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Evaluation of the HApS™ Method for the Enumeration of Aerobic Microorganisms and Coliforms in Retailed Meat Samples in Korea

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ABSTRACT – This study was designed to compare the effectiveness and applicability of the HApS™ (Hazard Analysis process System; HUKO, Seoul, Korea) based on Petrifilm™ (3M, St. Paul, MN, USA) with the AOAC (the Association of Official Analytical Chemists) standard total aerobic count (TAC) method and coliform count (CC) method for meat products. The comparisons were carried out using 230 meat samples collected from various retailers: 80 pork samples, 80 chicken samples, and 70 beef samples. In the comparison of the correlation coefficient (r) between conventional method and HApS™ method by a linear regression analysis, the correlation coefficients in total microorganism were 0.97767, 0.90712, and 0.95594 in pork, beef, and chicken samples, respectively. The correlation coefficients in coliform count were 0.82062, 0.94833, and 0.96839 in pork, beef and chicken samples, respectively. All the independent t-test on measurement values between conventional method and HApS™ method represented no significant differences in the means between two methods at the 0.05 of significance level(α=0.05). Based on the high correlation between HApS™ and the AOAC standard methods in the TAC and CC, it might be compatible to employ the HApS™ method to measure the microbial contamination in livestock products. HApS™ method was simpler and less time-consuming in sample preparation and procedures faster than the conventional method. These results suggested that the HApS™ method could be substitute for the conventional methods in the analysis of microbial contamination measurement in meat products.

Key words □ Total aerobic count, Coliform count, HACCP, HApS™

The food industry is spending increased effort to ensure food safety and develop faster and simpler methods to comply with HACCP (Hazard Analysis Critical Control Point) regulations because of growing interest of consumers and increasing demands of international regulation on the food safety and hygiene. The coliform count has been used to measure the safety of raw material and food manufacturing processes, while the total microorganism count has been used to measure microbial contamination during food processing. Therefore, food manufacturers can use both total microorganism and coliform counts to ensure processing hygiene and safety and to comply with HACCP regulations.

In general, both total microorganism and coliform counts have been measured with the methods developed by the Association of Official Analytical Chemists (AOAC)[11]. However, they are not convenient to use in the production facility because they require 2-3 days and substantial labor to obtain final results. To overcome such shortfalls in production facility applications, several time-efficient methods such as the ATP-Bioluminescence method and impedance method have been developed and applied[16].

Although such methods are simpler and more efficient than conventional methods, they are not completely accurate since they are indirect measurements. Also, they are not convenient to use in the production facility, due to requirement for a regression fit on the conventional methods for each measurement.

HApS™(Hazard Analysis process System) was also developed to be simpler and more efficient for production facility applications because of its easy sample

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preparations and more expedient measurement. HApS™ is based on 3M Petrifilm™ plate method and composed of incubator, scale, sterilized bag holder, sterilized bag stander, roller, roller plate, sterilized dilution water (90 ml; 25ea, 9 ml; 50ea), sterilized bags and sampling kit and equipments were gamma-sterilized.

Similar to conventional methods, HApS™ method is a direct measurement of microorganism incubated on a Petrifilm™ and does not require a regression fit on each measurement. The Petrifilm™ plate method which was adopted in HApS™ method has been compared with standard methods for the enumeration of the aerobic flora and coliforms in several different food products, including meat [1,5,7,9,14,15,17], poultry[1,7,15], fish[1], seafood[1,7], dairy products[1,14], vegetables[1,14], and other foods[1,14]. In addition, AOAC and APHA (American Public Health Association) have approved the Petrifilm™ plate method for the measurement of microbial contamination in the pasteurized and raw milk[8].

The purpose of this study was to demonstrate the compatibility of using the HApS™ method for production facility applications by comparing its accuracy and correlation of the total microorganism and coliform count to that of the AOAC conventional methods.

Materials and Methods

Sample collection

Samples were collected from various meat retailer stores in the suburbs of Seoul twice a month over 3 months of period (June through August). Sufficient freezer gel packs were added to maintain a temperature of approximately 4°C prior to transferring to the laboratory. All samples were transferred to the laboratory within 2 hours. A total of 80 pork samples, 80 chicken samples and 70 beef samples were collected for this study.

Total microorganism measurement

Aerobic plate counts were made by standard plate count agar (SPCA, DIFCO, Detroit, MI, USA) method. Meat samples (25 g) were mixed with 225 ml of buffered peptone water and homogenized (Seward 400, homogenizer, England). Using 10-fold dilution with 0.85% saline, the sample was diluted serially to 10^{-5}. One ml of each sample dilution in the range of 10^{-3} to 10^{-5} was inoculated in duplicate and mixed well with 12-15 ml of the SPCA. After solidification, it was overlaid with SPCA and incubated for 24-48 hours at 37°C and the total number of colonies was counted[11].

Coliform measurement

Using the dilution method described above, 1 ml of each sample dilution within the dilution range of 10^{-1} to 10^{-3} was inoculated in duplicate and mixed well with 12-15 ml of violet red bile agar (VRBA, Difco, Detroit, MI, USA). After solidification, the samples were overlaid with VRBA and incubated for 24-48 hours at 37°C and the number of coliform colonies were counted[11].

Measurements using HApS™ method

Using the equipments included in HApS™, Petrifilm ™ Aerobic and Coliform Count plates were inoculated with the diluted samples, according to the manufacturer's instruction. For total microbial counts, all colonies staining in various shades of red were counted. For ascertaining the number of coliform colonies, only red colonies with one or more gas associated with bubbles (within 1 colony diameter) were counted.

Statistical analysis

To ascertain the compatibility between the results of the conventional method and those of HApS™ method, a data were fitted to linear regression. The correlation coefficient (r) was calculated with the conventional method count as an independent variable and the HApS™ method count as a dependent variable (Microcal origin version 6.1, MA, USA). At the same time, each compatibility was tested with t-test.

Results and Discussion

Statistic data comparing the standard plate method with HApS™ method for enumeration of total microbial counts and coliform counts from pork, chicken, and beef are summarized in Table 1. Regression curves are shown in Fig 1. through Fig 6.

Total microorganism measurement

When the HApS™ method was compared with the AOAC method to measure total microbial contamination levels in meat products, a high correlation and com-

Table 1. Summary of linear regression, t-test on HApS™ method and the conventional AOAC method

		Pork		Chicken		Beef	
		TAC[a]/ PTC[b]	CC[c]/PCC[d]	TAC / PTC	CC/PCC	TAC / PTC	CC/PCC
Linear Regression	N[e]	80	80	80	80	70	70
	A[f]	-0.18691	0.84856	-0.33912	-0.02113	0.16689	0.14603
	B[g]	1.08003	0.72629	0.99188	0.95402	1.02366	0.93487
	r[h]	0.97767	0.82062	0.95594	0.96839	0.90712	0.94833
	SD[i]	0.21606	0.2973	0.38239	0.2006	0.55407	0.21519
	P[j]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
t-test	t[k]	1.22838	-0.5017	-1.87214	-1.47989	1.20809	-0.50473
	P[j]	0.22113	0.61657	0.06304	0.14089	0.22908	0.61456
	α[l]	0.05	0.05	0.05	0.05	0.05	0.05

a: Total Aerobic Count
b: Petrifilm Total Bacterial Count
c: Coliform Count
d: Petrifilm Coliform Count
e: Number of Sample
f: Intercept
g: Slope
h: Correlation Coefficient
i: Standard Deviation
j: P -value
k: T -value
l: Significance Level

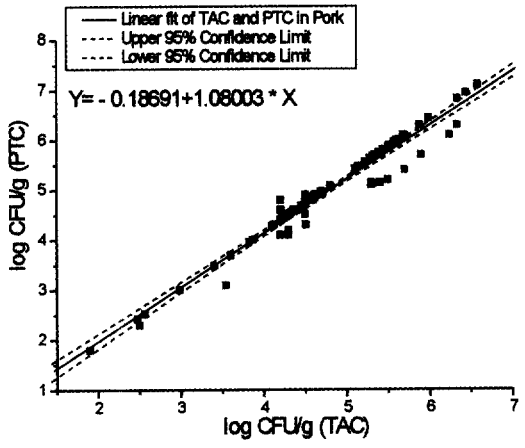

Fig. 1. Linear regression fit (solid line) of coliform count in pork meat with 95% confidence intervals (dashed line) for TAC vs PTC.

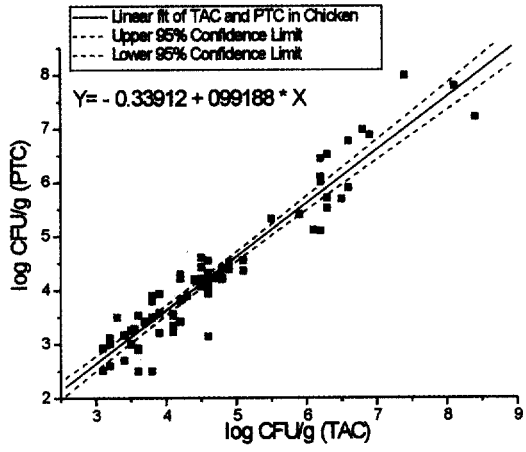

Fig. 2. Linear regression fit (solid line) of coliform count in chicken meat with 95% confidence intervals (dashed line) for TAC vs PTC.

patibility for data from total microbial counts from pork, chicken and beef samples were acquired. The correlation coefficients (r) were 0.97767, 0.90712, and 0.95594 for pork, beef, and chicken samples, respectively. These results showed that the total microbial count measurements were nearly identical for HApS™ and AOAC methods, especially, for the pork samples. These results were similar to previous studies, whose analysis of frozen and refrigerated food and meat samples. Those investigators demonstrated correlation coefficient 0.94 - 0.99 for the total microbial counts[1,3,12]. Independent t-test on HApS™ and AOAC methods represented that two means were not significantly different at the given

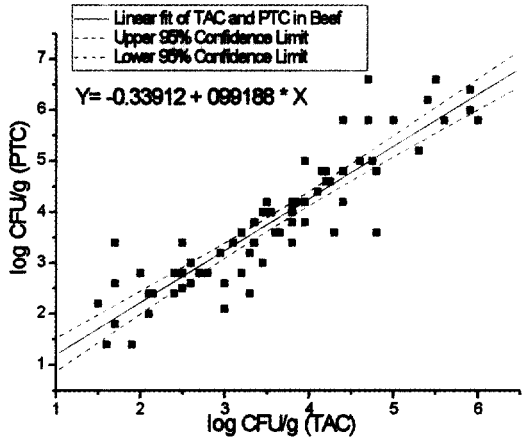

Fig. 3. Linear regression fit (solid line) of coliform count in beef meat with 95% confidence intervals (dashed line) for TAC vs PTC.

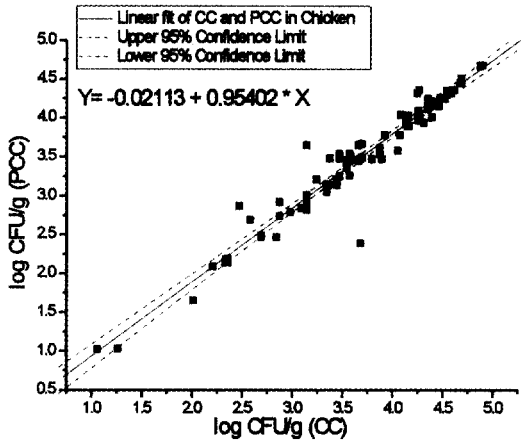

Fig. 5. Linear regression fit (solid line) of coliform count in chicken meat with 95% confidence intervals (dashed line) for CC vs PCC.

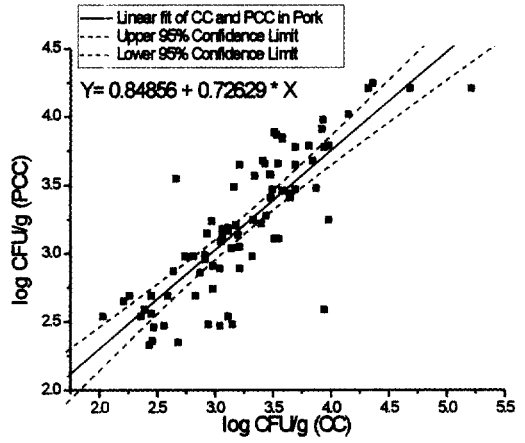

Fig. 4. Linear regression fit (solid line) of coliform count in pork meat with 95% confidence intervals (dashed line) for CC vs PCC.

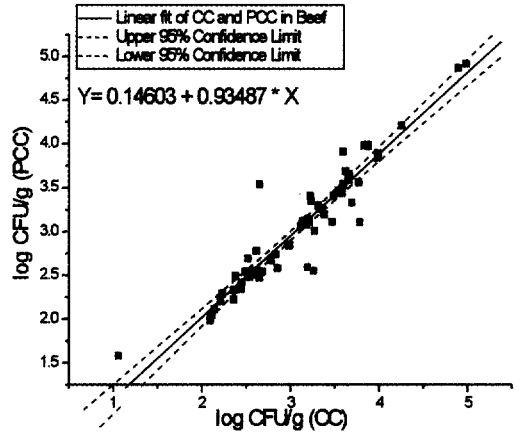

Fig. 6. Linear regression fit (solid line) of coliform count in beef meat with 95% confidence intervals (dashed line) for pour CC vs PCC.

significant level (α=0.05). Therefore, these combined results suggest that the HApS™ method could be reliably substitute for the AOAC method to quantify total microbial counts.

Coliform measurement

The correlation coefficient (r) for the pork, beef and chicken samples were 0.82062, 0.94833, and 0.96839 respectively. These results were similar to previous studies (Correlation coefficients of 0.87[1], 0.85[10], 0.86[14] and 0.93[13]). The correlation coefficients (r) from this study showed that the HApS™ method and standard VRBA method had a significant linear correlation. Independent t-test on HApS™ and AOAC methods represented that two means were not significantly different at the given significant level (α=0.05). Therefore, these results suggest that the HApS™ method is efficiently acceptable for counting coliforms. Coliform counts from the Petrifilm™ plate in the HApS™ method were lower than that of the AOAC method and it was more difficult to accurately perform these count using the Petrifilm™ plate because of the overlapping of some gas bubbles. Since the plates in the Petrifilm™ plate (50 mm) are smaller than the plate of the AOAC method (87 mm), the Petrifilm™ plate is more subject to colony overlap, resulting in lower counts.

The average time required for analysis of 20 samples

by the AOAC method, from sample preparation to incubation, was 2 hours compared to 45 minutes for the HApS™ using Petrifilm™ plate. If one includes the time to prepare incubation media for the AOAC method, the time saving is even more substantial.

In conclusion, the HApS™ method can replace the conventional AOAC method for faster measurement of microbial contamination in the meat samples without compromising accuracy.

국문요약

국내에서 시판중인 230점(돈육샘플80점, 계육샘플 80점, 우육샘플 70점)의 식육 샘플에 대하여 미생물 오염수준 측정시 표준방법으로 통용되는 AOAC (the Association of Official Analytical Chemists)의 standard total aerobic count method 및 coliform count method와 국내에서 개발된 HApS™ (Hazard Analysis process System) method를 비교하였다. 돈육, 계육, 우육을 샘플군으로 하여 총세균수 및 대장균수를 표준방법과 HApS™ 방법으로 각각 측정하여 그 두 방법간의 상관관계를 조사하였다. 선형회귀분석 및 t-검정법의 통계적 방법을 사용하여 각 샘플군에서의 두 방법간의 상관계수(r) 및 회귀직선식을 얻었고, 각 샘플군에서 두 방법에 의한 각각의 표본평균이 주어진 유의수준(α=0.05)에서 유의한 차이를 보이지 않았다. 즉, 본 실험에 사용된 샘플에 대하여 표준방법과 HApS™ 방법간의 유의한 차이를 발견하지 못하였다. 그러므로 표준방법에 비하여 실험시간 및 실험방법에서 신속성 및 간편성을 지니는 HApS™ 방법은 축산식품의 총세균 및 대장균군 오염도 측정시에 표준방법을 대신할 수 있는 유용한 방법이라고 할 수 있다.

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