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SHORT COMMUNICATION

# Using the mini-VIDAS® Easy *Salmonella* protocol to assess contamination in transitional and coastal waters

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**Abstract** Classical methodologies for Salmonella detection may be too long in time to assure public safety. Presently, one of the fastest assays for *Salmonella* detection using the mini-VIDAS® system is the Easy *Salmonella* protocol. This assay, developed for food matrixes analysis, was here assessed for the applicability on the detection of these bacteria in transitional and saltwaters. The presence of *Salmonella* was detected in 4.2 % of the samples studied. In these transitional waters, the proposed protocol presented an efficiency of 79.1 %, due to a high false positive rate (20.8 %), and a false negative rate of 0 %—implying reducing analysis time, the use of enrichment broths, and making it more cost effective. Despite the multitude of samples nature, the method here described revealed to be an efficient and promising tool for transitional waters analysis.

**Keywords** Environmental analysis · Enzyme-linked fluorescent assay · Microbiology safety · Fecal pollution indicators

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## Introduction

*Salmonella* is a bacterial pathogen excreted in animal feces, causing gastroenteritis and typhoid fever in humans. These microorganisms may be found in waters, when industrial, domestic and/or agricultural runoffs are present (originated mainly from piggeries, poultry breeding farms, etc.) (Willey et al. 2008). *Salmonella* presence was included in the 1976 European Bathing Water Directive (EEC 1976) as one of the evaluation parameters of bathing waters. The revised new Directive 2006/7/EC (EC 2006) excluded this parameter, and the microbiological pollution monitoring is based, since then, only in indicator bacteria (as *Escherichia coli* and intestinal enterococci), used as predictors of microbiological contamination of pathogens, such as *Salmonella*. Nevertheless, a clear relationship between these indicators and the presence of *Salmonella* (as well as the presence of other pathogens) is not always evident (e.g., Schets et al. 2008; Mansilha et al. 2010). In fact, the presence of *Salmonella* has been reported in the absence of fecal pollution indicators (Mansilha et al. 2010).

Classical methodologies for *Salmonella* detection (ISO 19250 2010) are time consuming (the standard protocol can last about 8 days, if presumptive *Salmonella* exists in the sample), which may be too long to prevent potential human contamination and assure public safety. The mini-VIDAS® auto-analyzer from bioMérieux (Marcy l'Etoile, France) allows the detection of *Salmonella* in food matrixes in about 2 h after enrichment, using the Vitek Immunodiagnostic Assay VIDAS® *Salmonella* system. A presumptive positive result pointed by the mini-VIDAS® assay implies further confirmation steps, and thus, the final result is postponed. This methodology, however, is particularly useful when *Salmonella* is absent from the sample. In this case,

the mini-VIDAS® assay can be concluded in less than 48 h, opposite to the standard procedure, where a negative result is achieved after 3–4 days. This assay, adopted by the Association of Official Analytical Chemists (AOAC), is an automated enzyme-linked fluorescent assay (ELFA), detecting *Salmonella*'s antigens, and which intensity of fluorescence is expressed in relative value of fluorescence (RVF) (AFNOR 2009).

The mini-VIDAS® system, using the dual selective enrichment protocol (AFNOR 1994), was used before for the surveillance of *Salmonella* in waters from rivers, beaches, coastlines, wells, streams and sewages (Ho and Tam 2000; Mansilha et al. 2010). Nevertheless, bioMérieux has developed new rapid mini-VIDAS® procedures for food analysis, as the Easy *Salmonella* protocol (AFNOR 2005) that allows a negative result to be known 1 day sooner comparing with the dual selective enrichment protocol tested by Ho and Tam (2000) and Mansilha et al. (2010). As far as the authors knowledge, the mini-VIDAS® Easy *Salmonella* protocol was never applied to transitional and saltwaters analysis. Furthermore, the characterization of the waters was not fully described by Ho and Tam (2000) and Mansilha et al. (2010). It is important to characterize the type and the degree of contamination, in order to be able to validate the methodology in different backgrounds.

Therefore, this study was conducted to evaluate the efficacy of the mini-VIDAS® system, applying the Easy *Salmonella* protocol, for detection of *Salmonella* in environmental samples of transitional and coast waters.

## Materials and methods

### Study area and sample collection

The estuary of S. Domingos River (Western Region, Portugal; Fig. SI 1) was used as a model system.

Four sampling stations were selected; The Station 1 (S1) was at the Molho Leste Beach (39°21′01.03″N; 9°22′07.59″W), in the open seawater. The Station 2 (S2) was located upstream, between the sea and the junction of the S. Domingos River and the Barradas River (39°21′03.62″N; 9°21′59.01″W). The Station 3 (S3) was located upwards, at the Barradas River (39°21′03.19″N; 9°21′32.49″W), presenting a water column of a few centimeters deep. The Station 4 (S4) was also upwards (39°21′05.44″N; 9°21′35.02″W), at the section resulting from the union of the S. Domingos River with the Ferrel River, showing a larger flow, with approximately 1 m water depth.

Every station was sampled six times, from April until mid-June during low tide. Water samples for microbiological and physical–chemical analysis were collected: 1.5 L for microbiological assessment, in a sterilized glass flask, and 1.5 L in a clean inert plastic bottle for physical–chemical quality assessment. The sampling, transport and storage of the samples were carried out according to APHA (2005). A total of 24 samples were used for microbiological and physical–chemical analysis. The sampling occurred always during the morning time of the day, in order to avoid daily variation bias in the data analysis.

### Microbiological procedures

#### *Salmonella detection*

*Salmonella's* presence/absence was assessed according to both the standard procedure (ISO 19250 2010) and the revised mini-VIDAS® Easy *Salmonella* method (AFNOR 2009) (Fig. SI 2). All media were supplied by Merck (Darmstadt, Germany), except for Rappaport–Vassiliadis–Soya broth (RVS, bioMérieux, Marcy l'Etoile, France) and Kristensen agar (Bio-Rad, Hercules, CA, USA).

In order to compare the procedures, the pre-enrichment step was common to both. As pre-enrichment, 1L of sample was concentrated by membrane filtration (0.45 µm) that was then incubated in buffered peptone water (BPW, Merck, Darmstadt, Germany) at 37 ± 1 °C for 18 h.

Regarding the mini-VIDAS® Easy *Salmonella* protocol, after the pre-enrichment (in BPW), 0.5 mL was transferred into 10 mL Salmonella Express 2 tubes (SX2, bioMérieux, Marcy l'Etoile, France) that were incubated for 22–26 h at 41.5 ± 1 °C. Following boiling (during 15 min) of 2 mL of the enrichment broth SX2, 0.5 mL of the heated sample was transferred into a mini-VIDAS® reagent strip (containing the SPR), and the sample was analyzed by the mini-VIDAS® system. VIDAS® immunoassays yielding RVF < 0.23 indicates the presumptive absence of *Salmonella* in the sample, ending the analysis of these samples. RVF ≥ 0.23 indicates the presumptive presence of *Salmonella* in the sample tested. Presumptive positives were confirmed following ISO 19250 (2010) using the remaining enrichment. The original culture (in SX2) was transferred to RVS, and after incubation at 41.5 °C for 24–48 h it was streaked onto both xylose lysine deoxycholate agar (XLD) and Kristensen agar and further incubated at 37 °C for 24 h. The suspicious colonies were purified on nutrient agar. Isolates from the mini-VIDAS® test (and isolates from the standard procedure) were screened biochemically with triple sugar iron agar (TSI) and API 20E as described by the manufacturer (bioMérieux, Marcy l'Etoile, France). Serological confirmation was performed according to ISO/TR 6579-3 (2014), when needed.

*Microbiological indicators*

The fecal indicators studied were coliforms and *E. coli* (using Colilert-18®/Quanti-Tray2000®) and intestinal enterococci (using Enterolert®/Quanti-Tray2000®). One hundred milliliters of water was tested using Colilert-18® and Enterolert® tests, according to the manufacturers' instructions (IDEXX Laboratories, Westbrook, Maine). The results were expressed as most probable number (MPN) of microorganisms per 100 mL.

Additionally, the aerobic heterotrophic bacteria growing on plate count agar at 22 and 37 °C (for 72 h) were assessed (according to ISO 6222 1999).

### Chemical–physical analysis

To characterize the sampled water: temperature, dissolved oxygen, salinity and pH were measured in situ, at all sample locations, using a portable multiparameter probe HANNA Hi 9828 (Woonsocket, USA). The water samples collected in each sampling site were immediately filtered (Whatman GF/C grade glass fiber filter: 1.2 μm), in the laboratory, and stored frozen at −18 °C until further analyses: following standard methods described in (1) Limnologisk Metodik (1992) for ammonia ($NH_4^+$) and phosphate ($PO_4^{3-}$), (2) in Strickland and Parsons (1972) for nitrite ($NO_2^-$) and in (3) Parsons et al. (1984) for chlorophyll a (phytoplankton chlorophyll *a* was extracted from the filters).

### Statistical analysis

To characterize sites and relate them according to their physical–chemical parameters, a principle component analysis (PCA) for all data from all sampling sites using the CANOCO version 4.0 package (ter Braak and Smilauer 1998) was conducted. The PCA allowed the detection of similarities and dissimilarities between the different samples as well as permitted to identify the main associations among environmental parameters that are responsible for the total variability of the studied data. The PCA model was built following standardization of environmental data, and full cross-validation was used to validate the model. Although only the results concerning the first two components are here presented, the others were also analyzed.

## Results and discussion

Here, the mini-VIDAS® Easy *Salmonella* protocol was investigated regarding its usefulness for evaluating the presence of *Salmonella* in saltwaters samples, thus providing a vaster array of uses and value this equipment present in widespread laboratories. Methodologies for the assessment of food meant for human consumption and for environmental samples that correspond to industrial surfaces and other industrial matrixes were validated in 2005 against the classical procedure (ISO 6579 2002). Since 2005, this test has been preferred by many food laboratories worldwide, due to its faster results when compared to the mini-VIDAS® dual enrichment protocol. In the present study, the RVF was higher than 0.23 in six samples; however, only one of these samples effectively presented *Salmonella* (Table 1).

**Table 1** Evaluation of mini-VIDAS® Easy *Salmonella* rapid method for detection of *Salmonella* from transitional waters (from river São Domingos)

| | Easy *Salmonella* | ISO 19250 (2010) |
|---|---|---|
| True positives | 1 | 1 |
| True negatives | 18 | 23 |
| False positives | 5 | 0 |
| False negatives | 0 | 0 |
| Total | 24 | 24 |

Thus, the false positive rate (defined as the presumptive positives that were further confirmed as negatives) of the mini-VIDAS® protocol was determined as being 20.8 % ($n = 24$: Table 1). This rate is higher than the 7 % obtained by Mansilha et al. (2010), which used the dual enrichment protocol for coastal, transitional and inland waters. The lower false positive rate can be explained by the similarities on the selectivity and specificity between the enrichment broths of the mini-VIDAS® protocol (dual enrichment) and the standard procedure (ISO 6340 1995) used by these authors. In fact, the dual enrichment protocol implies that after the pre-enrichment step (with BPW), an aliquot is transferred simultaneously into two enrichment broths (Muller–Kauffmann tetrathionate/novobiocin broth and RVS broth), which are the same enrichment broths recommended by ISO 6340 (1995). The ISO 19250 (2010) was the standard procedure chosen for this study, to compare the efficacy of the mini-VIDAS® Easy *Salmonella* protocol for analyzing transitional waters, because it is an updated revision of the early ISO 6340 (1995). The increased false positive rate, observed in the present work, is most likely due to the lack of selectivity of the SX2 broth (used by the Easy *Salmonella* protocol) compared to the RVS broth, part of the ISO 19250 (2010) (consult Fig. SI 2 for clearer schematics)—subject that may deserve specific studies in the future to better depict it.

A higher false positive rate, given by any alternative method, will imply several steps for the confirmation procedure, with the consequent expenses and delays. But, although not time consuming, false negative results lead into a false sense of safety and can imply serious health

consequences. In this work, the mini-VIDAS® Easy *Salmonella* presented a successful performance with no false negatives (Table 1). The efficiency (fraction of results correctly assigned) of the methodology was 79.1 % ($n = 24$) which is a lower value compared to other authors (e.g., 96.38 % obtained by Mansilha et al. 2010).

The Easy *Salmonella* assay demonstrated to be faster than the ISO 19250 (2010) procedure, due to the 2-day negative result feedback. Furthermore, it involves the use of only one enrichment broth, contrary to the two enrichment broths, plus one post-enrichment broth, as recommended by the mini-VIDAS® dual enrichment protocol. The elimination of these steps saves time, labur and other costs to the laboratory.

The new Directive 2006/7/EC (EC 2006) excluded the assessment of *Salmonella* in microbiological pollution monitoring and addresses indicator bacteria (as *E. coli* and intestinal enterococci) as predictors of microbiological contamination of pathogens, such as Salmonella. Despite the high level of biological contamination of these waters (depicted in Table SI 1—supplementary material), the presence of Salmonella was only confirmed in 4.2 % ($n = 24$) of the samples studied (Table 1), which agrees with several other reports (e.g., Schets et al. 2008; Mansilha et al. 2010) that point weaknesses to this relationship, endorsing additional necessary studies about the relationship between bacterial indicators and pathogens, such as *Salmonella*—while the reassessment of the use of this surrogate methods should be of concern.

To address the suitability of these methodologies to a wide array of conditions and characteristics, water bodies/samples were characterized—several microbiological indicators and physical–chemical parameters assessed (depicted in Table SI 1—supplementary material).

The S. Domingos and Ferrel Rivers water denoted some organic pollution, as nitrite presented high values, larger than the Maximum Recommended Concentration recommended by the European Water Frame Directive (EC 2000) and EPA (2001) for freshwater systems (0.03 mg $L^{-1}$), denoting sewage pollution. Also, the studied waters presented high numbers of *E. coli* and enterococci (depicted in Table SI 1—supplementary material) exceeding the maximum admissible (500 *E. coli* 100 $mL^{-1}$ and 200 enterococci 100 $mL^{-1}$) to coastal and transitional waters by the Directive EC (2006) and can be classified as of "poor quality," upon a 95-percentile evaluation.

The different sites/and sampling periods are characterized by different patterns of physical–chemical parameters (PCA analysis; see Figure SI 3—supplementary material). The only site that tends to group in this plot is the coastal site (Station 1), with the foreseen strong positive correlation with salinity and negative correlation with the nutrients and chlorophyll a, while all others (Stations 2, 3, 4) are randomly distributed with different characteristics/or group of parameters that characterize them. These results reinforce the suitability for use of the proposed methodologies either for costal waters (categorized by higher salinities) or for transitional water sites analysis, characterized by a vaster array, and combinations, of different physical–chemical parameters.

The use of the mini-VIDAS® Easy *Salmonella* protocol to detect *Salmonella* in a broad array of environmental water samples is in fact promising, as it revealed a 0 % false negative rate, although the improvement in the protocol in order to reduce the false positive rate is highly recommended. Likewise, a validation trial, which would benefit from inter-laboratory experimenting, is suggested for environmental transitional waters according to the reference method ISO 16140 (2003), with respect to the reference method ISO 19250 (2010). This validation would allow assessing the sensitivity and the specificity of the Easy *Salmonella* method, plus incentivizing many laboratories to broaden the application of their mini-VIDAS® systems onto transitional and saltwaters analysis and to a faster detection of *Salmonella* in this type of samples, adding value and providing a vaster array of assessments these facilities may provide.

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