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**TO:** American Meat Institute Foundation

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**RE :** **Final Report:** *Evaluation and Performance of the Premi-test®  
Salmonella System on Pork and Poultry Isolate from Commercial Sources*

## **Introduction**

*Salmonella* is the leading cause of human gastroenteritis and is responsible for 1.4 million cases in the United States (1). The rapid and accurate identification of *Salmonella* serotypes throughout the food chain is a critical factor in tracing the sources of outbreaks. Eggs, poultry and meat are frequent sources of transmission of *Salmonella* and other foodborne disease organisms, and are therefore highly regulated, continually monitored, and inspected. The Food Safety and Inspection Service have proposed that further analysis of *Salmonella* should include identification of serotypes frequently reported to cause human illness.

The *Premi®Test Salmonella* (PTS) serotyping system is a promising tool for rapid identification of *Salmonella* serotypes. The PTS is a DNA-based method that allows processing of samples within 9 hours with no need of highly trained personnel to perform the test. In addition, the chances of contamination are reduced. These could provide advantages over the traditional Kauffman-White method which is typically viewed as the gold standard for *Salmonella* serotyping. Rapid identification of *Salmonella* serotypes could potentially assist meat companies, the Food Net surveillance system, and government agencies in tracing sources of contamination, thus allowing for rapid corrective action when needed. A major outcome would be the decrease in the number of *Salmonella*-contaminated products reaching the consumer.

The following report discusses the use of the new *Premi®Test Salmonella* system to identify serotypes from both pork and poultry operations in the United States. Stored cultures obtained from the USDA along with a collection of fresh isolates were used to compare its ability to distinguish serotypes with that of traditional serotyping. One note on *Salmonella* nomenclature: by newer convention, names are retained only for subspecies *enterica* serovars, and these names are no longer be italicized. The first letter is a capital letter “S” followed by the serovar names of subspecies *enterica* (e.g. Typhimurium or Montevideo). This report follows the abbreviated modern naming system, i.e. *S.* Typhimurium rather than the more complete nomenclature *S. enterica*, subsp. *enterica* serovar Typhimurium.

### **Objective:**

The objective of this project was to evaluate the use of the *Premi®Test Salmonella* system as a serotyping tool to identify pork and poultry isolates obtained from vertically integrated operations and to compare the performance of the PTS system with traditional Kauffman-White (KW) serotyping methods.

### **Methodology**

#### **Stored Isolates**

Ninety *Salmonella* strains were obtained from the USDA–ARS–SPARC in College Station, TX, who generously allowed us to use them for this project. These cultures had been isolated using a

modified version of the USDA method, serotyped according to the traditional Kauffman – White scheme, and cryogenically stored. Isolates were shipped to the University of Nebraska-Lincoln (UNL) for typing using the PTS system for comparison. An additional 10 cultures were obtained from cryogenically stored cultures in the UNL Food Processing Center Laboratory's stock culture collection for a total of 100 isolates.

### **Fresh Isolates**

Fifty *Salmonella* strains from poultry and fifty from pork were isolated by investigators at Texas A&M using a modified version of the USDA method. Samples were collected from carcasses at different stages during the processing chain: live haul receiving, scalding, after evisceration, after chemical treatments, after cooling, and from final products. Following collection, samples were incubated overnight in buffered peptone water and then transferred to tetrathionate and Rappaport-Vassiliadis broth. After incubation overnight at 42°C, a loopful of the sample was streaked onto XLT4 and BGS agar. Samples showing typical colonies were screened for *Salmonella* using the GeneQuence® from Neogen (Lansing, MI). Samples with positive results for *Salmonella* from the GeneQuence® were confirmed using the API 20E biochemical system from BioMerieux. A subculture was then shipped to Mississippi State for serotyping according to the traditional Kauffman-White scheme, and to the University of Nebraska- Lincoln for typing by the *Premi*® *Test Salmonella* system.

Sponge samples were collected as follows: Samples were taken by pre-moistening a dry, sterile cellulose sponge (HydraSponge®; 3M, St. Paul, MN) with 25 ml of Butterfield's buffer (3M, St. Paul, MN). Using a sterile plastic glove, the sponge was removed from the sterile sample bag, all excess buffer expressed into the bag, and the sponge firmly rubbed against the surface of the animal, hide, carcass, or equipment approximately 10 times in the horizontal and 10 times in the vertical direction in approximately a 100-cm<sup>2</sup> area. The sponge was then turned over and the swabbing of the sample area repeated. For smaller pieces (e.g. ears and feet) and offal, the entire piece was swabbed. After sampling, the sponge was placed back into the sterile sample bag containing the expressed buffer and labeled. Labeled sample bags containing the sponge samples were packed into a cooler with cold packs for transport to the Food Microbiology Laboratory, Department of Animal Science, Texas A&M University, College Station, TX. Samples obtained from outside Texas were shipped overnight for next day delivery. Upon arrival at the laboratory, the temperature of samples was recorded and the samples prepared for analysis.

### ***The Premi*®*Test Salmonella* serotyping system**

#### Principle

The *Premi*®*Test Salmonella* system uses a methodology called multiplex ligation detection reaction (LDR) to generate a collection of circular DNA molecules which are subsequently PCR amplified. The test uses 25 DNA markers, three of which are generic markers used to verify that

the isolate belongs to the *Salmonella* genus, once the generic markers have confirmed the presence of *Salmonella*, the other 22 remaining markers are used to identify the serotype. The system creates a specific hybridization profile for each *S. enterica* serovar. A profile is generated by detecting positive hybridizations, each of which generates a spot. Each spot has a certain value assigned, thus the Genovar score is determined by adding up the spots in the pattern that those spots have formed. Once a certain serotype yields a specific genovar score at least three independent times, this serotype-genovar score is added to the PTS database and the software will indicate the serotype as well. In cases where the serotype-genovar association has not been found often enough, the software will only indicate the genovar score. However, the genovar score can still be useful in traceability.

The system allows processing three samples in one single tube because of the use of unique ZIP codes assigned to each LDR probe which are complementary to the oligonucleotides (cZIPcodes) immobilized in the microarray.

### **PTS procedures and equipment**

The PTS protocol has five steps:

1. Sampling
2. DNA extraction
3. PCR amplification
4. Hybridization
5. Reading

The major components of the system are shown in Figure 1. The reagents come in two separate boxes (Figure 1a). One box contains the set of reagents used for the PCR amplification and hybridization steps and is stored at -20°C. The second set of reagents is kept at room temperature and is used in the sampling, DNA extraction, and reading steps of the procedure.

The hardware needed to perform the test are commonly found in food microbiology testing laboratories, such as a heating block, a PCR system, standard pipetting devices with barrier tips, and an incubator for growing the cultures. Specialized equipment includes a single channel ATR03 reader connected to a standard computer, and the “Check points” software provided by the manufacturer. These are shown in Figures 1b through 1d. Typical DNA microarray pictures obtained with the ArrayTube® that is included in the kit. This format uses a DNA microarray chip fixed at the bottom of a micro-reaction vial, which can be seen in Figure 1d.

### **Results**

#### *Results from culture collection*

A total of 100 isolates from the USDA and UNL culture collections were tested using the PTS system and compared to the traditional Kauffman-White (KW) scheme. The results from these

tests are shown in Tables 1 through 4. Table 1 shows a comparison of KW versus the PTS system on serotypes isolated from **poultry** that were **not present** in the PTS database. The PTS system did not match KW serotyping on all 27 *Salmonella* serotypes that were tested. The system did respond with either a Genovar score or an alternative serotype, and correctly identified the isolates as *Salmonella* species 96% of the time. Table 2 shows the comparison of KW with the PTS system on serotypes isolated from **poultry** that **were present** in the PTS database. The PTS system matched KW serotyping on 45% of isolates tested. Again, the system did respond with either a Genovar score or an alternative serotype, and correctly identified the isolates as *Salmonella* species 96% of the time.

Table 3 shows a comparison of KW versus the PTS system on serotypes isolated from **pork** that were **not present** in the PTS database. Of the five that were tested, none matched the KW serotyping results. The system was able to correctly identify all isolates as *Salmonella* species, and produced either a Genovar score or alternative serotype. Table 4 shows the comparison of KW serotyping with the PTS system on serotypes isolated from **pork** that **were present** in the PTS database. The PTS system matched KW serotyping on 74% of the isolates tested. For the remaining isolates, a Genovar score or an alternative serotype was produced. The system also correctly identified all 27 isolates as *Salmonella* species.

#### *Results from the fresh isolates*

A total of 100 fresh isolates (50 from poultry, 50 from pork) were tested using the PTS system and compared to the Kauffman-White (KW) serotyping method. The results from these tests are shown in Tables 5 and 6. Table 5 shows a comparison of KW versus the PTS system on fresh isolates collected from **poultry** operations. The first column indicates the *Salmonella* serotyping result from the KW method, while the second column indicates the number of isolates of that serotype that matched the PTS system. The third column shows the locations that matching isolates were collected from. The last two columns show the alternative identifications produced by the PTS system and the locations where these isolates were found. The dominant serotype isolated was *S. Braenderup*, which comprised 52% of the total number of serotypes. Of these the PTS system matched the KW method in 78% of the isolates. The total match rate was 60% for all isolates. For those isolates that did not match, the system responded with either a Genovar score or an alternative serotype. The system also correctly identified the isolates as *Salmonella* species 100% of the time.

Table 6 shows a comparison of KW versus the PTS system on fresh isolates collected from **pork** operations. The information is outlined in the same format as Table 5 described above. The dominant serotype isolated was *S. Anatum*, which comprised 28% of the total number of serotypes. Of these the PTS system matched the KW method in 73% of the isolates. The total match rate was 66% for all isolates and the system correctly identified the isolates as *Salmonella* species 100% of the time. Again, for those isolates that did not match, the system responded

with either a Genovar score or an alternative serotype. One that was unknown (or untypable) by the KW method was given a Genovar score by the PTS method.

## **Discussion**

Overall, in tests with the USDA culture collection, the PTS results appeared to be reproducible independently of the source (pork or chicken). Sixty nine percent of the serotypes present in the PTS database matched traditional serotyping, and all were identified as *Salmonella*. Thirty one percent of the isolates present in the database were identified as *Salmonella* but did not match results from traditional serotyping. Further investigation may lead to discrepancies due to mistyping of the original isolates by the traditional method or overlaps with known serotypes. It has also been observed that serotypes can change over time depending on a number of factors such as storage and growth conditions (USDA, personal communication). Certain isolates not present in the PTS database were recognized as *Salmonella* Genovars, although the profile was unknown. It was difficult to decide whether these should be declared a “match” or not because the inherent limitations of the database preclude making this determination. Although some serotypes were not present in the database, the system did correctly identify these isolates as *Salmonella* species 96% of the time, indicating that the generic microarray markers were very accurate in determining species.

Among the 58 poultry isolates from the USDA collection, 56 different serotypes were represented; 27 serotypes were not present in the PTS data base and 29 were present. Thirty two USDA isolates from pork were evaluated, and 30 different serotypes were represented in this group. Serotypes which were not present in the PTS database are shown in Table 3. Most were assigned a different serotype from ones present in the database, except for *S. Uganda* which yielded a Genovar score. A majority of the serotypes present in the PTS database (Table 4) matched the serotyping results from the traditional method.

Serotyping of the fresh isolates yielded some interesting data. Both methods correctly identified the species as *Salmonella* 100% of the time. Two serotypes, *S. Kentucky* and *S. Braenderup* comprised 88 % of the total number of isolates found in chicken; while *S. Ohio* and *S. Anatum* made up 54% of the isolates found in pork (Tables 5 and 6). Among the fresh isolates only one serotype, *S. Johannesburg* from pork, was not included in the PTS database. Thirty out of fifty isolates from poultry fully matched with KW results which represent 60% of the total number of isolates from poultry; the remaining 40% yielded a Genovar score, a different serotype, or the report that the identification of the serotype was not possible (Table 5). However, the system was not able to identify *S. Kentucky*, although this serotype is claimed to be part of the database. Sixty six percent of the isolates from pork matched the results from the traditional method (Table 6).

A total of 200 isolates were evaluated using both the traditional Kauffman – White method and the Premi®Test *Salmonella* system. From the USDA isolates a wide variety of serotypes were assessed, with more than 60% of successful matches between the two methods occurring when the isolates were present in the database, if not, a Genovar score was generated. The presence of the genetic markers of the genus *Salmonella* were detected 100 % of the time. The results from serotypes present in the PTS data base that did not match the traditional method could be explained by a possible overlap with the profiles of those serotypes present in the database due to a close evolutionary relationship. It is also possible that *Salmonella* serotypes isolated in the United States have enough antigenic differences from their European counterparts to cause mismatches within the microarray, which was produced, manufactured, and validated in the Netherlands. According to a surveillance conducted on the world-wide distribution of *Salmonella* from 2000-2002, *S. Enteritidis* accounted for 85% of *Salmonella* cases, whereas *S. Typhimurium* was the most common human isolate (29%) in North America. In addition, the variety of serotypes in the U.S. was more evenly distributed with *S. Enteritidis* (21%), *S. Newport* (15%), and *S. Heidelberg* (10%) accounting for a sizeable proportion of the isolates (2).

*S. Ohio* and *S. Anatum* were the most frequently found serotypes isolated from pork sources, which represented over 50 % of the total number of fresh samples collected from pork processing plants. Similar results were observed by Rodriguez *et al.* (4). They reported on the prevalence of *Salmonella* in environmental farm samples and found *S. Anatum* to be the most commonly isolated serovar at 48.4% from 2,496 farm samples. The USDA reported that the five most frequently isolated *Salmonella* serotypes from swine collected from 1998 to 2000 were Derby, Typhimurium var. Copenhagen, Johannesburg, Infantis and Heidelberg (6). None of the most frequently isolated serotypes in this study fell into this group. Another interesting observation is that *S. Ohio* and *S. Anatum* are not listed among the top 20 most commonly reported serotypes from human sources (1). The results from this study indicate that although swine and poultry environments are reservoirs for *Salmonella*, the serotypes frequently reported in the literature to be most prevalent may not be representative of all plants and all regions of the United States. Much larger studies are needed corroborate these findings.

*S. Kentucky* and *S. Braenderup* represented 88% of the fresh samples isolated from poultry sources in this project. *S. Braenderup* is the 12th most often isolated serovar from human sources while *S. Kentucky* is not even listed as a human isolate. *S. Kentucky* appears to be the most prevalent *Salmonella* serovar in chicken (3, 5). Although this serovar does not cause invasive disease, some isolates have been shown to possess the MDR-AmpC multidrug resistance pattern (5). It is important to consider the possibility of other *Salmonella* serotypes acquiring resistance genes from *S. Kentucky* (5). Larger studies and increased sampling will help determine if the number of resistant strains is increasing in poultry processing plants around the country.

## Conclusions

The Kaufmann-White traditional serotyping method based on antibody-antigen reactions is considered the gold standard for typing *Salmonella* species. However, the method does possess deficiencies in that it is time-consuming, results are sometimes not reproducible, highly experienced personnel are required to perform the test, and the availability of sera can be limiting. The PTS system is a DNA based method which targets genetic information of different serovars for the purpose of identifying the serotype in addition to the genus *Salmonella*. The PTS system's processing time of 8-9 hours after enrichment and isolation is highly attractive for high-throughput laboratories. The PTS system is relatively simple to use and previous research has indicated a high specificity for the 100 serotypes present in the data base (7). The procedures are standardized and therefore should be more easily reproducible from laboratory to laboratory. The use of this system has the potential of increasing the accuracy of serotyping and decreasing the time to result of analysis, which are important factors when responding to outbreaks or when monitoring sanitary controls in flocks or slaughter operations. Although complete differentiation between all serotypes is not yet possible in this system, future releases of the PTS software should include new identifiers that will expand the database.

The PTS system has tremendous potential for additional growth, expansion, and research even though the results of this study indicate that it does not yet possess the discriminatory power necessary to replace traditional serotyping. It is recommended that companies and research institutions interested in this technology maintain links to traditional serotyping methodologies to verify the instrument and work hand-in-hand with the manufacturer to identify difficult serotypes. Genovar scores should be analyzed in greater depth to find if they correspond to unique serotypes in different locations. Also, specific practical and technological issues need to be addressed. For example, the price of the kits is quite high (\$3,200 for 72 samples), and the incubation time for the final detection step seemed to be inconsistent from sample to sample. Although a 15 minute incubation time is recommended, sometimes the reader would not produce any result or a correct result until a longer incubation time was used. Sometimes the reader would report one serotype, and then a few minutes later would report a different one when it was read a second time. Correcting this issue will help avoid discrepancies in the future. The system also had trouble in identifying *S. Kentucky*, a common serotype in poultry in the U.S. There seems to be some evidence that the use of pure DNA extracts is better than crude extracts in increasing the accuracy of the device, therefore this may lead to more reliable results.

As the system and method evolves, it should continue to undergo rigorous testing on as many isolates from as many sources as possible that originate from all parts of the world. If perfected, this new technology could provide a means of rapid surveillance of *Salmonella* serotypes in the food chain and in epidemiological investigations.



## References

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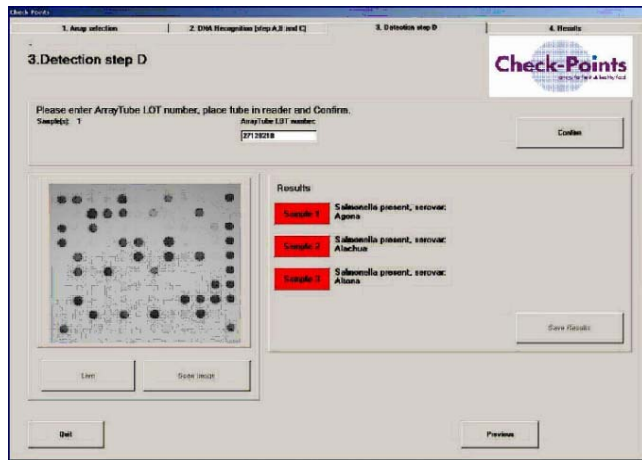
Figure 1. Major Components of the *Premi*®*Test Salmonella*



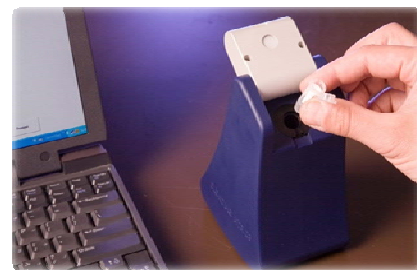
a. Kit contents



b. Heating block and Thermocycler



c. Checkpoints Software



d. Microarray tube and reader

TABLE 1. Comparison of Kaufmann-White (KW) and PTS results from USDA isolates collected from POULTRY, *NOT PRESENT* in the PTS database

<b>KW</b>	<b>PTS Results</b>
S. G22-,23+	Genovar 3171
S. Bere	Genovar 3303
S. 4, 12:i:-	Genovar 3997
S. 4,12:-:1,2	Genovar 13487
S. 4,5:2:-	S. Typhimurium
S. 4,5:d:-	S. Schwarzengrund or Grupensis
S. 4,5:i:-	S. Typhimurium
S. 6,7: nonmotile	Genovar 7604
S. 6,7:-:1,5	S. Muenster or Montevideo
S. 6,7:-:1,6	Muenster or Reading 14958.F
S. 6,7:k-	S. Brandenburg
S. Alachua	S. Cubana
S. Cape	S. Thompson
S. Essen	Derby
S. Fresno	S. Ouakam or Meleagridis
S. Gaminara	S. Typhimurium
S. Kiambu	Genovar 15533
S. Menston	S. Oranienburg
S. Mississippi	Genovar 16013
S. Molade	Genovar 10299
S. Norwich	Genovar 3104
S. Remo	S. Schwarzengrund or Grupensis
S. roughO:y:1,7	S. Pomona
S. Thomasville	S. Orion
S. Truro	S. Typhimurium
S. Try Z29	No Salmonella
S. Uganda	Genovar 13487
Total Match	0/27 (0%)
Salmonella species confirmed	26/27 (96%)

TABLE 2. Comparison of Kaufmann-White (KW) and PTS results of USDA isolates collected from POULTRY, *PRESENT* in the PTS database.

<b>KW</b>	<b>PTS Results</b>
S. Havana	Genovar 3171
S.1,4,5,12:i:-*	S. 1,4,(5),12:l
S.1,4,5,12:i:-*	S. 1,4,(5),12:l
S. Agona	S. Montevideo
S. Braenderup*	S. Braenderup
S. Colindale	S. Montevideo
S. Cubana*	S. Cubana
S. Derby*	S. Derby
S. Enteritidis	Salmonella suspected
S. Hadar*	S. Hadar
S. Heidelberg*	S. Heidelberg
S. Infantis	S. Heidelberg
S. Kentucky	Genovar 10299
S. Kentucky	No Salmonella
S. Lille	Genovar 14537
S. Litchfield	S. Ouakam
S. Livingstone	S. Lille
S. Meleagridis*	S. Meleagridis
S. Montevideo*	S. Montevideo
S. Muenchen*	Montevideo or Muenchen
S. Muenster	Genovar 14948
S. Oranienburg	S. Monschau
S. Orion,var, 15, 34*	S. Orion
S. Quakam*	S. Quakam
S. Senftenberg*	S. Senftenberg
S. Stanley	S. Muenchen
S. Schwarzengrund	Serovar cannot be identified
S. Tennessee	S. Ouakam
S. Thompson*	S. Thompson
S. Typhimurium	No Salmonella
S. Worthington	Genovar 14377
Total Match*	14/31 (45%)
Salmonella species confirmed	30/31 (96%)

TABLE 3. Comparison of Kaufmann-White (KW) and PTS results from PORK, *NOT PRESENT* in the PTS database

KW	PTS
3,10:L,W-Monophasic	S. Meleagridis
S. Johannesburg	S. Urbana
S. Menhaden	S. Give
S. New Brunswick	S. Give
S. Uganda	Genovar 13487
Total	0/5 (0%)
Salmonella species confirmed	5/5 (100%)

TABLE 4. Comparison of Kaufmann-White (KW) and PTS results from PORK, *PRESENT* in the PTS database

KW	PTS
1,4,5,12:l-*	S. 1,4,5,12:i
S. Agona*	S. Agona
S. Anatum*	S. Anatum
S. Braenderup*	S. Braenderup
S. Derby*	S. Derby
S. Havana	Genovar 9610
S. Heidelberg*	S. Heidelberg
S. Infantis*	S. Infantis
S. Javiana*	S. Javiana
S. Livingstone	Genovar 14537
S. Mbandaka*	S. Mbandaka
S. Meleagridis*	S. Meleagridis
S. Montevideo	S. Schwarzengrund or Grupensis
S. Muenchen	S. Newport
S. Muenster*	S. Muenster
Multiple Serotypes*	S. 1,4,5,12:i
S. Newport*	S. Newport
S. Orion*	S. Orion
S. Schwarzengrund*	S. Schwarzengrund or Grupensis
S. Tennessee	Genovar 56
S. Thompson*	S. Thompson
S. Typhimurium*	S. Typhimurium
S. Typhimurium*	S. Typhimurium
S. Typhimurium*	S. Typhimurium
Untypable	S. Meleagridis
S. Urbana*	S. Urbana
S. Worthington	S. San Diego
Total Match*	20/27 (74%)
Salmonella species confirmed	27/27 (100%)

Table 5. Comparison of Kaufmann-White (KW) and PTS results of fresh isolates collected from POULTRY

Salmonella serotype (KW)	Salmonella PTS RESULTS			
	Complete Match	Location	Other I.D. (# Isolates)	Location
S. Braenderup	26	Carcass, rinse feathers on, outside beetle, inside beetle, soil inside, soil outside, water, feed, ceca, booty, beetle, larvae	Genovar 9614 (2)	Ceca, litter
			Genovar 9646 (1)	Water
			Genovar 11658 (1)	Soil inside
			Manhattan (2)	Inside beetle, booty
			Unidentified (1)	Feed
S. Kentucky	0		Genovar 10299 (6)	Scalder, live chicken loader, chicken after picking
			Genovar 102983 (1)	Chicken feet
			Genovar 14907 (1)	Live chicken loader
			Genovar 15423 (1)	Feet chute
			Ohio (1)	Inedible barrel evisceration
			Unidentified (1)	Chicken after picking
S. Newport	3	Carcass rinse feathers on	Genovar 13502 (1)	Booty
S. Anatum	1	Booty		
S. Seftenberg	0		Genovar 2156 (1)	Scalder/picker inedible barrel
TOTAL MATCH	30/50 (60%)		20/50 (40%)	
Salmonella species confirmed	50/50 (100%)			

Table 6. Comparison of Kaufmann-White (KW) and PTS results of fresh isolates collected from PORK

Salmonella Serotype ( KW)	Salmonella PTS Results			
	Complete Match	Locations	Other I.D. (# Isolates)	Locations
S. Anatum	14	Inedible cart, head, head without hide, feces, hide puller, ears, foot, hide conveyor, ground pork, offal	Genovar 16111 (5)	Offal, hide, foot, ears, head
S. Ohio	6	Inedible cart, offal, hide puller chain, foot, inedible conveyor belt, post evisceration conveyor	Genovar 16077 (1)	hide
			Unidentified (1)	foot
S. Typhimurium var. Copenhagen	0		Genovar 11935 (1)	Stomach
			Unidentified (1)	Chunk trim meat
S. Derby	1	tongue	Unidentified (1)	head
			Adelaide (1)	inedible
S. Heidelberg	3	Inedible cart, hide puller		
S. Mbdanka	2	Ears, head	Genovar 11949 (1)	Hide puller
S. Adelaide	1	Stomach		
S. Agona	0		Altona (1)	Head rack
S. Bovis-morbificans	0		Genovar 15607 (1)	offal
S. Manhattan	1	offal		
S. Newport	0		Unidentified (1)	Inside barrel
S. Saint Paul	0		Unidentified (1)	hide
S. Johannesburg	0		Urbana (1)	offal
S. Typhimurium	2	Ground meat, head		
Not Salmonella	2	Ground pork, feces		
Unknown by KW	1		Genovar 7540	Pen feces
TOTAL MATCH	33/50 (66%)		17/50 (34%)	
Salmonella species confirmed	50/50 (100%)			