test results. In other words, although single sera, which have *Salmonella* antibody concentrations around the cut-off point, can be recognized as positive with one test and negative with the other test or vice versa, the three tests can be used, since less than 10% of the sera tested over years in Germany, have *Salmonella* antibody concentrations around the cut-off point (around 70% have very low titers, and about 15% have quite high titers, which are always correctly identified by all three tests as negative or positive). This means that the risk that a set of sera is categorized differently by using another test out of the three tests is so low that it can be neglected in the light of the overall goal of the German Salmonella Monitoring and Reduction Programme: Identifying via an ongoing semi-quantitative estimation the herds with the relatively highest risk of introducing zoonotic *Salmonella* spec. into the food production chain to be able to implement measures for a) reducing the cross-contamination of *Salmonella* spec. in the slaughter plant and b) reducing the salmonella load of swine herds identified as high risk herds. Finding the herds with the relatively highest salmonella risk, at which cut-off point ever, will be performed by all three tests at a comparative herd sensitivity level.

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ABBREVIATED IDENTIFICATION SCHEME FOR *ESCHERICHIA COLI* IN SWINE FECES

O 32

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Summary: Antimicrobial susceptibility profiles of *Escherichia coli* (EC) are often used to monitor the effect of antimicrobial use regimens on the antimicrobial resistance (AR) reservoir in animal species. Epidemiological studies of AR may involve the identification of thousands of bacterial isolates, so complete biochemical identification of EC can be prohibitively expensive and time consuming. In this study an abbreviated biochemical scheme using colony phenotype and the indole test results in a sensitivity and specificity of 91.7% and 100% respectively for identification of EC as compared to a commercial biochemical identification kit. This abbreviated scheme results in over US$500 savings per 100 candidate EC isolates identified. These savings have significant benefits to the economics of conducting epidemiologic investigations of AR.

Keywords: Indole, Antimicrobial Resistance, API 20E, Sensitivity, Specificity

Introduction: The gram negative fecal flora represents a reservoir of antimicrobial resistance (AR) genes transferable to foodborne pathogens. *Escherichia coli* (EC) are often used to define the reservoir of AR. Epidemiological studies may involve testing thousands of isolates, so complete biochemical identification of EC can be prohibitively expensive. The goal of this project was to develop an inexpensive and effective method of identification of EC. Previous investigators (Hyatt et al., 2002) determined that 99.4% of colonies isolated from cattle feces that had typical EC phenotype on MacConkey agar and were positive for indole production were EC. According to the Manual of

Clinical Microbiology (Farmer, 1999), 95% of active EC ferment lactose and 98% of active EC are positive for indole production. Of inactive EC, 25% ferment lactose and 80% are indole positive.

Materials and Methods: In order to determine if the abbreviated protocol would be effective for swine feces we used the following methods. As part of a separate investigation (Funk et al., 2003) fecal samples from 154 randomly selected market age pigs representing 3 different farms were collected by a free catch method with a clean glove used for every sample. The samples were then placed in sterile Whirl-pak bags (Nasco, Ft. Atkinson, WI, USA) and transported to the lab. Samples were processed as previously described (Funk et al., 2003). One bacterial colony was selected from each sample for storage at -80° C for further AR testing. Stored isolates were streaked onto MacConkey agar (Becton Dickinson, Sparks, MD USA) and incubated at 37^{0}C for 18-24 hours. Following incubation colonies were phenotypically compared to that of the control strains. Those colonies that were phenotypically consistent with EC were then inoculated into 4mL of DEV Tryptophan broth (EM Science, Gibbstown, NJ USA) for the detection of indole production. Following incubation at 37^{0}C for 18-24 hours, 3-5 drops of Kovac's reagent was added to each tube. A red ring at the top of the tube represented the presence of indole. All indole positive isolates were identified as EC by the abbreviated method. All isolates were also inoculated onto an API 20E strip according to manufacturer's instructions (BioMeriux, Hazelwood, MI USA).

Results: Of the 154 isolates tested, 144 were identified as EC by the API 20E. The other 10 isolates were *Klebsiella* (2), *Salmonella* (2) or unidentifiable (6). One hundred thirty-two isolates were identified as EC by the abbreviated scheme. When the unidentified isolates were coded as non-EC using the gold standard, the sensitivity and specificity of the abbreviated scheme was 91.7% and 70.0% respectively (Table 1). The positive predictive value was 97.8%, and the negative predictive value was 37.0%. When isolates unidentifiable by the API 20E were not included in the analysis, the sensitivity remains 91.7% and the specificity improves to 100%. The positive predictive value becomes 100% and the negative predictive value becomes 25.0% (Table 2). As a consequence of the results of this study, our lab protocol is as follows: Isolates are struck onto MacConkey agar and incubated at 37°C for 18-24 hours, those isolates that phenotypically appear to be EC are then inoculated in 4mL of DEV Tryptophan broth and incubated at 37°C for 18-24 hours. Following incubation isolates were tested for the presence of indole, using Kovac's reagent. All isolates that are not identified as EC by the abbreviated scheme are identified using the API 20E strips.

Discussion: In epidemiological investigations of AR, there is a need to identify large numbers of bacteria. When looking at large numbers of samples there are often two limiting factors, time and finances. In adopting this method of identification a substantial amount of money can be saved with very little labor cost as compared to traditional biochemical identification systems. Using the gold standard method there would be a cost of US$541.00 for every 100 isolates. Using the method described above there is a cost of US$34.65 for every 100 isolates, assuming 95% of the isolates are EC. There is a savings of US$506.35 for every 100 isolates. These savings have significant impact of the financial resources necessary for conducting epidemiological studies.

Table 1. *Sensitivity and specificity of abbreviated scheme as compare to the API 20E for biochemical identification of E. coli. Isolates unidentified by API are coded as non-E. coli.*

		Gold Standard (API 20E)		
		Positive	Negative	
Abbreviated Scheme	Positive	132	3	PPV=132/135=97.8%
	Negative	12	7	NPV=7/19=36.9%
		Sensitivity=132/144 =91.7%	Specificity=7/10 =70%	

Table 2. *Sensitivity and specificity of abbreviated scheme as compare to the API 20E for biochemical identification of E. coli. Isolates unidentified by API are not included in analysis.*

		Gold Standard (API 20E)		
		Positive	Negative	
Abbreviated Scheme	**Positive**	132	0	PPV=132/132=100%
	Negative	12	4	NPV=7/19=25%
		Sensitivity=132/144 =91.7%	Specificity=4/4 =100%	

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Isolation of *Salmonella* enterica in seropositive classified finishing pig herds

O 33

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Summary: The aim of this study was to assess the probability of detecting *Salmonella* from pen faecal samples in seropositive classified finishing pig herds. The study involved 77 herds from Denmark (20), the Netherlands (20), Greece (17) and Germany (20). The serological herd status was determined by the blood- sampling of 50 finishing pigs. Bacteriological sampling was performed by 20 pen faecal samples per herd. Over-all, 47 % of the blood samples had an OD% larger than 10 and 23 % larger than 40. *Salmonella* was isolated from 135 (9.3 %) pen faecal samples in 32 herds (42 %). Twenty-eight of these herds (87.5 %) had a within-herd seroprevalence larger than 50% at sample cut-off OD%>10. A correlation coefficient of 0.62 was found between the proportion of culture positive- and seropositive samples in a herd at cut-off OD % > 10 and of 0.58 at cut-off OD % > 40. Due to the low sensitivity of culture methods, apparent 'false positive' serological results may well represent real infections not detected by bacteriological testing. In this study, there was an increasing probability of recovering *Salmonella* with increasing within-herd seroprevalence.

Keywords: pig-bacteria, herd-status, serology, bacteriology, epidemiology,