Liquillizer Does Not Interfere with Galactomannan Antigen Detection in Contrast to Dithiothreitol and *N*-Acetyl-L-Cysteine

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To improve the recovery rate of fungal culture, it is recommended to liquefy viscous lower respiratory tract secretions with reducing agents such as dithiothreitol (DTT) or *N*-acetyl-L-cysteine (NALC) (1). Samples for fungal culture are often submitted to additional analytical tests, for example, galactomannan (GM) enzyme-linked immunosorbent assay (ELISA). Detection of *Aspergillus* GM in bronchoalveolar lavage (BAL) fluid samples is a well-established criterion for diagnosing probable invasive pulmonary aspergillosis (IPA) (2). Recently, Prattes et al. reported about a significant decline of GM levels in BAL fluid samples after pretreatment with a commercially available DTT reagent (Sputasol; Oxoid Microbiological Products) (3). This effect was confirmed by Gils et al. with another DTT reagent, Copan SLSolution (Copan, Italia SPA) (4).

We investigated whether other, disulfide-cleaving mucolytic agents impair the detection of GM as well. For that purpose, we analyzed the impact of a pretreatment with NALC or Liquillizer (Miacom Diagnostics, Düsseldorf, Germany) on GM detection and compared these results with the effect of DTT on GM detection.

We included in our study 37 respiratory specimens from 29 patients. They were reanalyzed after a storage at -20°C up to 12 months. All samples were tested without pretreatment (naive) and after pretreatment at a ratio of 1:1 with DTT, NALC, or Liquillizer. Additionally, we treated the samples with saline solution at a ratio of 1:1 (NaCl) in order to assess the dilution effect on GM level detection.

Treatment with mucolytic agents routinely liquefied viscous secretions. GM detection was performed using Platelia *Aspergillus* ELISA (Bio-Rad Laboratories, Marnes-la-Coquette, France). Moreover, we assessed cultural recovery of *Aspergillus* species after Liquillizer pretreatment as this information was not provided by the manufacturer.

Cultural detection of *Aspergillus* is not affected by Liquillizer (data not shown). A total of 23 naive samples from 19 patients were GM positive with an optical density index (ODI) of ≥ 0.5 . A total of 14 naive samples from 14 patients were GM negative with an ODI of < 0.5 . After pretreatment with DTT or NALC, GM levels declined significantly compared with those seen with naive samples (Table 1). However, there was no significant difference between samples liquefied by Liquillizer and samples without pretreatment or NaCl-diluted samples (Table 1). Of note, Liquillizer on its own generated no significant signal in the GM assay (data not shown).

Four GM-negative naive samples had a positive value after Liquillizer pretreatment. We judged these values to represent true evidence of *Aspergillus* in the samples, because *Aspergillus fumigatus* was detected in all four samples by culture and/or nucleic acid amplification testing. We hypothesize that liquefaction of viscous respiratory

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TABLE 1 Galactomannan optical density indices in naive and pretreated respiratory samples^a

Sample category	Median ODI (range)				
	Naive	NaCl	NALC	DTT	Liquillizer
GM negative (<i>n</i> = 14)	0.14 (0.02–0.34)	0.08 (0.01–0.3)	0.02 ^{b,d} (0.01–0.34)	0.015 ^{b,c,d} (0.01–0.08)	0.09 (0.02–1.19)
GM positive (<i>n</i> = 23)	3.99 (0.53–5.6)	2.13 (0.23–5.6)	0.05 ^{b,c,d} (0.02–0.32)	0.03 ^{b,c,d} (0.01–0.15)	2.26 (0.11–5.6)

^aA total of 23 GM-positive and 14 GM-negative respiratory samples were examined. Data are shown as medians and ranges. Statistical analysis was performed with the Kruskal-Wallis test with the Dunn *post hoc* test. Abbreviations: ODI, optical density index; naive, no pretreatment; NaCl, pretreatment with NaCl (1:1) (dilution control); NALC, pretreatment with *N*-acetyl-L-cysteine (1:1); DTT, pretreatment with dithiothreitol (1:1); GM, galactomannan.

^b*P* < 0.05 (versus naive).

^c*P* < 0.05 (versus NaCl).

^d*P* < 0.05 (versus Liquillizer).

samples results in the release of additional GM from the mucous sample matrix, facilitating GM detection by ELISA.

In GM-positive specimens, the ODIs seen after Liquillizer pretreatment were about half the level seen with the naive specimens and were comparable to those seen with the dilution controls (ODIs after dilution with saline solution). Two naive specimens and the corresponding NaCl-pretreated specimens had ODIs between 0.5 and 0.75. However, Liquillizer pretreatment of these specimens resulted in ODIs below 0.5 even after taking the dilution factor into account. ODIs of 0.5 to 1.0 in respiratory samples have a low positive predictive value (according to the manufacturer of the Platelia *Aspergillus* ELISA). Moreover, our retrospective study design did not allow classification of the cases according to the European Organisation for Research and Treatment of Cancer (EORTC) classification for IPA (2). Therefore, it remains unclear whether those low positive GM levels represent nonspecific signals. Hence, further prospective studies which include the EORTC classification for IPA and follow-up monitoring of the cases are needed.

In conclusion, our retrospective data suggest that pretreatment with NALC impairs GM detection as reported for DTT earlier (3, 4) and that Liquillizer can be used to liquefy respiratory samples without significant impairment of either GM detection or cultural recovery of *Aspergillus* species.

REFERENCES

- McGowan K. 2015. Specimen collection, transport and processing: mycology, p 1944–1954. In Jorgensen JJ (ed), *Manual of clinical microbiology*, vol 2. ASM Press, Washington, DC.
- De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WE, Herbrecht R, Hope WW, Kibbler CC, Kullberg BJ, Marr KA, Muñoz P, Odds FC, Perfect JR, Restrepo A, Ruhnke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaoutis T, Bennett JE; European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. 2008. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 46:1813–1821. <https://doi.org/10.1086/588660>.
- Prattes J, Koidl C, Eigl S, Krause R, Hoenigl M. 2015. Bronchoalveolar lavage fluid sample pretreatment with Sputasol((R)) significantly reduces galactomannan levels. *J Infect* 70:541–543. <https://doi.org/10.1016/j.jinf.2014.11.005>.
- Gils S, Maertens J, Lagrou K. 2016. Pretreatment of bronchoalveolar lavage fluid samples with SLsolution leads to false-negative *Aspergillus* galactomannan levels. *J Clin Microbiol* 54:1171. <https://doi.org/10.1128/JCM.02968-15>.