RESEARCH ABSTRACT

PORK SAFETY

Title: Comparative Evaluation of Rapid Methods for *Salmonella* Detection in Pork – **NPB #08-120**

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Scientific Abstract: The goal of this study was to develop an assay which combined all of the steps of conventional culture of *Salmonella* into a single well of a 48-deep well microtiter plate. The second goal was to screen hog carcass samples and ground pork to refine the assay as it was being developed. Finally, the specificity and sensitivity of the assay was compared to conventional microbiological *Salmonella* testing methods. Conventional culture includes pre-and selective enrichments followed by plating to a selective agar, such as XLT-4, on which *Salmonella* appear as black colonies. The components used for conventional *Salmonella* isolation were consolidated into each well (5 ml/well capacity): XLT-4 (400ul) was first added to each plate and allowed to solidify followed by addition of Modified Semisolid Rappaport-Vassiliadis (MSRV) with novobiocin (800ul) and the selective enrichment broth (900 ul). The 10% suspension of the test sample to be screened was added as the final upper layer (100 ul). Plates were sealed, incubated (42C, 48 hrs) and scored as positive if the lower XLT-4 agar layer turned black. The assay is called the RX plate because it incorporates Rappaport-Vassiliadis modified semisolid agar (R) and XLT-4 agar (X). Pure cultures were initially screened to ensure that the assay could detect *Salmonella*. Hog carcass lymph nodes harvested after the deep chill were screened (n=264) to ensure that non-specific blackening of the wells due to the tissue matrix did not occur. Lymph nodes harvested prior to the chiller (n=733), hog fecal samples (n=80) and retail pork sausage (n=240) were also evaluated. The original RX tube method offered the potential to replace conventional culture in a single tube with a reported 96.9% sensitivity and 93.1% specificity and a limit of detection of 1 *Salmonella* even in the presence of competing microflora. The RX microtiter format achieved 100% specificity and sensitivity only for pure cultures and the carcass swine lymph nodes recovered post deep chill. For retail purchased ground pork (n=210 samples), when compared to conventional culture, the estimated sensitivity (67%) and specificity (80%) of the RX reflect the unacceptable high number of false-positives. Despite repeated modifications, including incorporating antibiotics specifically inhibiting *Pseudomonas*, *Citrobacter*, and *Proteus*, the number of false positive reactions persisted. The time needed to transfer the RX blackened wells to XLT-4 for *Salmonella* confirmation realized little time savings when compared with conventional bacteriological isolation. The percentage of *Salmonella* positive samples detected in the BAX real-time PCR format was as expected slightly higher than that achieved by culture. In conclusion, the RX format, while theoretically attractive, failed to achieve the either the specificity or sensitivity of conventional microbiological culture.