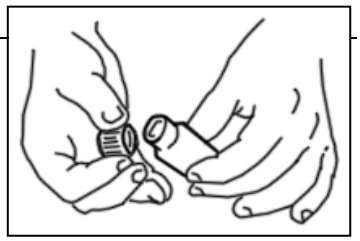


Place the vial in a microbiological incubator set at the temperature of interest (30°C, 37°C or 44°C).

3.2.2 Liquid samples



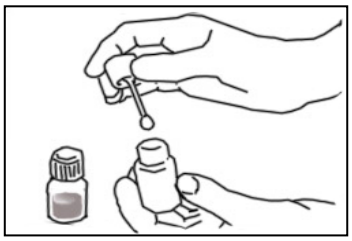
After gently inverting for 10-15 times the sample in its original container, transfer 1 ml of the sample with a sterile sucker (available upon request) and inject it into the reaction vials Close the reaction vial using the reaction plastic cap provided in the kit. Be sure to pick up the cap by its upper side avoiding any kind of

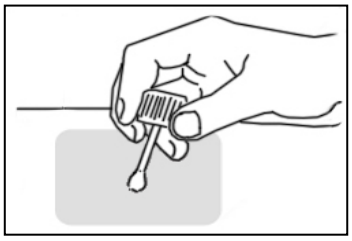


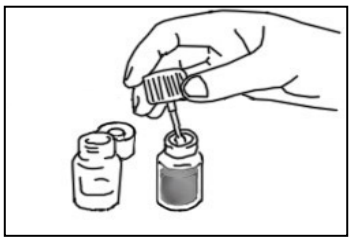
contamination.

Thoroughly shake the vial by inverting it several times.

Place the vial in a microbiological incubator set at the temperature of interest (30°C, 37°C or 44°C).







3.2.3 Swabs for surface analysis

Remove the swab vial (TM-A14) from the blister. Open the vial and rub the swab on the surface of interest trying to cover a square area of around 10 cm per side. Insert the swab into the selected reaction vial (previously prepared as described in 3.1).

Thoroughly shake the vial by inverting it several times.

Place the vial in a microbiological incubator set at the temperature of interest (30°C, 37°C or 44°C).

3.3 Interpretation of results

Results using the **MBS-HACCP&WATER Easy test – Basic system** are obtained by visual inspection of the color change of the reaction vials.

For each reagent, a **Quality Control Sheet** is available and downloadable at https://www.emmebiesse.net/en/schede-controllo-qualita/. Reagent-specific correlation tables can be used to obtain, from the time of color change of the reaction vials, the microbiological concentration of the analyzed samples (Colony Forming Units CFU per g, ml, 100cm^2), according to the type of matrix (Water, Meat, Fish, Dairy products, Vegetables, Surfaces and Other).

The interpretation of results varies according to the analysis of interest, either quantitative or qualitative.

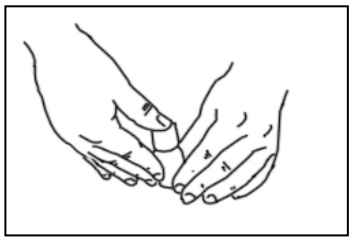
A **quantitative analysis** is performed to quantify the microbiological concentration of the sample as CFU/g, ml or 100cm^2. In this case, visual inspection of the reaction vials

should be performed approximately every three hours until detection or, if detection does not occur, until the maximum analysis time (corresponding to 0 CFU/g, ml or 100cm^2 in the correlation table). A positive result, i.e. presence of bacteria, is indicated by a complete color change of the vial (see Quality Control Sheets); while a negative result, i.e. absence of bacteria, is indicated by the persistence of the initial color (see Quality Control Sheets). In the presence of a positive result, the time for color change must be related to the concentration of bacteria (CFU/g, ml or 100cm^2) using the reagent-specific correlation table.

A **qualitative analysis**, instead, is performed to detect if the microbiological contamination of the sample is higher or lower than a set concentration value. In this case visual inspection of the reaction vials should be performed only once, at the time of detection corresponding to the concentration limit of interest, attainable by the reagent-specific correlation tables. A positive result, i.e. concentration of bacteria higher than the set limit, is indicated by a complete color change of the vial (see Quality Control Sheets); results are report as CFU/g, ml or 100cm^2 > than the set value. A negative result, i.e. concentration of bacteria lower than the set limit, is indicated by the persistence of the initial color (see Quality Control Sheets); results are report as CFU/g, ml or 100cm^2 < than the set value.

In order to facilitate this procedure, Quality Control Sheets already include a table reporting the generally accepted limits (based on EU and International regulations) and the recommended time of observation of the reaction vials, according to the type of analysis and the type of matrix.

3.4 Post-analysis sterilization



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

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

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

Note: After sterilization, the vial may be disposed as waste EWC 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

Food matrices can display peculiar physical-chemical characteristics (eg: low specific weight, strong acidity / alkalinity, high viscosity, marked coloring ...) that may interfere with the analysis method in a case-specific way. Moreover, such features can be responsible for non-homogenous distribution of microorganisms within the matrix. For these reasons, it is recommended to perform a preliminary homogenization of the sample. For this purpose, weigh 10g of sample in 90 ml of sterile diluent and homogenize following standard guidelines. Perform analyses inoculating 1ml of the food homogenate in the reaction vials.
For quantitative analysis, in order to obtain the bacterial concentration of the undiluted sample it is necessary to consider the dilution factor according to ISO 7218:2007.

Example 1

Food: Salad

Type of analysis: *Escherichia coli* (EC-L22), quantitative

Dilution factor: 10; diluting 10g of food in 90ml of diluent

Result: 10^3 CFU/g

Sample *E. coli* concentration: **10^4 CFU/g** = 10^3 x **10** (dilution factor).

Also, for qualitative analysis the dilution factor must be taken into consideration when choosing the appropriate concentration value and detection time of interest. Since the analyzed sample is 10 times less concentrated than the undiluted sample, the

concentration limit to consider for the analysis must be adjusted accordingly. The final result must be however always referred to the undiluted sample.

Example 2

Food: Salad

Type of analysis: Coliforms (CO-L02), qualitative

Concentration limit: 10^3 CFU/g

Detection time: 16,35 hours

Dilution factor: 10; diluting 10g of food in 90ml of diluent

Concentration limit: 10^2 CFU/g = 10^3 : 10

Detection time: 20,50 hours

Result: CFU/g > 10^3 CFU/g or CFU/g <10^3

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.

5 ANNEX I - SALMONELLA ENRICHMENT BROTH

According to UNI EN ISO 6579:2008 detection of *Salmonella* spp. must be performed in 25 g of sample and results must be expressed as presence or absence in 25 g. The operating procedure requires several enrichment steps to ensure an accurate detection, improving the growth of *Salmonella* spp. and removing the background microflora.

Detection of *Salmonella* spp. using the **MBS-HACCP&WATER Easy Test** is performed in SL-L06 – *Salmonella* spp. reaction vials. In order to allow the analysis of 25 g of sample and to improve the detection of *Salmonella* spp. the enrichment broth ESL-A32 – *Single dose preparation of Salmonella selective enrichment broth* has been developed in order to perform a selective enrichment of the sample.
The enrichment requires the following materials:

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

The following operating procedure must be applied:
Step 1. Dissolve the ESL-A32 vial content in 225 ml of sterile saline solution and shake until complete dissolution.
Step 2. Weigh 25 g of sample in a sterile plastic bag and add the enrichment broth prepared as above mentioned.
Step 3. Mix or homogenize for about 2 minutes;
Step 4. Incubate at 37°C ± 1°C for 18 h ± 2 h.
Step 5. Inoculate 1 ml of the enrichment medium in the SL-L06 reaction vials, and proceed with the analysis following the standard operating procedures for qualitative analysis, previously described.

After maximum 48 hours results can be:

- the reaction vial turns yellow, indicating the presumptive **presence** of *Salmonella* spp.;

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

Note: according to ISO 6579:2008, in the case of a positive result, i.e. presumptive presence of Salmonella spp., such result has to be confirmed using validated tests.