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# Performance characteristics and estimation of measurement uncertainty of two plating procedures for *Listeria monocytogenes* enumeration in chicken meat

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**Abstract:** The objectives highlighted in the present study were to determine the estimates of measurement uncertainty associated with PALCAM and CHROMagar™ Listeria media, to compare the efficacy between both media in relation to their measurement uncertainties. In addition, this study was carried out to assess the performance characteristics of spread and spiral plating procedures based on the comparison of *Listeria monocytogenes* enumeration between PALCAM and CHROMagar™ Listeria media. This work involved pure culture experiment, artificially contaminated samples experiment and naturally contaminated samples experiment. In pure culture experiment, PALCAM performance was relatively inferior to CHROMagar™ Listeria medium for both plating procedures. From the artificially contaminated samples, the results revealed that the values of repeatability, reproducibility, and measurement uncertainty at 95% confidence interval were comparable between both media under evaluation. However, at the level of naturally contaminated samples, the performance of CHROMagar™ Listeria medium was refutable as the presence of high number of competitive microorganisms reduced the clarity of the medium. The current emphasis in ensuring microbiological safety which requires use of accredited laboratories has led to measurable need for measurement uncertainty to ensure reliability of test results for global acceptance.

**Keywords: *Listeria monocytogenes*, food safety, enumeration, repeatability, reproducibility, measurement uncertainty, ISO**

## Introduction

It has been 70 years since *Listeria monocytogenes* is recognized as an animal pathogen (McLauchlin, 1997). Increased attention has been paid to *L. monocytogenes* since it was recognized as a foodborne pathogen responsible for human listeriosis (Jemmi and Keusch, 1994). Foodborne listeriosis continues to be of major concern to the food industry and the general public because of its rate of lethality (more than 25%) and its economic impact (McLauchlin *et al*., 1991). The high prevalence of *L. monocytogenes* in food and the high fatality rates of listeriosis suggest that this pathogen represents an important hazard to human health. *L. monocytogenes* has been reported from a wide variety of food types and clinical samples in various countries of the world (Cordano and Rocourt, 2001; Rocourt and Cossart, 2001; Dhanashree *et al*., 2003). Since the onset of quantitative microbial risk assessment, the need for quantitative data on the concentration of *L. monocytogenes* in chicken meat has increased dramatically. For the enumeration of *L. monocytogenes*, protocols mentioned in the FDA and USDA-FSIS methods can be used (Hitchins, 1998).

The international reference method imposes only the use of PALCAM agar for the enumeration of *L. monocytogenes*. PALCAM medium is recommended by APHA and AFNOR for use in the detection of *L. monocytogenes* in foods (Ryser and Donnelly, 2001). According to Van Netten *et al*. (1991), PALCAM agar is a selective medium used for the differentiation and isolation of *L. monocytogenes* from foods and environmental samples that are even highly contaminated. It gives satisfactory results, producing highly typical *Listeria* colonies at the same time as inhibiting almost all other contaminating bacteria. CHROMagar™ Listeria is one of the medium recommended in the Bacteriological Analytical Manual (Hitchins, 2001), and was validated by AFNOR in 2001. It facilitates the detection and differentiation of *L. monocytogenes* from other *Listeria* spp. directly at the isolation step.

During the past few years, considerable attention has been paid to the estimation of measurement uncertainty in microbiology. The precision of a quantitative risk assessment lies in its ability to reflect

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and evaluate the 'variability' and the 'uncertainty' of the risk estimate separately (Lammerding, 1997; Vose, 2000). It is an essential prerequisite that sources of variability (components of uncertainty) in test methods and subsequent results should be identified (ISO/ IEC 17025, 1999, 2005; EURACHEM, 2000; Horwitz, 2003).

The estimation of uncertainty in microbiological measurements is based mainly on the repeatability (r) and reproducibility (R) data generated by intra-laboratory and inter-laboratory testing. The precision of a measurement which is the closeness of agreement between independent test results (Anon, 2002) can be expressed as the repeatability and reproducibility. The value of repeatability standard deviation is denoted by $S_r$ and the reproducibility standard deviation is denoted by $S_R$. Both the repeatability standard deviation, $S_r$ and the reproducibility standard deviation, $S_R$ tend to increase with the nominal level. The aim of present study is to determine the estimation of measurement uncertainty (U), repeatability (r) and reproducibility (R) as well as the performance characteristics of two plating media which are PALCAM and CHROMagar™ Listeria by using spread plating and spiral plating techniques for *L. monocytogenes* enumeration in chicken meat.

## Materials and Methods

*Pure culture experiment*

A working culture from two pure strains of *L. monocytogenes* was revived using Tryptic Soy Broth (TSB). A part of this day zero working culture (D0 WC) was used immediately for serial dilution and plating, while the rest was stored at 5°C under an aerobic condition for 72 hr to achieve day 3 working culture (D3 WC). Each of the two laboratory analysts working in parallel prepared two sets of 10-fold serial dilutions from D0 WC. A 1 ml of inoculum ($10^{-1}$) was transferred to a tube containing 9 ml of TSB for second dilution ($10^{-2}$). The solution was mixed well and 1ml of inoculum from this tube was transferred to another 9 ml of LEB for the third dilution ($10^{-3}$). The procedures were repeated to make dilution factors 4, 5, 6 and 7. The two analysts inoculated, each in parallel using their own prepared serial dilutions set, the surface of triplicate plates of PALCAM and CHROMagar™ Listeria agar, by aseptically transferring 0.1 ml from dilutions $10^{-6}$ and $10^{-7}$ and spread it to dryness. Besides, the suspensions of both dilutions were plated using mechanical spiral platter (WASP 2, Don Whitley Scientific, UK) for spiral spreading over surface of triplicates of PALCAM and CHROMagar™ Listeria agar. The agar plates were incubated for 48 hr at 30°C. The colony forming units (cfu) of *L. monocytogenes* were counted and recorded.

*Artificially contaminated samples experiment*

Fifteen chicken meat samples were used. These samples were purchased from the supermarket and stored in laboratory freezer (-21°C) and used within two weeks. The freezing step before artificial contamination was purposely done to initiate extreme physical stress conditions capable of declining, if any present, inherent *L. monocytogenes* in inoculated samples.

The fresh chicken meat sample was cut into small pieces. As the sample unit was a solid, a representative analytical unit was obtained by taking a portion from several locations within the sample unit. A 10 g portion was homogenized with 90 ml of *Listeria* Enrichment Broth Base (LEB; acc. to FDA/IDF-FIL) into a sterile stomacher bag and then pummeled in the stomacher for 120 s. Day three working cultures (D3 WC) of *L. monocytogenes* strain were used to artificially contaminate broiler chicken meat samples. For each sample, 1 ml of D3 WC was spiked into the sterile chicken meat sample and homogenized in the stomacher bag.

Aseptically, 1 ml of inoculum ($10^{-1}$) from stomacher bag was transferred to a tube containing 9 ml of LEB for second dilution ($10^{-2}$). The solution was mixed well and 1 ml of inoculum from second tube was transferred to another 9 ml of LEB for the third dilution ($10^{-3}$). Further 10-fold dilutions were performed to obtain $10^{-4}$ and $10^{-5}$ dilution factors. By transferring 0.1 ml from dilution $10^{-4}$, inoculation onto duplicates of PALCAM agar and CHROMagar™ Listeria was carried out using spread plating and the same step was repeated for dilution $10^{-5}$. Also, suspensions of both dilutions were plated using mechanical spiral platter (WASP 2, Don Whitley Scientific, UK) for spiral spreading over the surface of duplicates of PALCAM and CHROMagar™ Listeria agar. Agar plates were incubated for 48 hr at 30°C. The colony forming units (cfu) of *L. monocytogenes* were counted and recorded and the comparisons between the performance characteristics of each agar and plating technique were made.

*Naturally contaminated samples testing*

A total of 20 samples of fresh raw chicken meat products were purchased from a wet market. The samples were analyzed within 3-4 hours upon purchasing. Representative 10 g portion was aseptically weighed and homogenized with 90 ml LEB. From the stomacher bag, 1 ml of inoculum

($10^{-1}$) was transferred to a tube containing 9 ml of LEB for second dilution ($10^{-2}$). By using spread plating, 0.1 ml from each dilution was inoculated to PALCAM agar and CHROMagar™ Listeria. Also, suspensions of both dilutions were plated using mechanical spiral platter (WASP 2, Don Whitley Scientific, UK) for spiral spreading over the surface of duplicates of PALCAM and CHROMagar™ Listeria agar. Incubation of agar plates for 48 hr at 30°C was conducted and the colony forming units (cfu) of *L. monocytogenes* were counted and recorded. The performance characteristics of each agar and plating technique were compared.

*Statistical analysis*

All cfu results, per gram or millilitre, were transformed to $\log_{10}$ counts before statistical analysis in order to stabilize the reproducibility variance over the contamination levels (Anonymous, 2006). As distribution of cfu on Petri dishes follows a Poisson series, the significant differences (P-value ≤ p0.05) between counts obtained using the two plating media and between plating procedures were tested using generalized linear models (GLMs) starting with Poisson regression analysis.

The standard deviations for repeatability ($S_r$) and reproducibility ($S_R$) were calculated based on the following equation:

*Repeatability*

$$\text{Between replicate variance for Sample 1} = \frac{(\text{Replicate 1 log count} - \text{Mean})^2 + (\text{Replicate 2 log count} - \text{Mean})^2}{\text{n-1}}$$

Where n = number of duplicate pairs

$$\text{Standard deviation of repeatability, } S_r = \sqrt{\frac{\sum \text{Between replicate variance}}{n}}$$

Where n = number of samples

*Reproducibility*

$$\text{Standard deviation of reproducibility, } S_R = \sqrt{\sum_{i=1}^{n} \frac{(y_{iA} - y_{iB})^2}{2n}}$$

Where $y_{iA}$ and $y_{iB}$ are the $\log_{10}$-transformed results of parallel tests by different analysts on a sample, and *i* is the number of samples from 1 to n.

*Measurement uncertainty*

Measurement uncertainty (U) was obtained using the reproducibility standard deviation ($S_R$) of $\log_{10}$ transformed data, by applying the following formula (Anonymous, 2006):

$$U = \log_{10}(c) \pm [k \times S_R]$$

Where c is the $\log_{10}$ count; $S_R$ is the reproducibility standard deviation; and k is the appropriate coverage factor which is usually 2 (for 95% confidence level) to obtain the expanded uncertainty.

Statistical methods of Shapiro-Wilks Normality test, Independent T-test, Poisson regression analysis and Bland-Altman plot were performed to compare the two plating methods and performance characteristics of plating media. All statistical analyses were carried out with IBM SPSS Statistics 19.

## Results

*Pure culture experiment*

Table 1 summarizes the results of pure culture experiment. Enumeration was successful for PALCAM and CHROMagar™ Listeria agar because all results were countable. For both spread and spiral plating techniques, it could be observed that CHROMagar™ Listeria agar showed a better performance characteristic and a higher degree of accuracy (lower SD) than PALCAM agar. Higher enumeration in both media were obtained using spiral plating technique (Augustin and Carlier, 2006).

**Table 1.** Descriptive statistics for *L. monocytogenes* pure culture experiment

| Plating Method | Media | Count based on analyst 1 (Mean ± SD) | Count based on analyst 2 (Mean ± SD) |
|---|---|---|---|
| Spread | PALCAM | 9.23 ± 0.14 | 9.05 ± 0.07 |
| | CHROMagar™ Listeria | 9.28 ± 0.01 | 9.19 ± 0.01 |
| **Count based on spiral plater machine** | | | |
| Spiral | PALCAM | 9.63 ± 0.12 | |
| | CHROMagar™ Listeria | 9.69 ± 0.01 | |

Note: Counts are presented as $\log_{10}$ cfu/g.

At 95% confidence, Poisson regression analysis indicated that there was a significant difference (P<0.000) between the two plating techniques. To be specific, Poisson regression analysis indicated that for every one unit colony count increase in spiral plating, there will be 0.01 unit colony count increase in spread plating (Wald Chi-Square, p<0.00). This relationship can be parameterized as, Spiral = (0.01) (Spread) + 9.59.

*Artificially contaminated chicken matrix*

Parameter values were calculated according to ISO/TS 19036:2006 (Anonymous, 2006). Values of repeatability, reproducibility and measurement uncertainty are presented in Table 2. For spread

**Table 2.** Performance parameters of two plating procedures for the enumeration of *L. monocytogenes* on artificially contaminated chicken meat

| Plating Method | Media | $S_R$ ($log_{10}$) | R ($log_{10}$) | U ($log_{10}$) | $S_r$ ($log_{10}$) | r ($log_{10}$) | Mean count ($log_{10}$ cfu/g) |
|---|---|---|---|---|---|---|---|
| Spread | PALCAM | 0.052 | 0.119 | 0.104 | 0.0545 | 0.124 | 8.34 |
| | CHROMagar™ Listeria | 0.031 | 0.071 | 0.062 | 0.0399 | 0.091 | 8.38 |
| Spiral | PALCAM | 0.134 | 0.306 | 0.268 | 0.134 | 0.306 | 8.55 |
| | CHROMagar™ Listeria | 0.030 | 0.068 | 0.060 | 0.030 | 0.060 | 8.61 |

Note:
(*r*) repeatability limit: is the value less than or equal to which the absolute difference between two test results obtained under repeatability conditions is expected to be with probability of 95%, Repeatability limit, $r = 2.28 \times S_r$
(*R*) Reproducibility limit: is the value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions is expected to be with probability of 95%, Reproducibility limit, $R = 2.28 \times S_R$.

plating, the two evaluated media showed a comparable repeatable, reproducible and measurement uncertainty performance. For plating on PALCAM and CHROMagar™ Listeria, the estimated *r* values were 0.124 $log_{10}$ and 0.091 $log_{10}$ respectively while the *R* values were 0.119 $log_{10}$ and 0.071 $log_{10}$ respectively for PALCAM and CHROMagar™ Listeria. The measurement uncertainty associated with PALCAM and CHROMagar™ Listeria media were estimated as ±0.104 $log_{10}$ cfu/g and ±0.062 $log_{10}$ cfu/g respectively.

In retrospect, for spiral plating method, bigger discrepancy of repeatability (*r*), reproducibility (*R*) and measurement uncertainty (U) between both media were observed and higher values were associated with PALCAM media. The *r* values for PALCAM and CHROMagar™ Listeria media were estimated as 0.306 $log_{10}$ and 0.060 $log_{10}$ respectively; *R* values were 0.306 $log_{10}$ and 0.068 $log_{10}$ respectively; U values were ±0.268 $log_{10}$ cfu/g and ±0.060 $log_{10}$ cfu/g respectively. Mean counts using spiral plating were higher for both media. According to Bland-Altman plot in Figure 1, since not all the differences lie within the mean ± 1.96 SD, the PALCAM and CHROMagar™ Listeria methods may not be used interchangeably for artificially contaminated chicken matrix.

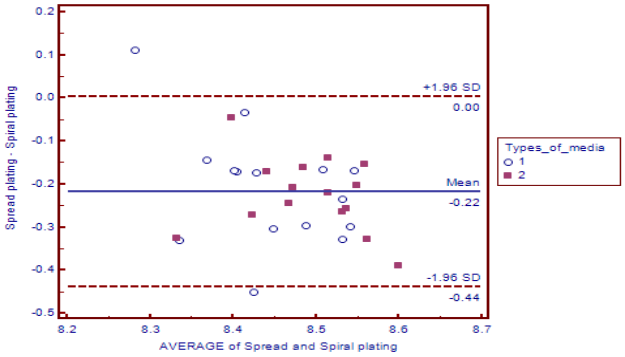


**Figure 1.** Difference between counts on PALCAM (1) and CHROMagar™ Listeria (2) is plotted against their mean value, with upper and lower horizontal lines showing the 95% limits of agreement using spread and spiral plating technique

*Naturally contaminated samples testing*

When comparing the measurement uncertainty (U) between PALCAM and CHROMagar™ Listeria media for both plating procedures, CHROMagar™ Listeria media demonstrated higher values estimated as ±0.628 $log_{10}$ cfu/g for spread plating and ±0.510 $log_{10}$ cfu/g for spiral plating (Table 3). Overall, for both media, higher accuracy was associated with spiral plating technique as it gave lower values of repeatability, reproducibility and measurement uncertainty. Mean counts using spiral plating were higher than spread plating for both media under evaluation. According to Bland-Altman plot in Figure 2, since not all the differences lie within the mean ± 1.96 SD, the PALCAM and CHROMagar™ Listeria methods may not be used interchangeably for naturally contaminated samples.

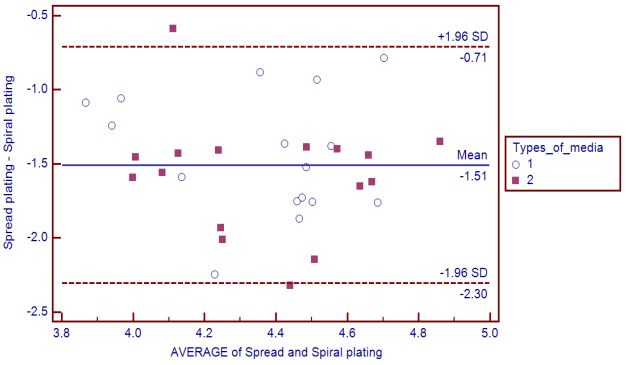


**Figure 2.** Difference between counts on PALCAM (1) and CHROMagar™ Listeria (2) is plotted against their mean value, with upper and lower horizontal lines showing the 95% limits of agreement using spread and spiral plating technique

## Discussion

The present study provided an intra-laboratory evaluation of the performance characteristics, and measurement uncertainty associated with the two selective plating media. Intra-laboratory $S_R$ is preferable as it provides a measurement uncertainty value that is linked to the laboratory, thus nearer to the basic measurement uncertainty definition which relates measurement uncertainty even to one individual test result (Lombard, 2006). In this study

**Table 3.** Performance parameters of two plating procedures for the enumeration of *L. monocytogenes* on naturally contaminated chicken meat

| Plating Method | Media | $S_R$ ($log_{10}$) | R ($log_{10}$) | U ($log_{10}$) | $S_r$ ($log_{10}$) | r ($log_{10}$) | Mean count ($log_{10}$ cfu/g) |
|---|---|---|---|---|---|---|---|
| Spread | PALCAM | 0.227 | 0.518 | 0.452 | 0.238 | 0.543 | 3.64 |
| | CHROMagar™ Listeria | 0.314 | 0.716 | 0.628 | 0.292 | 0.666 | 3.58 |
| Spiral | PALCAM | 0.205 | 0.467 | 0.410 | 0.212 | 0.483 | 5.08 |
| | CHROMagar™ Listeria | 0.255 | 0.581 | 0.510 | 0.255 | 0.580 | 5.16 |

Note:
(*r*) repeatability limit: is the value less than or equal to which the absolute difference between two test results obtained under repeatability conditions is expected to be with probability of 95%, Repeatability limit, $r = 2.28 \times S_r$
(*R*) Reproducibility limit: is the value less than or equal to which the absolute difference between two test results obtained under reproducibility conditions is expected to be with probability of 95%, Reproducibility limit, $R = 2.28 \times S_R$.

also, the '*top-down*' approach was used. It is usually derived from inter- or intra-laboratory trials (Anon 2003, 2006) in which the trueness, accuracy and precision of a laboratory procedure are estimated. Review of technical standards reveals that, ideally, at least 30 sets of paired counts (paired counts = count set obtained from two analysts working in parallel under reproducibility conditions) should be used, but a reasonable estimate may be obtained using 10 sets of paired counts (Anonymous, 2005, 2006). In artificially contaminated level, 15 sets of paired counts were used whilst 20 sets of paired counts were used in naturally contaminated level.

Estimates of precision in the enumeration of *L. monocytogenes* in pure culture and artificially contaminated level were found to be better when using CHROMagar™ Listeria (in aspect of media performance) and spiral plating technique (in aspect of plating procedure) as depicted by lower standard deviations of reproducibility. With respect to the performance characteristics of PALCAM and CHROMagar™ Listeria media under repeatability and reproducibility conditions, Independent T-tests hypothesized that there was no significant difference ($P>0.05$). Meanwhile, interestingly, Poisson regression analysis revealed that there was a significant difference between the two plating procedures. Similarly, Bland-Altman plot analysis stated that both plating techniques cannot be used interchangeably. The estimation of measurement uncertainty of both plating procedures can hence be arguably deduced to be significantly different from each other. The overall mean counts of *L. monocytogenes* from pure cultures, artificially and naturally contaminated levels were notably higher by spiral plating procedure than by spread plating method irrespective of the type of medium used. This interesting finding was in agreement with the previous statistical evidence (Augustin and Carlier, 2006; Habib *et al*., 2008).

The overall measurement uncertainty was much greater for the spread plating technique. The manipulation of spread plating technique may result in the adherence of a proportion of the inoculum when being spread across the surface of the agar, thereby giving a falsely low colony count. On the other hand, the use of mechanical spiral plating reduces the errors of dilution since an increasingly reducing inoculum is distributed across the surface of the agar plate in the form of an Archimedes spiral to give the equivalent of a 3-log dilution of organisms on a single plate (Gilchrist *et al*., 1973). It is interesting to find in this study that the use of spiral plating procedure produces data with a reasonably better reproducibility than the spread plating method. The difference probably reflects the lower level of serial dilutions required for spiral plating counts (Hedges, 2002). The spiral plate method, in many cases, can replace advantageously any of the other conventional methods as spiral plating results in significant savings in cost, time, space and labour.

It is noticeable that the CHROMagar™ Listeria plating medium was relatively more efficient than the PALCAM medium for the isolation of *L. monocytogenes*. Higher mean counts and lower measurement uncertainties were associated with CHROMagar™ Listeria medium. In artificially contaminated level where real contamination was mimicked, it was evident that the presence of high level of background microflora was not a problem with CHROMagar™ Listeria medium as indicated by low values of measurement uncertainty. In retrospect, higher uncertainties were associated with PALCAM agar and the estimated U-values for spread and spiral plating on PALCAM were $\pm 0.104$ $log_{10}$ cfu/g and $\pm 0.268$ $log_{10}$ cfu/g respectively. Several authors have mentioned that *Listeria innocua* (a non-pathogen species for humans) can mask the presence of *L. monocytogenes* on PALCAM agars because of differences in growth rates of *Listeria* spp. in selective broths (Petran and Swanson 1993; Mc Donald and Sutherland 1994; Carles *et al*. 1997). Thus, species identification from a definite number of colonies per plate can be biased. Besides, the lower mean counts obtained using PALCAM medium also reflected the lower degree of sensitivity of the medium since its ability to support growth of the *L. monocytogenes* was relatively inferior compared to CHROMagar™

Listeria medium.

The inspection of measurement uncertainties at naturally contaminated level, however, found that CHROMagar™ Listeria agar was associated with higher U-values, estimated as ±0.628 $\log_{10}$ cfu/g for spread plating and ±0.510 $\log_{10}$ cfu/g for spiral plating, as shown in Table 3. This could be explained by the fact that the presence of relatively high number of competitive microorganisms was intolerable in CHROMagar™ Listeria and the coalescence of microorganisms had resulted in a higher degree of opacity which masked the white halos rendering uncompromising identification and counting of colonies. The confluence of colonies also occurred on PALCAM agars and had impacted on the high measurement uncertainties as precise counts were made difficult due to the relatively indistinguishable olive-green colonies owing to the dark brown-black background of the medium.

Enumeration errors were prevalent in all colony count techniques. The common errors include dilution error, errors in pipetting volumes of diluted sample and errors of counting. When evaluating the effect of reproducibility condition on measurement uncertainty, it appears clear that different operators play a measurable role in contributing to the varied uncertainties that arise from pipetting to counting. Compared to spread plating, the Archimedes spiral plating stops before the meniscus of the agar is reached. Thus, colonies are counted only on the clear, level portion of the agar. This is an important factor in visual counting. By counting to a predetermined colony level, the distribution error is kept constant, thereby ensuring comparability in precision between replicate series of plates. Hedges *et al.* (1978) have shown that the spiral plating technique showed higher precision.

The expression of uncertainty is relevantly important. The information could be reported as a confidence interval or as confidence limits, once the expanded uncertainty data have been antilogged (Jarvis, 1989).

Uncertainty is a measure of variability in a test result and is therefore a measure of the lack of precision in the result rather than an estimate of the precision of the method itself. Generally, uncertainty may be reduced by further experimental or sampling investigations. This study shows that method validation data (repeatability and reproducibility) can be used to provide a good estimation of measurement uncertainty.

The ultimate goal of a method validation in microbiology is to ensure that every future measurement in routine analysis will be close enough to the unknown true value (Gonzalez and Herrador, 2007). The objectives of validation are not simply to obtain estimates of trueness or bias and precision but also to evaluate those risks that can be expressed by the measurement uncertainty associated with the result (Gonzales *et al*., 2005; Gonzalez and Herrador, 2007).

## Conclusion

It is clear that the estimates of uncertainty are influenced by the choice of medium and plating procedures. In separate contexts (pure cultures, artificially inoculated, or naturally contaminated samples), obtaining an accurate estimation of measurement uncertainty, repeatability and reproducibility was not feasible as drawing a conclusion set based on one context would warrant misled and premature indication. This multilevel evaluation strategy in validation of alternative quantitative methods or assessment performance of standard methods in food microbiology is therefore of paramount importance. With respect to the efficacy between PALCAM and CHROMagar™ Listeria media in terms of their uncertainty, repeatability and reproducibility, CHROMagar™ Listeria medium was relatively better and gave more precise enumerations.

In comparison of the two plating procedures, spiral plating technique gave systematically higher colony counts and was shown to be more reliable. Statistical Independent T-tests showed that there was no significant difference ($P > 0.05$) of performance characteristic (*L. monocytogenes* enumeration) between PALCAM and CHROMagar™ Listeria media neither under repeatability nor reproducibility conditions in both artificially and naturally contaminated level. Meanwhile, Poisson regression analysis indicated that there was a noteworthy difference between the performance characteristic of two plating procedures and Bland-Altman plot depicted that the two plating methods could not be used interchangeably. The estimation of measurement uncertainty for quantitative determinations is a pragmatic approach, particularly in food microbiology. Measurement uncertainty estimation will bring an added value in the analytical field of food microbiology. In future, the performance of CHROMagar Listeria medium is worth to be investigated in further studies considering its higher sensitivity and accuracy in isolating *L. monocytogenes*.

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