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Identification of *Mycobacterium avium* subsp. *paratuberculosis* (MAP) via VOC analysis over in vitro cultures using differential ion mobility spectrometry (DMS)

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Objectives

To investigate if VOC analysis using GC-IMS is capable of identifying in vitro MAP growth.

Conclusions

This study provides strong evidence that identification of MAP positive samples is possible by IMS analysis of headspace over bacterial cultures, even much earlier than with current standard methods.

Results

- VOC´s over bacteria-free control slants differed clearly from VOC patterns of control slants inoculated with heat-inactivated MAP.
- Positive samples showed a high proportion of significant differences to all groups of controls. The longer the incubation time, the higher the proportion of clusters with significant differences.
- A positive sample can be identified after just one week of incubation.
- Using principle component analysis, the groups could be very well separated just by the first two factors.

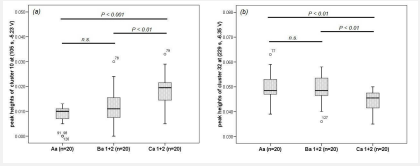

Figure 1: Two representative clusters with significant differences between control samples

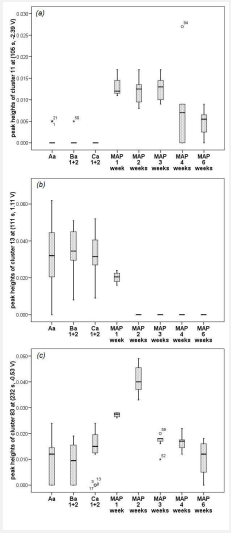

Figure 2: Differences in the peak intensity of three representative VOC clusters (a, b, c) between controls and MAP positive samples (Dilution 10^0)

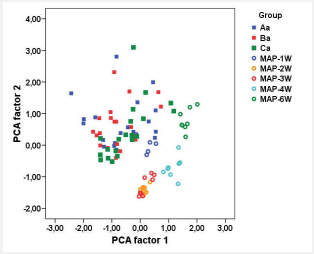

Figure 3: Position of controls (Aa, Ba, Ca) and MAP positive samples (dilution 10^0) in coordinates of first two PCA-factors

Table 1: Cross-validated discriminant analysis (first 20 clusters) between control groups and positive samples (Dilution 10^0, Week 1)*

		Actual Group	Predicted Group Membership		Total
			Aa/Ba/Ca (1+2)	MAP-1W	
Cross-validated	Count	Aa/Ba/Ca (1+2)	60	0	60
		MAP-1W	0	4	4
	%	Aa/Ba/Ca (1+2)	100.0	.0	100.0
		MAP-1W	.0	100.0	100.0

*100.0 % of cross-validated grouped cases correctly classified

Materials and Methods

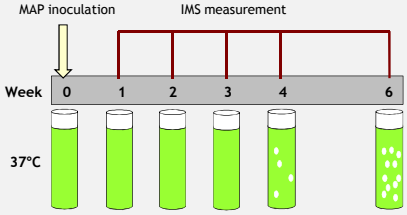

Figure 4: Experimental setup

Table 2: Samples and measurements

			Week				
			1	2	3	4	6
	Pure medium (Aa)		2 x 2	2 x 2	2 x 2	2 x 2	2 x 2
Con-trols		Sterile-filtered culture broth (Ba)	2	2	2	2	2
		Heat-inactivated (Ca)	2	2	2	2	2
Live MAP	MAP (DSM 44133/ JII-1961	Dilution 10^0	2	2	2	2	2
		Dilution 10^0 (re-opened)	0	2	2	2	2
		Dilution 10^{-4}	2	2	2	2	2
		Dilution 10^{-4} (re-opened)	0	2	2	2	2

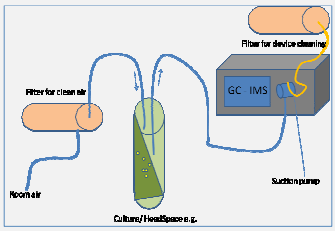

Figure 5: Principle of IMS-measurement unit

Table 3: Colony counts of the inocula

MAP strain	Dilution	Colony counts/mL
DSM 44133	10^0	3.23×10^7
	10^{-4}	7.2×10^3
JII-1961	10^0	2.21×10^7
	10^{-4}	5.1×10^3

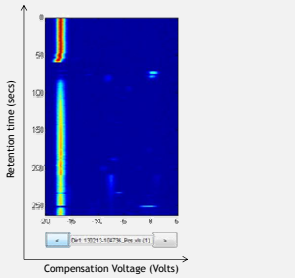

Figure 6: One original real-time Heatmap (positive IMS-Scan)

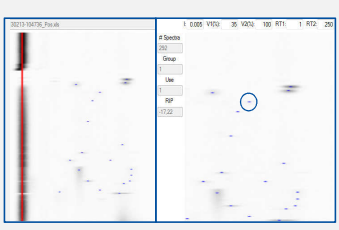

Figure 7: Transformation of the Heatmap for noise-reduction and cluster identification (Peaks with the same position in all measurements, i.e. the same VOC)