Statement of Inactivation of Mycobacteria and *Nocardia* during Sample Processing

Viability studies for mycobacteria and *Nocardia* were performed by bioMérieux's Research and Development department to assess and verify there is no biological risk to the user when handling test samples or slides after the inactivation portion of the sample preparation protocol has been performed.

Fresh cultured strains from a panel of different mycobacteria and *Nocardia* species (Table 2) were inactivated as part of the sample preparation protocol developed by bioMérieux. The test panel included mycobacteria (32 strains tested by the bead beater method in the initial study[1], 28 strains tested by the vortex method in a follow-up study, see Table 2) as well as *Nocardia* (23 strains by each method). Testing encompassed both antibiotic susceptible and antibiotic resistant strains of *Mycobacterium tuberculosis*, as well as other non-tuberculous mycobacteria. Organisms were first grown on solid media. To ensure inactivation of cell mass encountered during routine testing, the inactivation studies were performed using a higher cell mass (i.e. $1.3x10^9$ CFU) of mycobacteria than normally encountered in a clinical microbiology laboratory (Table 1) using different media types.

Table 1

Inactivation Study	Approximate Colony Forming Units (CFU) in Routine Testing*	
	Solid Media	Liquid Media
$1.3x10^9$ CFU	$\leq 2.1x10^8$ CFU	$\leq 3.1x10^8$ CFU

* Based on internal bioMérieux enumeration studies

After performing the inactivation steps as outlined in the *Mycobacterium/Nocardia* Kit package insert, the bacterial cells were then inoculated onto appropriate solid and liquid culture media for detection and recovery. **NOTE:** *Nocardia* were tested on solid media only. The cultures were incubated at 37°C and monitored for growth for 42 days. No growth was seen for the 32 mycobacteria and 23 *Nocardia* demonstrating that no organisms were viable after the inactivation steps of the sample preparation protocol.

This sample preparation protocol consists of the sample being subjected to mechanical disruption with 0.5 mm glass beads in 70% ethanol. Mechanical disruption is achieved by using a bead beater for 5 minutes or by using a vortex-type mixer with a horizontal position adaptor for 15 minutes[2,3]. The sample is then incubated for 10 minutes at room temperature in the same tube for complete inactivation. Both mechanical disruption methods were tested in the inactivation study with no viable organisms observed.

As these are highly pathogenic organisms, safe handling practices, which may include working in a Biosafety Level 3 (BSL3) environment, are recommended prior to inactivation. These steps are described in detail in the package insert for the VITEK® MS *Mycobacterium/Nocardia* Kit. Based on the bioMérieux R&D studies, this preparation procedure has been shown to repeatedly inactivate mycobacteria and *Nocardia* species if followed exactly as described. Once the mycobacteria and *Nocardia* are no longer viable, the user can perform any subsequent steps including protein extraction, spotting, and handling of the VITEK® MS-DS target slide outside of a Biological Safety Level 3 (BSL3) environment.

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Table 2

Mycobacteria Species Tested	# of Strains Tested (Bead Beater)	# of Strains Tested (Vortex)
Mycobacterium tuberculosis **(antibiotic susceptible)**	**5**	**2**
Mycobacterium tuberculosis **(antibiotic resistant)**	**4**	**3**
Mycobacterium fortuitum	**3**	**3**
Mycobacterium senegalense	**1**	**1**
Mycobacterium abscessus	**3**	**3**
Mycobacterium intracellulare	**3**	**3**
Mycobacterium kansasii	**3**	**3**
Mycobacterium avium	**2**	**2**
Mycobacterium chelonae	**2**	**2**
Mycobacterium gordonae	**1**	**1**
Mycobacterium scrofulaceum	**1**	**1**
Mycobacterium smegmatis	**1**	**1**
Mycobacterium genavense	**2**	**2**
Mycobacterium haemophilum	**1**	**1**
Total	**32**	**28**
Nocardia **Species Tested**	**# of Strains Tested (Bead Beater)**	**# of Strains Tested (Vortex)**
Nocardia cyriacigeorgica	**9**	**9**
Nocardia farcinica	**4**	**4**
Nocardia kruczakiae	**2**	**2**
Nocardia nova	**7**	**7**
Nocardia otitidiscavarum	**1**	**1**
Total	**23**	**23**

In conclusion, these inactivation studies show that mycobacteria (from solid and liquid media) and *Nocardia* (from solid media) are no longer viable after performing the inactivation steps if the user adheres to the protocol provided by bioMérieux. As a general safety precaution, all users should wear protective powder-free gloves when handling the VITEK® MS-DS target slide.

Note: For further information about the inactivation study for the mycobacteria and *Nocardia, please refer to the following publications:*

[1] Dunne WMJ, Doing K, Miller E, et al. Rapid Inactivation of Mycobacterium and Nocardia Species before Identification Using Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry. *Journal of Clinical Microbiology*. 2014;52:3654-3659.

[2] Deol P, Girard V, Hyman J, et. al. Identification of Mycobacteria by VITEK® MS Matrix-Assisted Laser Desorption Ionization –Time of Flight Mass Spectrometry. MSACL, San Diego, CA, 9-13FEB2013.

3 Totty H, Miller E, Moreno E, et al. Comparison of mechanical disruption techniques for the rapid inactivation of Mycobacterium and Nocardia species before identification using MALDI-TOF mass spectrometry, submitted.