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UK Standards for Microbiology Investigations

Review of Users' Comments received by
Working Group for Bacteriological Identification and Test Procedures

ID 16 Identification of Enterobacteriaceae

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PROPOSAL FOR CHANGES

Comment Number	1		
Date Received	06/12/2013	**Lab Name**	AusDiagnostics
Section	3.5		
Comment			
The section on RT-PCR refers to the work of Hernandez-Guijarro et al. (1).These authors state that that 'multiplex PCR is cumbersome and sometimes lacks reproducibility between laboratories because of the specific conditions needed for simultaneous amplification of several regions'. While the development of multiplex PCR assays is not trivial, there are now wide ranges of CE marked multiplex-PCR IVD systems available, see eg (2), (3), (4), (5). Many of these are not cumbersome, and show excellent sensitivity and selectivity. Could this blanket statement be removed?			
Evidence			
(1) Hernandez Guijarro, K., Feingold, S. & Terzolo, H. R. A Single Nucleotide Polymorphism on rpoB Gene Allows Specific Identification of *Salmonella enterica* Serotype Typhimurium. Res. J. Microbiol. 344-352 (2012). (2) Stanley, K. K. & Szewczuk, E. Multiplexed tandem PCR: gene profiling from small amounts of RNA using SYBR Green detection. Nucleic Acids Res. 33, e180 (2005). (3) Anderson, T. P. et al. Comparison of four multiplex PCR assays for the detection of viral pathogens in respiratory specimens. J. Virol. Methods 191, 118-121 (2013). (4) Touati, A., Benard, A., Hassen, A. Ben, Bébéar, C. M. & Pereyre, S. Evaluation of five commercial real-time PCR assays for detection of *Mycoplasma pneumoniae* in respiratory tract specimens. J. Clin. Microbiol. 47, 2269-71 (2009). (5) Pillet, S. et al. Comparative evaluation of six commercialized multiplex PCR kits for the diagnosis of respiratory infections. PLoS One 8, e72174 (2013).			
Recommended Action	**ACCEPT** The subsection on Real time PCR has been updated to reflect that “However, multiplex PCR for *Salmonella* serovars identification may be cumbersome (due to its limitation on specificity) and sometimes lack reproducibility between laboratories because of the specific conditions needed for simultaneous amplification of several regions” {Guijarro, 2012 37259 /id; Park, 2009 37257 /id}. This sentence is specific for *Salmonella* serovars identification and not the other *Enterobacteriaceae.*		