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Research Note

Comparison between VIDAS Automatic Enzyme-Linked Fluorescent Immunoassay and Culture Method for *Salmonella* Recovery from Pork Carcass Sponge Samples

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ABSTRACT

VIDAS *Salmonella* (VIDAS-SLM) is an automated system that uses the enzyme-linked fluorescent assay method to detect *Salmonella* species. This study evaluated the efficacy of the VIDAS-SLM method in detecting *Salmonella* species in pork carcass sponge samples gathered from 10 slaughter plants in Taiwan. Two hundred fifty-seven pork carcass sponge samples were screened by the VIDAS-SLM method and by the culture method in parallel. While 18 sponge samples were found to test positive by both methods, the VIDAS-SLM method detected four additional positive samples for which the culture method failed to recover *Salmonella.* The specificity of the VIDAS-SLM method was found to be 0.98, and its sensitivity was 1.0, since no false-negative results occurred. Artificially inoculated *Salmonella* at concentrations as low as 5.0×10^0 CFU/ml was detected in the heat-inactivated sponge sample in the presence or absence of 5.0×10^4 CFU of *Citrobacter freundii* per ml. Thus, the VIDAS-SLM method is a rapid screening method and a potential alternative to the time- and labor-intensive culture method.

Conventional methods of recovering *Salmonella* can take 3 to 4 days to yield a negative result and require up to 7 days for a positive result *(13).* A high level of technical skill is required to perform these tests. Because of the time-consuming nature of these procedures, several rapid methods for detecting *Salmonella* have been developed, including enzyme-linked immunosorbent assays (ELISAs) *(12),* immunodiffusion methods *(8, 18),* immunomagnetic bead ELISAs *(3),* nucleic acid hybridization methods *(7),* and polymerase chain reaction methods *(1, 9, 11, 14).* The development of these rapid methods is crucial, particularly when the isolation and identification of *Salmonella* are routine for clinical laboratories.

The VITEK immunodiagnostic assay system (VIDAS; bioMérieux, Marcy l'Etoile, France) constitutes a fully automated enzyme-linked fluorescent immunoassay method. The VIDAS-SLM test is used for the detection of *Salmonella.* Previous studies involving various types of food have revealed a close correlation between the VIDAS system and conventional culture methods *(4, 5).* VIDAS has been used to detect salmonellae from poultry and milk *(5, 17).* However, data on the use of these methods for the detection of salmonellae on pork carcasses are lacking.

The Food Safety and Inspection Service issued a landmark rule, "Pathogen Reduction: Hazard Analysis and Critical Control Point (HACCP) Systems; Final Rule," on 25 July 1996. This rule sets pathogen reduction performance standards for *Salmonella* that must be met by slaughter plants and plants producing raw ground meat products. To improve the hygiene of the slaughtering process and set performance standards for *Salmonella,* the Bureau of Animal and Plant Health Inspection and Quarantine of Taiwan adopted this framework for the Nationwide Microbiological Baseline Collection Program. This program was designed by the Food Safety and Inspection Service to gather data to provide a microbiological profile of specific carcass pathogens. Consequently, it is important to identify accurate and time-efficient methods for detecting pathogens on carcasses. The objectives of this study were to compare the effectiveness the VIDAS system with that of the culture method in detecting *Salmonella* in naturally contaminated pork carcass sponge samples and to determine the sensitivity of the VIDAS system.

MATERIALS AND METHODS

Sample collection. Ten slaughter plants, located in Taipei, Taoyuan, Hsinchu, Miaoli, Taichung, Changhua, Nantou, Chiayi, Kaohsiung, and Pingtung Counties, Taiwan, were chosen for this investigation in January and June 2001. Each slaughter plant was visited once, and 25 pork carcasses from each plant except the plant in Taipei County were randomly selected and sampled after the final wash and prior to refrigerated storage. Thirty-two carcasses were collected from the slaughter plant in Taipei County. Each pork carcass sample was obtained by swabbing an area (ca. 10 by 10 cm) with a 0.1% peptone–moistened sterile sponge (Whirl-Park Speci-Sponge, NASCO, Fort Atkinson, Wis.). A template (10 by 10 cm) was used as a reference for the chosen sample size. The locations of the sampling sites were the belly, ham, and

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jowl areas, and each site was swabbed five times horizontally and five times vertically. Samples were individually bagged and placed in an insulated cooler capable of maintaining refrigeration temperatures, after which they were either shipped via express delivery to the laboratory or brought back from the slaughter plants by laboratory personnel.

Culture method. The culture method presented below is routinely used in our laboratory to screen for *Salmonella* on pork carcass sponge samples and is adopted, with slight modifications, from the U.S. Department of Agriculture Food Safety and Inspection Service Microbiological Laboratory guidebook. Peptone suspension from each sample was squeezed from the sponge, and 0.5 ml was inoculated into 4.5 ml of buffered peptone water and then preenriched at 35°C for 18 h *(6).* In a second enrichment step, 0.5 and 0.1 ml of peptone suspension were transferred into 10 ml of tetrathionate broth and 10 ml of Rappaport-Vassiliadis (RV) broth, respectively, and the samples were cultured at 42°C for 18 h *(16).* A loopful of tetrathionate or RV broth was then streaked onto the chromogenic culture medium, *Salmonella* detection and identification medium (SM-ID) agar (bioMérieux). *Salmonella* colonies were indicated by red coloration on SM-ID agar. Presumptive *Salmonella* colonies were streaked onto MacConkey agar (Difco Laboratories, Sparks, Md.) and biochemically and serologically tested, with API 20E (bioMérieux) and *Salmonella* antisera (Difco) being used for confirmation.

VIDAS *Salmonella.* The automated VIDAS was used to screen for the presence of *Salmonella* on the carcass surface with a VIDAS-SLM assay. Preenriched buffered peptone water samples (50 μl) were subcultured into 5 ml of RV broth and cultured at 42°C for 8 h. In parallel, 0.5-ml preenriched buffered peptone water samples were transferred into 4.5 ml of selenite cystine broth and incubated at 37°C for 8 h. Subsequently, 0.5 ml from each of the selective enrichment broths was added to separate tubes containing 4.5 ml of M-broth (Merck, Darmstadt, Germany) and incubated at 42°C for 18 h with constant agitation. One milliliter of the M-broth resulting from the RV and selenite cystine broths was mixed in another tube and boiled for 15 min. The heated M-broth (0.5 ml) was loaded into a VIDAS-SLM reagent strip and analyzed according to the manufacturer's instructions. A relative fluorescence value (RFV) of ≥0.23 for a sample was considered a presumptive positive result for the VIDAS-SLM assay. To confirm presumptive positive results, M-broth cultures were streaked onto SM-ID agar and processed according to the culture method described above.

Sensitivity test. Various controls were used to test the sensitivity levels of the VIDAS method and the culture method. Peptone suspension preparations from the carcass surface sponge samples that were confirmed to contain no *Salmonella* cells by the culture method were mixed and used for inoculation. Fifty milliliters of the peptone suspension from the mixture was heat treated at 60°C for 2 h to kill viable bacteria. Meanwhile, a pure culture of *Salmonella* Typhimurium ATCC 14027 (5.0×10^6 CFU/ml) suspended in sterile saline was used for inoculation. The suspension was serially diluted in 0.1% peptone to concentrations of 5.0×10^5, 5.0×10^4, 5.0×10^3, 5.0×10^2, and 5.0×10^1 CFU/ml. Heat-treated *Salmonella*-free suspension samples were then inoculated with different concentrations of cells to obtain final concentrations ranging from 5.0×10^4 to 5.0×10^0 CFU/ml. Another set of controls consisted of 5.0×10^4 to 5.0×10^0 CFU of *Salmonella* Typhimurium per ml, with 5.0×10^4 CFU of *Citrobacter freundii* ATCC 8090 per ml in each preparation. This control was intended to test the sensitivity of both methods in detecting *Salmonella* cells in the presence of non-*Salmonella* flora. Finally, 0.5 ml of each suspension was inoculated into 4.5 ml of buffered peptone water, preenriched, and tested by the culture method and the VIDAS method as described above. The false-positive rate (f_p) was defined as the number of samples that were VIDAS-positive but culture-negative divided by the total number of culture-negative samples, while the false negative rate (f_n) was defined as the number of samples that were VIDAS-negative but culture-positive divided by the total number of culture-positive samples. Sensitivity was defined as $1 - f_n$, and specificity was defined as $1 - f_p$.

TABLE 1. *Comparison between results obtained with the culture method and those obtained with the VIDAS method for naturally contaminated pork carcass surface samples*[a]

	Culture results		
VIDAS results	Positive	Negative	Total
Positive	18	4	22
Negative	0	235	235
Total	18	239	257

[a] Kappa value = 0.89.

Statistical analysis. In order to compare the results of the two different tests, the Cohen kappa value was determined *(15).* Only a sample for which VIDAS-SLM presumptive-positive (RFV ≥ 0.23) and confirmation-positive results were obtained was considered positive, while a presumptive-positive result coupled with a confirmation-negative result was considered a negative result.

RESULTS AND DISCUSSION

A total of 257 pork carcass sponge samples gathered from 10 slaughter plants located in 10 counties in Taiwan were screened for *Salmonella* by the VIDAS-SLM assay and the culture method. *Salmonella* was recovered from 18 samples by the culture method (Table 1). In comparison, the VIDAS-SLM approach not only detected the 18 confirmed positive samples, but also gave four false-positive results for samples for which no *Salmonella* was recovered from either the M-broth or the RV broth (Table 1). A total of 235 samples tested negative by both methods (Table 1). The reason four samples tested negative with the culture method but positive with the VIDAS-SLM method is unknown. Blackburn et al. *(2)* reported that some *C. freundii* isolates cross-reacted in the VIDAS-SLM assay. However, our study demonstrated that VIDAS-SLM did not cross-react *C. freundii, Citrobacter amalonaticus, Citrobacter diversus, Pseudomonas aeruginosa, Yersinia enterocolitica,* and *Enterobacter cloacae* (data not shown). Overall, the false-positive problem is minor, as the culture method failed to detect the presence of *Salmonella* for only 4 (18%) of the 22 samples that had tested positive. Should the VIDAS-SLM method be used routinely to screen carcass samples for *Salmonella,* further detailed investigation is required only for positive samples, thus saving the time and labor costs of culturing VIDAS-negative samples. No false-negative results were obtained in this study, but that does not necessarily guarantee that these samples were free of *Salmonella.* The number of *Salmonella* cells may be too small for resuscitation by the preenrichment procedure. The spec-

ificity of the VIDAS-SLM method was 97.67%, while its sensitivity was 100%, since no false-negative results were obtained. The specificity and sensitivity of the VIDAS-SLM method obtained in this investigation are very close to those reported by Curiale et al. *(4)* for tests involving a variety of food sources. For Curiale et al.'s study, sensitivity ranges from 95 to 100%, while specificity ranges from 84 to 100% *(4).* The kappa value (0.89) demonstrated very good agreement between the results of the culture method and those of the VIDAS method *(15).*

For the sensitivity test, the VIDAS-SLM method gave positive results for samples containing artificially inoculated *Salmonella* with an initial inoculum of 5.0×10^4 to 5.0×10^0 CFU/ml. When the non-*Salmonella* flora *C. freundii* was present at a concentration of 5.0×10^4 CFU/ml in the samples, *Salmonella* at 5.0×10^4 to 5.0×10^0 CFU/ml was also screened out by the VIDAS-SLM method. Typical red colonies of *Salmonella,* along with dark purple colonies of *C. freundii,* were present on SM-ID agar when the M-broth was streaked from the VIDAS-SLM–positive samples. In this study, chromogenic SM-ID agar, rather than conventional media such as xylose lysine desoxycholate or Hektoen enteric agar, was used to reduce the workload created by the unnecessary identification of suspected colonies. The major disadvantage of employing conventional media in the detection of *Salmonella* is the generation of false-positive results, such as those obtained with *Citrobacter* and *Proteus* species. In addition, the specificity of SM-ID was shown to be higher than that of conventional media *(10).* From our experience, we find that it is easy to misinterpret the agglutination result if *Salmonella* colonies grown on SM-ID agar are mixed with antiserum on a glass slide, and therefore colonies on SM-ID agar are routinely restreaked on MacConkey agar for serological and biochemical tests in our laboratory.

Heat was used to inactivate all of the vegetative cells before inoculating *Salmonella* or *Citrobacter* to attempt to remove any microflora that might possibly compete with target microorganisms and at the same time retain any substances present in the sponge sample. The presence of *Citrobacter* at a concentration of 5.0×10^4 CFU/ml in the sponge sample did not interfere with the growth of *Salmonella,* which could then grow to the VIDAS detection level following the preenrichment and selective enrichment steps. Any debris or other substances collected while swabbing did not appear to impede the level of *Salmonella* detection after dilution in the subsequent broths. This test provides a rough estimate of the sensitivity of the VIDAS-SLM method in detecting *Salmonella* from pork carcasses; however, the design of this test could not completely replicate the actual components of the sponge samples obtained from slaughter plants. Generally, the artificially contaminated samples failed to reproduce the ecological and physiological differences encountered in naturally contaminated samples *(5).*

Overall, the VIDAS-SLM assay requires less technical training than the culture method and reduces the time needed to obtain presumptive positive and negative results. Consequently, the VIDAS-SLM assay is a very feasible tool for use in clinical laboratories such as ours, which screen over 1,000 pork carcass sponge samples for *Salmonella* annually.

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