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Performance of testing laboratories in Proficiency testing in *Salmonella* sp. detection in seafood

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***Abstract* – Proficiency testing (PT) results from PT schemes in *Salmonella* sp. detection in octopus powder (ICSM-2022-01), and in shrimp powder (ICSM-2023-01) were described. Participants' performance was assessed based on accurate determination of presence or absence of the target organism. Reported results from all 29 participants in ICSM-2022-01 achieved 93.10% sensitivity and 86.21% specificity. Out of 29 laboratories, 23 received satisfactory evaluation while six received unsatisfactory evaluation. In ICSM-2023-01, reported results from all 28 participants obtained 96.43% sensitivity and 78.57% specificity. Of the 28 laboratories, 21 received satisfactory rating while seven received unsatisfactory rating. In both PT rounds, three laboratories had repeatedly received unsatisfactory rating mainly due to false positive results. Possible sources of errors might be from cross-contamination and/ or confusion during confirmatory tests. Laboratories with unsatisfactory performance were advised to conduct root cause analysis and corrective action, if necessary.**

I. INTRODUCTION

Participating in PT helps laboratories ensure accurate, reproducible results, demonstrates the effectiveness of their quality systems, and serves as a tool for monitoring overall service quality [1]. A study conducted by Abdelmassih et al., showed that the regular participation to PT schemes, when supported by proper feed-back and corrective actions, led to improvement in analysis and confidence in carrying-out laboratory analysis which, in turn, would provide better laboratory results [2]. A separate report summarizing discussions from the 6th International Conference on Food Safety and Regulatory Measures emphasized that proficiency testing (PT) schemes play a vital role in quality control for food analysis [3].

One example of a PT scheme is the *Salmonella* proficiency testing scheme, which involves a wide range of matrices, strains, and contamination levels, with or without background flora. This complexity is necessary due to the nature of *Salmonella* spp., which includes over 2,500 known serovars, making it impossible to detect all variants with a single testing method across all sample types. *Salmonella* is also a pathogenic organism and a leading cause of foodborne outbreaks globally [4]. For instance, in 2021, a multistate outbreak investigation led by the U. S. Food and Drug Administration (FDA), Center for Disease Control and Prevention (CDC), and state agencies identified *Salmonella* Weltevreden as the cause of illnesses linked to frozen, precooked shrimp imported from India [5]. Data from CDC also showed that *Salmonella* is the leading cause of seafood-related outbreaks in the United States, with 26 outbreaks reported—10 linked to crustaceans (224 cases), 14 to fish (852 cases), and 2 to molluscan shellfish (13 cases) [6]. In contrast, the European CDC and European Food Safety Authority (EFSA) had reported no seafood-related *Salmonella* outbreaks in the past decade, with most incidents in Slovakia, Spain and Poland were linked instead to eggs [7], while the most recent was from alfalfa sprouts [8].

Contamination of seafood with *Salmonella* spp. can occur at various points such as during rearing, processing, and storage and transportation [6], [9]. This presents a significant risk for imported products as contamination can lead to rejection at borders, trade restrictions, or public health alerts. In fact, under FDA's Import Alert 16-81, titled "Detention Without Physical Examination of Seafood Products Due to the Presence of *Salmonella*" and updated on April 30, 2025, numerous seafood products from various countries were placed on the "Red List" and detained at the U.S. border. The Philippines was among the countries affected, with seafood products from 68 manufacturers or producers denied entry into the U.S. market. Notably, this included 12 octopus products and nine shrimp and prawn products, highlighting the serious trade implications of *Salmonella* contamination in export goods [10].

According to the 2023 Philippine Fisheries Profile, octopus and shrimp ranked 6th and 10th, respectively, among the Philippines' top ten fishery exports by volume and value. The Philippines exported 7,048 MT of octopus products worth USD 39.03 million, and 3,046 MT of shrimp products valued at USD 14.87 million, with major importers including South Korea, Japan, and the USA [11].

While shrimp and prawn exports are supported by established national standards—such as PNS/BAFS 297:2020 Code Practice for the Processing of Shrimps and Prawns, and PNS/BAFS 70:2023 Fresh Chilled and Fresh Frozen Shrimps and Prawns – Product Standard—which help ensure compliance with international food safety requirements and reduce the risk of export rejection [12], [13], no equivalent standards currently exist for octopus products, despite octopus being among the Philippines' top seafood exports. This lack of specific guidelines leaves a critical gap in quality assurance, potentially increasing the risk of contamination, regulatory non-compliance, and trade disruptions, such as those reflected in *Salmonella*-related detentions under FDA's Import Alert 16-81.

Quality assurance ensures that required parameters meet established standards before products are released to the market. An effective National Quality Infrastructure (NQI) is therefore crucial in guaranteeing that products meet both national and international quality requirements. As one of the key pillars of the NQI, the National Metrology Laboratory has recognized the need to support the seafood industry by expanding its services to include microbiological proficiency testing (PT) schemes in seafood products. These PT schemes help testing laboratories maintain and evaluate their technical competency, ultimately enabling food manufacturers and producers to deliver safe, high-quality products fit for human consumption.

This paper describes the organization of the two PT schemes focusing on detecting *Salmonella* sp. in octopus and shrimp powder.

II. METHODOLOGY

A. Microbial strains

For ICSM-2022-01, in-house isolates, *Salmonella enterica*, as target strain, and *Proteus mirabilis*, as background microflora, were used. For traceability, the identification of these organisms was established through automated biochemical identification system (Vitek 2.0 Compact), and by 16S rDNA sequence analysis.

For ICSM-2023-01, *Salmonella enterica* subsp. *enterica* serovar Typhimurium ATCC 14028 and *Proteus mirabilis* ATCC 12453 were used.

B. Preparation of PT materials

In ICSM-2022-01, the matrix was prepared from raw octopus samples sourced from a local supplier and processed into a free-flowing sterile powder. The analytes, *Salmonella enterica* (target) and *Proteus mirabilis* (background flora), were preserved through cell immobilization, then pulverized and sieved to obtain a uniformly sized powder. A similar procedure was followed for the shrimp samples and analytes used in ICSM-2023-01.

C. Homogeneity and stability tests

The PT materials were assessed for homogeneity and stability in accordance to ISO Guide 35:2017. Homogeneity was statistically assessed using one-way analysis of variance (ANOVA) by determining the F-test value. Outliers was also determined using Cochran's Test for Outliers for duplicate results, based on The International Union of Pure and Applied Chemistry (IUPAC) Harmonized Protocol [14], was conducted to determine the presence of outlying data.

Stability testing was statistically evaluated using linear regression analysis (univariate linear model). Following the classical sampling approach, randomly selected units were stored at -20 to -18 °C and 4 to 8 °C. At each predetermined time interval, two samples were tested. Stability was monitored from Day 0 onward until the *t*-test indicated a statistically significant change, at which point the samples were considered no longer stable.

D. Organization of PT schemes

The Interlaboratory Comparison Scheme for Microbiology (ICSM) – 2022-01 was held from July to October 2022, and ICSM-2023-01 from April to September 2023. Each proficiency testing (PT) program included activities from invitations to the issuance of the final report and collection of evaluation forms. Invitations were sent via email, with 29 laboratories participating in *Salmonella* detection in octopus powder (ICSM-2022-01) and 28 in shrimp powder (ICSM-2023-01). Participants received instructions to participants, forms, and related documents by email and had 30 days to complete the analysis using their chosen test methods. Each laboratory was assigned a unique code for confidentiality. Results were collected, evaluated, and consolidated, and a final report detailing the PT materials, submitted results, and performance evaluation was issued to participants, along with customer survey forms. These forms were also collected and consolidated.

E. Distribution of PT materials

In each PT scheme, samples were arranged into two sets: one with freeze-dried food matrix spiked with powdered freeze-dried *S. enterica* cells and a jar of powdered freeze-dried *P. mirabilis* cells, and the other set contained sterile food matrix and a jar of *P. mirabilis.* The samples were labeled, wrapped in a bubble wrap, and packaged in polystyrene foam boxes or thermal bag, along with refrigerants and dry ice. These were then dispatched

to each participant's registered address via courier, or personally received by a representative.

D. Evaluation of participants' performance

Results for each set were reported by participants by accomplishing the provided forms and, submitted a copy via email. As a qualitative measurement, the participants were required to indicate the presence or absence of *Salmonella* sp. in each set. Their performance in each set was then evaluated satisfactory or unsatisfactory based on their reported result.

III. RESULTS AND DISCUSSION

All laboratories reported that they received the PT samples in good condition, though some reported damaged polystyrene foam boxes due to courier mishandling.

A. Homogeneity and stability test of PT materials

Each batch of PT materials was tested for homogeneity and stability. These two tests were conducted to ensure that the produced PT materials were fit for their intended use.

Satisfying the test criterion for homogeneity test assumed that there was no significant statistical difference, at 95% confidence level. Also, Cochran's Test for outliers indicated that outlying data were significantly absent at 95% confidence level. Thus, the PT materials were considered sufficiently homogenous.

For the stability test, the PT materials from ICSM-2022-01 showed that *S. enterica* and *P. mirabilis* remained stable at -20 to -18 ºC for 70 to 74 days, respectively. *S. enterica* also maintained stability at 4 to 8 ºC for 7 days. In ICSM-2023-01, *S. enterica* was stable at -20 to -18 ºC for 70 days and at 4 to 8 ºC for 28 days, while *P. mirabilis* demonstrated extended stability of 150 days at -20 to -18 ºC. All materials exceeded the 30-day duration allotted to the participants from sample receipt until conduct of analysis.

B. Evaluation of participants' performance

Participants were required to detect the presence or absence of the target organism. Laboratories with correct results received a satisfactory rating; others were rated unsatisfactory.

In ICSM-2022-01, Set 1 contained *Salmonella*, while Set 2 had only *P. mirabilis.* Of 29 participants, 27 correctly detected *Salmonella* in Set 1 and 25 accurately confirmed its absence in Set 2, yielding a sensitivity of 93.10% and specificity of 86.21% for this PT round. Overall, 23 received satisfactory rating in both sets while six were rated unsatisfactory. Table 1 shows the summary of results of participating laboratories in this PT round.

Table 1. Results and performance rating of participants in ICSM-2022-01.

Lab Code	Set 1 (target)	Set 2 (background)	Rating[1]
ICSM 043	Present	Absent	S
ICSM 049	Present	Absent	S
ICSM 093	Absent	Absent	U
ICSM 168	Present	Absent	S
ICSM 201	Present	Absent	S
ICSM 224	Present	Absent	S
ICSM 243	Present	Absent	S
ICSM 272	Present	Present	U
ICSM 375	Present	Absent	S
ICSM 407	Absent	Absent	U
ICSM 425	Present	Absent	S
ICSM 450	Present	Absent	S
ICSM 486	Present	Absent	S
ICSM 539	Present	Absent	S
ICSM 590	Present	Absent	S
ICSM 615	Present	Absent	S
ICSM 618	Present	Absent	S
ICSM 646	Present	Absent	S
ICSM 663	Present	Absent	S
ICSM 687	Present	Absent	S
ICSM 694	Present	Present	U
ICSM 695	Present	Absent	S
ICSM 717	Present	Absent	S
ICSM 739	Present	Absent	S
ICSM 774	Present	Present	U
ICSM 840	Present	Absent	S
ICSM 842	Present	Present	U
ICSM 861	Present	Absent	S
ICSM 901	Present	Absent	S

[1]*S, satisfactory; U, unsatisfactory*

In ICSM-2023-01, Set 1 contained only *P. mirabilis*, while Set 2 was spiked with *Salmonella*. Among 28 participating laboratories, 22 accurately reported the absence of *Salmonella* in Set 1, and 27 correctly detected its presence in Set 2, resulting in a sensitivity of 96.43% and specificity of 78.57%. A total of 21 laboratories received a satisfactory rating in both sets, while seven were rated unsatisfactory. Table 2 shows the summary of results in this PT round. Also, shown in Figure 2 is the overall performance of laboratories in both PT schemes.

In both PT schemes, three laboratories: Lab 1, coded as ICSM 093 and ICSM 712; Lab 2 coded as ICSM 272 and ICSM 104; and Lab 3 coded as ICSM 694 and ICSM 264, were observed to have repeatedly received unsatisfactory rating mainly due to false-positive results.

Table 1. Results and performance rating of participants in ICSM-2023-01.

Lab Code	Set 1 (background)	Set 2 (target)	Rating[1]
ICSM 020	Absent	Present	S
ICSM 047	Absent	Present	S
ICSM 065	Absent	Present	S
ICSM 104	Present	Present	U
ICSM 105	Present	Present	U
ICSM 106	Absent	Present	S
ICSM 230	Absent	Present	S
ICSM 264	Absent	Absent	U
ICSM 309	Absent	Present	S
ICSM 360	Absent	Present	S
ICSM 374	Absent	Present	S
ICSM 389	Absent	Present	S
ICSM 395	Absent	Present	S
ICSM 464	Present	Present	U
ICSM 495	Absent	Present	S
ICSM 557	Present	Present	U
ICSM 607	Absent	Present	S
ICSM 627	Absent	Present	S
ICSM 667	Absent	Present	S
ICSM 712	Present	Present	U
ICSM 730	Absent	Present	S
ICSM 771	Absent	Present	S
ICSM 778	Absent	Present	S
ICSM 795	Absent	Present	S
ICSM 890	Absent	Present	S
ICSM 923	Absent	Present	S
ICSM 971	Present	Present	U
ICSM 998	Absent	Present	S

[1]*S, satisfactory; U, unsatisfactory*

Fig. 1. Overall performance of participating laboratories in ICSM-2022-01 and ICSM-2023-01.

Sensitivity refers to the proportion of true positive results correctly identified by the laboratory out of the total expected positive results [16]. On the other hand, specificity is the proportion of the true negatives out of the total expected negative results [17]. Both PT rounds had low specificity rates due to reporting of false positive results. This indicates that a careful review on the methods, procedures, and training of laboratory analysts is necessary.

S. enterica and *P. mirabilis* share similar biochemical characteristics—such as negative indole production, positive acid production (methyl red test), negative acetylmethyl carbinol production (Voges-Proskauer test), and positive citrate utilization—making P. mirabilis a suitable background flora to challenge participants' analytical accuracy. Most laboratories used conventional *Salmonella* detection methods (Fig. 2 and 3) that rely on biochemical confirmatory tests, such as those outlined in FDA-BAM Chapter 5. For example, both organisms produce similar results on Triple Sugar Iron agar—red slant, yellow butt, and black precipitate—due to glucose fermentation and H_2S production [16], [17]. However, they differ on Lysine Iron Agar: *S. enterica* shows a purple slant and butt (lysine decarboxylation and H_2S production), while P. mirabilis shows a red slant and yellow butt due to lysine deamination [18], [19]. These subtle differences highlight the need for skilled analysts to accurately distinguish between the two organisms.

Among the laboratories that reported false positives in both PT rounds, potential causes include misinterpretation during confirmatory testing and cross-contamination, which can occur regardless of the testing method used. These issues highlight the importance of careful observation, strict adherence to aseptic techniques, and maintaining a controlled testing environment. Additionally, conducting a thorough root cause analysis and regularly reviewing laboratory procedures is essential—regardless of performance ratings—to ensure ongoing accuracy and reliability in results.

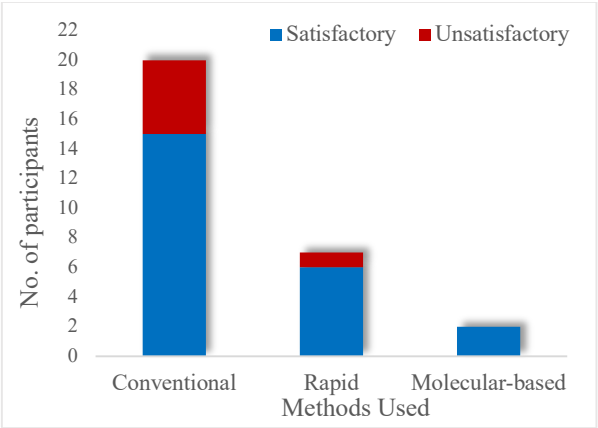

Fig. 2. Overall performance of participating laboratories in ICSM-2022-01 in relation to methods used.

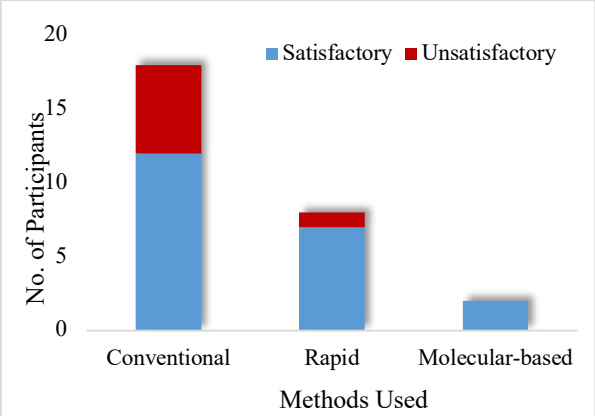

Fig. 3. Overall performance of participating laboratories in ICSM-2023-01 in relation to methods used.

IV. CONCLUSION

This study outlines the implementation of two proficiency testing (PT) schemes focused on the detection of *Salmonella* spp. The PT materials used demonstrated acceptable homogeneity and stability throughout the program. In ICSM-2022-01, approximately 79% of participating laboratories achieved a satisfactory rating, while 75% did so in ICSM-2023-01. The majority of unsatisfactory outcomes were attributed to false-positive results, suggesting potential issues related to methodological interpretation, cross-contamination, or procedural lapses during confirmatory testing. These findings underscore the need for targeted interventions, including regular method validation, technical training, and root cause analysis to address recurrent performance issues. The results provide significant insights on the analytical capabilities of microbiological laboratories in the Philippines.

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