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Evaluation of the Performance and Stability of the VRBG Culture Medium Prepared In-House at the Laboratory—Senegal

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Abstract

Violet Red Bile Glucose Agar (VRBG) is used for the enumeration of Enterobacteriaceae in food microbiology. According to ISO 11133:2014, its maximum usable time after preparation is four hours, unless its stability and performance are validated by the laboratory. Thus, the objective of our study was to evaluate the microbiological performance and stability of VRBG medium prepared in-house at the Laboratory of Food Safety and Environmental Hygiene (LSAHE) of the Pasteur Institute over a two-month period. Five independent batches of VRBG medium were prepared and subjected to physicochemical and microbiological tests conducted on Day 0, Day 15, Day 30, Day 45, and Day 60. The physicochemical tests involved measuring the pH and visually inspecting the medium, while the microbiological tests assessed sterility, productivity, and selectivity. The microbiological tests were performed using reference strains adapted to the requirements of ISO 11133:2014. The results showed that the VRBG medium maintained physicochemical characteristics within the acceptance criteria throughout the sixty days of storage. The microbiological tests confirmed that sterility was maintained, along with satisfactory productivity—with productivity ratios exceeding the acceptance threshold set by the standard, and good selectivity against non-target microorganisms. These results suggest that the in-house prepared VRBG medium can be used for a period of two months under the storage and testing conditions evaluated, provided that its stability and performance are validated by the laboratory in accordance with the requirements of ISO 11133:2014.

Keywords

VRBG, Enterobacteriaceae, Stability, Microbiological Performance, ISO 11133:2014

1. Introduction

Food safety is a major public health issue worldwide. According to the World Health Organization (WHO), nearly 866 million people fall ill after consuming contaminated food each year, resulting in approximately 1.52 million deaths [1]. These illnesses are primarily caused by food contamination with pathogenic microorganisms or microorganisms indicative of poor microbiological quality. Microbiological analyses are used to detect and identify microorganisms in food and to assess food safety and quality [2].

Among indicator microorganisms, Enterobacteriaceae play a significant role in the microbiological evaluation of food. Their presence indicates contamination and poor hygiene during the production or handling of food products [3]. The enumeration of these microorganisms is widely used to assess the microbiological quality of food products.

The enumeration of *Enterobacteriaceae* was performed according to the colony-count technique described in ISO 21528-2:2017 [4]. The microbiological performance of the VRBG medium was evaluated according to the principles and performance criteria specified in ISO 11133:2014 [5].

In food microbiology laboratories, either ready-to-use culture media or media prepared in-house from dehydrated powders are used. Many laboratories opt for the latter option to reduce procurement costs and minimize the impact of potential delivery delays [6]. However, in accordance with ISO 11133:2014, VRBG medium must be used within a maximum of four hours after preparation [5].

It is nevertheless possible to extend this timeframe if the laboratory demonstrates, through internal validations, that the medium's performance is maintained beyond the recommended time limit [5]. Given that laboratories sometimes have limited financial, material, and human resources, this approach is of significant interest.

Previous studies have evaluated the performance and formulation of VRBG and other culture media [7] [8]. However, they do not directly address the stability of VRBG prepared in-house and stored under defined laboratory conditions over an extended period. Therefore, laboratory-specific validation is necessary to assess its stability and microbiological performance during prolonged storage.

The objective of this study is to evaluate, over a two-month period, the physicochemical stability and microbiological performance of the VRBG medium prepared in-house and stored under laboratory conditions, in accordance with the requirements of ISO 11133:2014.

More specifically, the study aims to prepare five independent batches of VRBG

medium and then conduct periodic monitoring of the different batches at 15-day intervals by evaluating their performance through physicochemical (pH and visual inspection) and microbiological (sterility, productivity, and selectivity) tests.

2. Materials and Methods

2.1. Biological Materials and Reagents

Our study was conducted at the Pasteur Institute in Dakar, specifically in the Food Safety and Environmental Hygiene Laboratory.

Dehydrated VRBG medium was used to prepare agar plates for stability and microbiological performance testing. Tryptic Soy Agar (TSA) served as the reference medium for productivity tests, while Tryptone Salt (TS) medium was used to prepare bacterial suspensions and decimal dilutions. A sodium hydroxide (NaOH) solution was used to adjust the pH during the preparation of the VRBG medium.

Microbiological tests were conducted using the reference strains *Escherichia coli* ATCC 8739, *Salmonella* Typhimurium ATCC 14028, and *Enterococcus faecalis* ATCC 19433, in accordance with the requirements of ISO 11133:2014.

2.2. Preparation and Storage of VRBG Medium

Five separate batches of VRBG medium were prepared on different days in accordance with the manufacturer's recommendations and the requirements of ISO 11133:2014 [5] [9]. After weighing, the dehydrated powder was dissolved in purified water. The pH of the preparation was then adjusted to 7.4 ± 0.2 before heating in a water bath until the agar was completely dissolved. The melted medium was then distributed into five glass bottles, with one bottle dedicated to each scheduled storage point (Day 0, Day 15, Day 30, Day 45, and Day 60) which were hermetically sealed for microbiological testing, as well as into five separate aliquots used for physicochemical testing at the corresponding storage point. The various batches were stored at 5˚C ± 3˚C until evaluation.

2.3. Preparation of Reference Strains and Microbial Suspensions

Reference strains were subcultured on TSA one day before each control. Microbial suspensions were prepared in 9 mL of Tryptone Salt (TS) from isolated colonies and adjusted to approximately 2 McFarland (≈10^9 CFU/mL). Serial decimal dilutions were then prepared to obtain approximately 10^2 CFU/mL for the productivity tests with *Escherichia coli* ATCC 8739 and *Salmonella* Typhimurium ATCC 14028, and 10^5 CFU/mL for the selectivity test with *Enterococcus faecalis* ATCC 19433.

For microbiological testing, 1 mL of inoculum was used for deep inoculation. The same target inoculum was used for both VRBG and TSA.

For each microorganism and test condition, two plates were prepared, with one plate used for the reported data and the second retained as a reserve in case of a problem with the first plate.

The first layer consisted of approximately 15 mL of VRBG medium, in accordance with ISO 21528-2:2017 [4]. After solidification, approximately 5 mL of

VRBG medium was added as a second layer. The covering layer was used to limit colony spreading and provide semi-anaerobic conditions during incubation.

Productivity was assessed on VRBG using TSA as the reference medium and expressed as:

$$Pr = \text{Number of colonies on VRBG/Number of colonies on TSA}$$

A $Pr \geq 0.5$ was considered acceptable. Selectivity was assessed using *E. faecalis* ATCC 19433, with absence of growth considered satisfactory. VRBG and TSA plates were incubated at 37˚C for 24 h.

2.4. Performance and Stability Evaluation

The stability of the VRBG medium was evaluated through physicochemical and microbiological tests conducted every fifteen days, including the day of preparation (D0, D15, D30, D45, and D60), over a two-month period.

At each scheduled control point, the bottle corresponding to the storage time was used for microbiological testing. The stored VRBG medium was regenerated in a water bath at 95˚C until complete regeneration and then transferred to a cooling bath at 45˚C, before inoculation.

The physicochemical tests included pH measurement as well as a visual inspection to check for color, clarity, the presence of precipitates, undissolved particles, and syneresis.

Microbiological performance was evaluated in accordance with the requirements of ISO 11133:2014 through sterility, productivity, and selectivity tests. Productivity was determined by calculating the productivity ratio (Pr), obtained by dividing the number of colonies observed on the VRBG medium by the number of colonies obtained on the reference medium (TSA). According to ISO 11133:2014, this ratio must be greater than or equal to 0.5. The acceptance criteria applied to the various tests are those defined by this standard.

For the sterility test, one uninoculated plate was examined for each batch at each scheduled storage point, and the absence of growth was considered satisfactory. For the selectivity test, complete absence of growth of *Enterococcus faecalis* ATCC 19433 on VRBG was considered satisfactory. Sterility and selectivity were assessed at each scheduled storage point (D0, D15, D30, D45, and D60).

2.5. Data Analysis

The data obtained were entered and processed using Microsoft Excel. The results are presented in tables and graphs to assess changes in the physicochemical and microbiological parameters of the VRBG medium over the two-month storage period.

3. Results and Discussion

3.1. Physicochemical Stability of the VRBG Medium

Changes in pH

The change in the pH of the VRBG medium over the 60-day storage period is

shown in Figure 1.

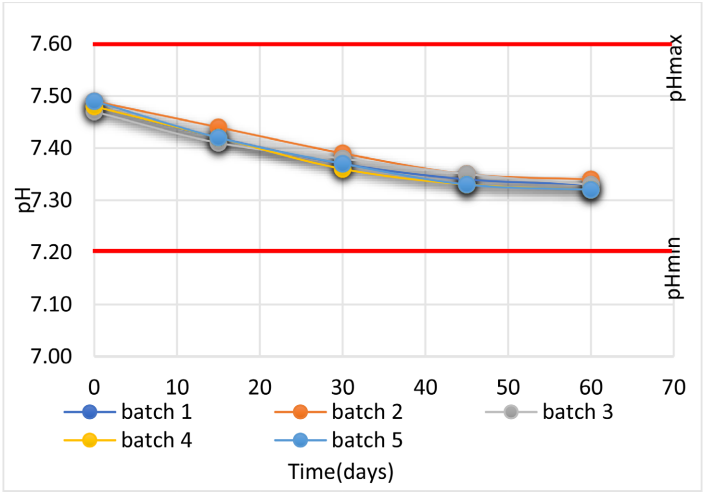

Figure 1. pH trend curve for VRBG batches over 60 days.

The pH of the VRBG medium showed a slight decrease over the 60-day storage period. However, the values remained within the acceptance range specified by ISO 11133:2014 (7.2 to 7.6), thus demonstrating good physicochemical stability of the medium throughout the study period.

A slight decrease in the pH of culture media during storage was also reported by Ulisse *et al.* (2006), who evaluated physicochemical and microbiological parameters during the shelf-life assessment of culture media [10]. Despite this observed decrease, no impact on the microbiological performance of the VRBG medium was detected during this study.

Visual Inspection

The results of the visual examination of the VRBG medium during storage are presented in Table 1.

Table 1. Results of the visual examination of the VRBG medium during storage.

Parameter	Observation
Color	Within specifications
Transparency	Compliant
Precipitates	None
Undissolved particles	None
Syneresis	None

No macroscopic changes were observed during the 60-day storage period. The VRBG medium retained its color and transparency and showed no precipitates, undissolved particles, or syneresis. These observations demonstrate the physical stability of the VRBG medium and comply with the requirements of ISO 11133:2014.

The visual appearance of the VRBG medium after storage is shown in Figure 2.

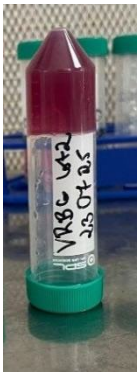

Figure 2. Visual appearance of the VRBG medium after storage.

3.2. Microbiological Performance of the VRBG Medium

Sterility

Figure 3 shows a representative sterility test result obtained on Day 60 after 24 h of incubation.

Figure 3. Sterility test of VRBG medium after 24 hours of incubation on Day 60.

The sterility test performed on the various batches of VRBG medium showed no microbial contamination. No growth was observed in the plates incubated without inoculum after 24 hours of incubation. No growth was observed at any of the scheduled storage points. These results indicate that no detectable microbial contamination occurred during the preparation, packaging, and storage of the medium under the conditions evaluated.

Similar results were reported in guidelines and recommendations concerning the quality assurance and storage of microbiological culture media [11] [12]. These recommendations emphasize the importance of appropriate preparation, storage conditions, and quality control to minimize the risk of microbial contamination during storage. In the present study, the absence of contamination up to Day 60 demonstrates that the in-house-prepared VRBG medium retained its microbiological integrity throughout the entire study period.

Productivity

The results of the productivity tests conducted with *Escherichia coli* and *Salmonella* Typhimurium strains are presented in Table 2 and Table 3.

Table 2. Results of the productivity ratio (Pr) of the *E. coli* strain on VRBG medium.

Days \ Batch	1	2	3	4	5
0	1.04	1.00	0.70	0.88	0.99
15	1.06	0.93	0.99	0.99	1.04
30	1.00	1.00	1.00	0.82	1.08
45	0.87	0.94	1.07	0.92	1.02
60	0.94	1.15	0.86	0.95	0.95

Table 3. Productivity ratio (Pr) results for the *S.* Typhimurium strain on VRBG medium.

Days \ Batch	1	2	3	4	5
0	1.00	0.99	1.12	1.04	1.00
15	1.07	0.94	0.92	1.40	1.13
30	1.00	1.14	0.98	0.84	0.85
45	0.92	0.95	0.96	0.97	1.00
60	1.04	0.98	1.00	0.98	0.98

The change in the productivity ratio over the 60-day storage period for the *E. coli* and *Salmonella* Typhimurium strains is illustrated in Figure 4 and Figure 5, respectively.

The results show that the productivity ratios remain above the acceptance threshold set by ISO 11133:2014 (Pr ≥ 0.5) for all batches and testing time points. These findings are consistent with previous studies evaluating the performance of VRBG and other selective media for the recovery of Enterobacteriaceae [8] [13].

These fluctuations may be attributed to the biological variability of the reference strains, the preparation of the bacterial suspensions, or the seeding and

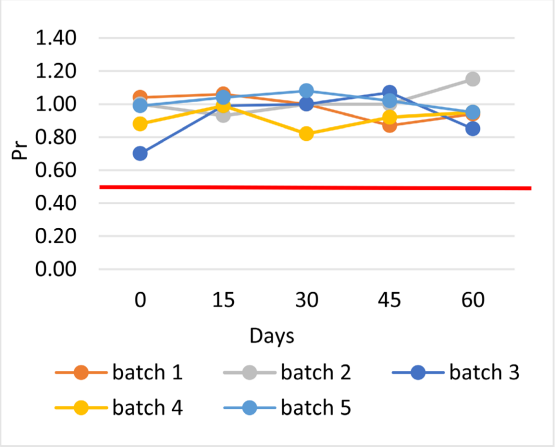

Figure 4. Curve showing changes in the productivity ratio over 60 days of storage for the *E. coli* strain.

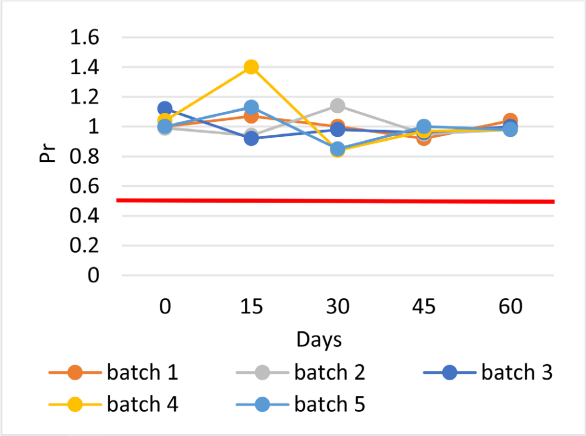

Figure 5. Productivity ratio trend curve over 60 days of storage for the *S. Typhimurium* strain.

incubation conditions.

Selectivity

Figure 6 shows a representative selectivity test result obtained on Day 60 after 24 h of incubation.

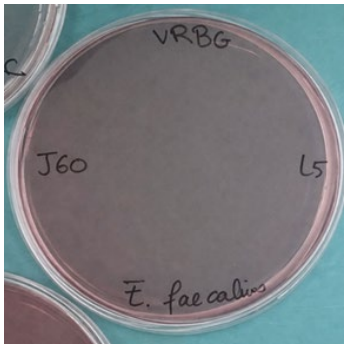

Figure 6. Selectivity test of the VRBG medium after 24 h of incubation on Day 60.

The selectivity test performed with *E. faecalis* showed a complete absence of growth on VRBG medium. This result was observed for all batches and at all testing time points.

The VRBG medium contains inhibitory agents, notably bile salts and crystal violet, which limit the growth of Gram-positive bacteria [14]. The absence of growth *of Enterococcus faecalis*, a Gram-positive bacterium, therefore supports the maintenance of the selective properties of the medium under the conditions evaluated.

These observations are consistent with previous studies evaluating the microbiological performance and selectivity of VRBG and other selective media [8] [14].

4. Conclusions

Culture media play an essential role in food microbiological analyses, as they directly contribute to the reliability of analytical results. This study focused on evaluating the microbiological performance and stability of the VRBG culture me-

dium prepared in-house at the Laboratory of Food Safety and Environmental Hygiene (LSAHE) of the Pasteur Institute in Dakar.

Five independent batches of VRBG medium were prepared and then subjected to physicochemical and microbiological tests conducted every fifteen days over a 60-day period. The physicochemical tests (pH measurement and visual inspection) showed results that complied with the requirements of ISO 11133:2014. Microbiological tests for sterility, productivity, and selectivity confirmed that the medium maintained its microbiological performance throughout the entire storage period.

Under the storage and testing conditions evaluated in this study, the in-house-prepared VRBG medium maintained acceptable physicochemical characteristics and microbiological performance for up to 60 days. However, this extension of the shelf life is contingent upon the implementation of an internal validation protocol that complies with the requirements of ISO 11133:2014, as well as regular monitoring of the medium's performance.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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