LUMEN ET VIRTUS
ISSN: 2177-2789

STUDY AND DESCRIPTION OF MICROBIOLOGICAL ASSAYS USING THE COMMERCIAL COMPACT DRY KIT

https://doi.org/10.56238/levv15n41-110

Submitted on: 30/09/2024 **Publication date:** 30/10/2024

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ABSTRACT

The objective of this research was to study and describe microbiological assays performed with the commercial kit Compact Dry. The studies and description of new methods of microbiological analysis require a description and characterization of the relationship to a standard method, this process of evaluation and characterization is called validation. The results of the technical studies are relevant and contribute to the analysis of the performance of the kit, as well as being satisfactory and effective according to the parameters analyzed and described by studies, research institutions and validation bodies. Thus, the data collected and described contemplate technical criteria of the analysis and description with the new commercial kit - Compact Dry, showing to be efficient in the microbiological analysis of water.

Keywords: Chromogenic Kit. Escherichia coli. Analysis. Water.

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INTRODUCTION

According to Carneiro (2022), the identification of microorganisms is traditionally based on microbiological cultures and biochemical processes. Technological advances indicate that, historically, microbiology has been responsible for major advances in physiological and molecular studies, among which the development of selective and differential culture media, virology, microbial genetics and microbial metabolism stands out (Borin; Martins; Taketani, 2021). Additionally, according to the authors, *op. cit*., strain identification kits have been improved by the Era of MMR - Rapid Microbiological Methods, especially in the food industries and medical clinics, which have been presenting considerable advances with new technologies.

According to Brito *et al.* (2021), in microbiological analyses, there are official reference methods (gold standard) for the detection of microorganisms, as well as their metabolites, for different types of samples. In addition, they are mostly long-term, involving numerous steps that sometimes require qualified human resources and laboratory infrastructure.

With the advent of new scientific techniques, more and more methodological procedures have emerged that have enabled the detection of faster, more practical and low-cost analytes, with equal or superior sensitivity in the results (Kumar *et al.*, 2020; Jiang *et al*., 2021; Yang *et al*., 2021).

Normally, the development and advances of new detection techniques also occur in the development of new commercial products such as kits or other devices, which are occasionally submitted to validation studies by regulatory agencies in the country (Brito *et al.*, 2020; Lemos *et al*., 2020; Brito *et al.*, 2021).

Of the technological innovations designed to carry out, in ready-made plates, technical-scientific tests aimed at the detection and microbiological quantification in raw materials, food and the environment, as an alternative to conventional practices, the Compact Dry plates of the Cap-Lab laboratory stand out. In these, the analysis corresponds to a quick and simple method, consisting of disposable material kits (Brandão, 2015). In addition, the culture medium present in the plates is made up of chromogenic inhibitor enzymes that promote the identification of microorganisms in specific colors; it has high sensitivity and provides greater efficiency when compared to conventional methods (Cesarotti; Paula; Rossi, 2007). In view of the above, this research aimed to carry out the study and description of microbiological assays using the Compact Dry kit in water samples from the stream of the Igarapé da Penal de Porto Velho – RO.

MATERIALS AND METHODS

Initially, it is a descriptive study based on the guidelines of Bastos and Keller (1995) with regard to "scientific research that is a methodical investigation [...]" and Lakatos and Marconi (2003) and Gil (2002) for bibliographic research. Thus, the surveys were prepared on free scientific research platforms available on the Global Network of Interconnected Computers – Internet such as *google* scholar, *Scielo*, Ministry of Health - MS; Scientific journals such as *Biosensors & Bioelectronics*; Free Platform of University and Research Institutes, as well as that of the Technology and Innovation Laboratory: Cap-Lab, available at: <https://cap-lab.com.br/produto/compact-dry/>, among others consulted between the years 2023 and 2024.

In the second stage, the samples were taken to the microbiology and biotechnology laboratory – MicroBiotec of the Federal Institute of Rondônia, Porto Velho Calama campus, in 2024, where microbiological tests were carried out with samples from water from the Igarapé da Penal de Porto Velho and deionized water produced in the *campus* laboratory, in accordance with technical standards and with the use of the Compact Dry Chromogenic Kit, lot: R057876121. Which presents, in addition to the nutritive substrate, the following enzymatic substances: Magenta-Gal and X-Gluc, specific for the identification of *E. coli* and coliforms, according to the manufacturer's guidelines.

As for the description of the microbiological tests, observed in the description and flowchart, below, carried out with samples for water analysis, they followed, with adaptations, the manufacturer's guidelines and technical protocols. Thus, in the tests, the following materials and equipment were used: transparent glass bottle; Styrofoam box for transporting the sample; bacteriological greenhouse; sterile pipettes/tips; Bunsen burner; Compact Dry EC Kit.

The technical procedures carried out, already specified and guided by the manufacturing laboratory, were developed in the MicroBiotec laboratory, with adaptations, following the following steps:

a- The kit was opened the laminated package containing the plates to separate them (Figure 2 a);

b- The identification of the plates was performed (Figure 2 c);

c- The sample was diluted according to the technical procedures of the laboratory for serial dilution;

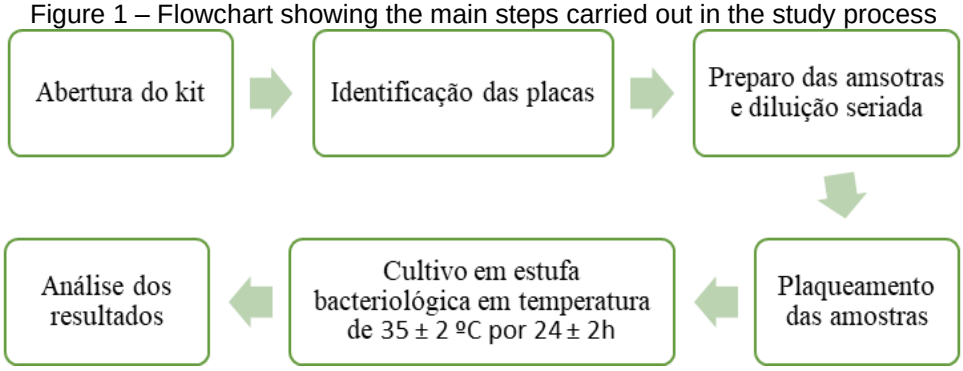
d- The cap was removed and 1mL of the sample was inoculated into the plate containing the dehydrated medium (Figure 2 b);

e- He added the kit plate and incubated in a bacteriological incubator at 35 ± 2 ºC

for 24 ± 2 hours according to a specific technical procedure;

f- After the incubation period, mentioned above, the plate was removed and analyzed observing the chromogenic aspects of the colonies.

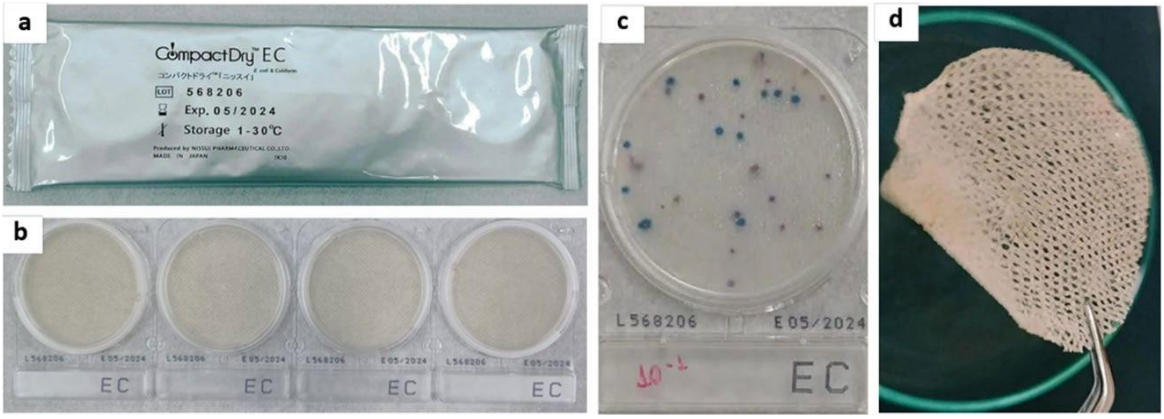
Figure 1 – Flowchart showing the main steps carried out in the study process



RESULTS AND DISCUSSIONS

The studies resulted in the characterization of the Compact Dry kit in sample microbiological assays. The commercial kit is presented in packaging characterized in a sachet of synthetic polymers, sealed, with silver-gray tones, consisting of 4 rigid polymeric plates with internal reservoirs of 50 mm and matte space for notes. In this reservoir, there is a nutrient medium with two types of enzymatic chromogenic substrates soaked in dry sheets, Magenta-GAL and X-Gluc (Figures 2b, c and d).

Figure 2 - Compact Dry kit used in microbiological tests in 2024



Source: Image bank of the authors.

The images show the characteristics of the Compact Dry Kit. In **(a)** sachet, in (**b)** polymeric plates, in (**c)** plate evidencing the activities of the chromogenic substrate and, in (**d**) dehydrated sheets constituting the medium.

Considering the relevance of the kits used in microbiological identification, Ferreira (2017) mentions that in industries, especially pharmaceuticals, the following are mostly used: a manual method that uses API galleries (profile analytical index) and an automated method that uses the Vitek system. In addition, these methods are used due to the easy

and fast execution of the technique, and also because they are easily adapted to the routine of a laboratory for microbiological analysis of products.

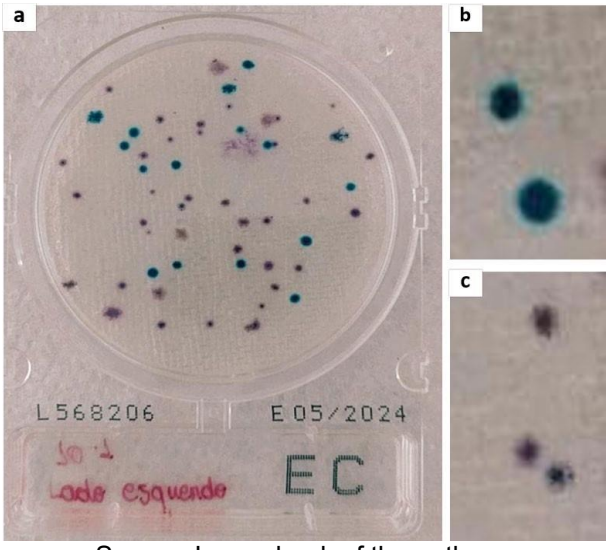
Thus, the observations showed a compact kit with the following dimensions: 77 mm high and 58 mm wide. It has, in its structure, apparatus for the operational purposes proposed by the manufacturing laboratory. Among them, in addition to the identification of the lot, the validity of the kit and the analyte, the presence of a reservoir with subtly raised edges, a lid for the culture medium and a space for notes (Figure 2c) stands out.

One of the relevant aspects of the kits is, in addition to economy and operationality, in microbial identification. Data show that, in these sets of substrates, it is possible to classify an isolated strain according to its family, genus and species, as well as to carry out more complete investigations, determining its origin, the nature of specific contamination events and knowing the bioburden present in that process to take the necessary measures according to compliance with regulatory and legal requirements (Easter, 2005; Brito, 2019; Challener, 2019).

Regarding the morphological and chromogenic aspects observed in the colonies in the tests performed, both for total coliforms and for *E. coli*, they mostly presented rounded shapes with approximate dimensions of 0.41 mm to 1.48 mm in diameter and colors that varied in predominantly red, magenta and blue tones (Figure 3a). The colonies identified as *E. coli* are characterized by blue to purple (Figure 3b) and those of total coliforms with reddish and/or magenta color (Figure 3c).

Colonies of total coliforms and *E. coli* are usually observed due to the association of metabolic processes mediated by the chromogenic substrate enzymatic, organic substances characterized as chemoorganotrophs, can be expressed in different colors, under appropriate cultivation conditions, depending on the species, as cited by Bouzon, Gargioni and Ouriques (2010) and indicated in the references for other tests (BRASIL, 2013).

Figure 3 - Plate showing colonies of *E. coli* and total coliforms with colors and characteristics observed in the assays with raw water samples



Source: Image bank of the authors.

In figures 3a, b and c, on the kit plate, above, the growth of *E. coli colonies* and total coliforms obtained with serial dilution at 10-1 are observed. Data from the literature indicate that the results with conventional tests may be slightly different from those obtained with rapid biochemical test systems due to the use of other more sensitive indicators, such as those already described in the works of Över, Tüç and Söyletir (2000).

However, in recent kits, such as the one used in this study and made available by the Cap-Lab laboratory (2024), they mention that the method allows a perfect absorption of inoculated samples, which makes it possible to identify and quantify most of the bacteria of interest to the food industry. Additionally, in the substrate there are two types of enzymatic chromogenic compounds, Magenta-GAL and X-Gluc, which act by allowing the expression of clusters of bacteria with reddish (Magenta-GAL) and bluish (X-Gluc) colors. Thus, in addition to the stains already described, in the process of counting the colony-forming units - CFU, the author (*op. cit*.), describes that the total number of coliform clusters is the sum of the total number of red and blue colonies combined, as observed in the tests carried out in the water analyses of this study.

As for Magenta-Gal (C14 H15 BrClNO6), it is characterized by being a compound used as an alternative to X-Gal. Normally, in the metabolic process, it is hydrolyzed by β-galactosidase to produce a red precipitate that is insoluble in alcohol and xylenes, making it suitable for immunoblotting and immunocytochemical assays. In this way, bacterial colonies, such as those of total coliforms, containing *active β-galactosidase produce red tones when cultured in plates containing Magenta-Gal, as described by GoldBio (2024) and observed in the results of the assays carried out (Figures 3a and c).*

For the chromogenic substrate X-Gluc (X-Glucuronide CHA Salt), it is used in several applications for the detection of the enzyme β-glucuronidase, identifying the GUS gene (*β*-glucuronidase), an accurate indicator of the presence of *E. coli* in drinking water samples, its full name is cyclohexylammonium salt of 5-bromo-4-chloro-3-indolyl-β-D-glucuronic acid (C20H26BrClN2O7). It is an internationally accepted compound in the identification of *E. coli* and reported as a good reducer of false positives and negatives found with other methods. In addition, the substrate detects β-glucuronidase and that, due to the biochemical characteristics of the substrate and the cells, after reduction, X-Gluc, produces a localized color, making it useful in identifying the presence of the GUS gene, as already characterized by X-Gluc Direct (2024) and observed its phenotypic expression in the present study.

The studies and operational sample tests carried out enabled the technical description of the procedures with the Compact Dry kit. It was structured, based on the manufacturer's guidelines and technical specifications, with properties already specified in the works of Ferreira Júnior (2002), Cesarotti, Paula and Rossi (2007) and with results characteristic of those present in other studies such as those of Hosakawa and Kodaka (2010) and Cardoso *et al.* (2019).

The chromogenic substrate is notably an option for the method of performing bacteriological examinations of the water and falls on one of the procedures that best suits the conditions of the laboratory (Brasil, 2013). However, the methodologies, frequencies and interpretation of results established and recommended by the legislation in force should be adopted as a standard.

Thus, the kit presented itself as a promising resource in the sample evaluations of effluents, with the identification of total coliforms and enterobacteria, especially *Escherichia coli*.

CONCLUSION

This study and description of microbiological assays using the Compact Dry EC kit structured data with operational dimensional characteristics, as well as demonstrated that the step-by-step process for performing the tests is efficient.

In the kit, the chromogenic substrate present in the culture medium made it possible to efficiently identify and count *E. coli* and also total coliforms, compatible with the data present in the literature.

In the trials, there was no need to use complementary or auxiliary equipment for secondary or complementary tests. In addition, the cultivation time indicated by the manufacturer (24 ± 2 h) was sufficient for the analyses.

Thus, it is concluded that the tests with the Compact Dry EC Kit use a recently developed technology using chromogenic substrates soaked in dry sheets, being an alternative for routine microbiological analysis in water.

ACKNOWLEDGMENT

To the Federal Institute of Rondônia – IFRO, to DEPESP, DAPE and DEPEX of the Porto Velho Calama Campus and to all members of the Laboratory of Microbiology and Biotechnology – MicroBioTec, for their support and collaboration.

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