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Strategy for Overcoming Serum Interferences in Detection of Serum (1,3)-β-D-Glucans

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The (1,3)-β-D-glucan (BG) is a cell-wall polysaccharide of most fungi. Regular measurement of BG levels in serum is recognized as a useful diagnostic marker for invasive fungal diseases in immunocompromised and intensive care unit (ICU) patients (1). Previous studies have identified several sources of false-positive reactions, such as BG in fungus-derived antibiotics (2, 3) or serum glucans associated with bacteremia caused by *Pseudomonas aeruginosa* (4). In addition, high serum concentrations of hemoglobulin, bilirubin, and triglycerides may also be mentioned by the manufacturer of detection kits as interfering in the detection of glucanemia (3). We and others have also observed that high levels of proteinemia may prevent proper

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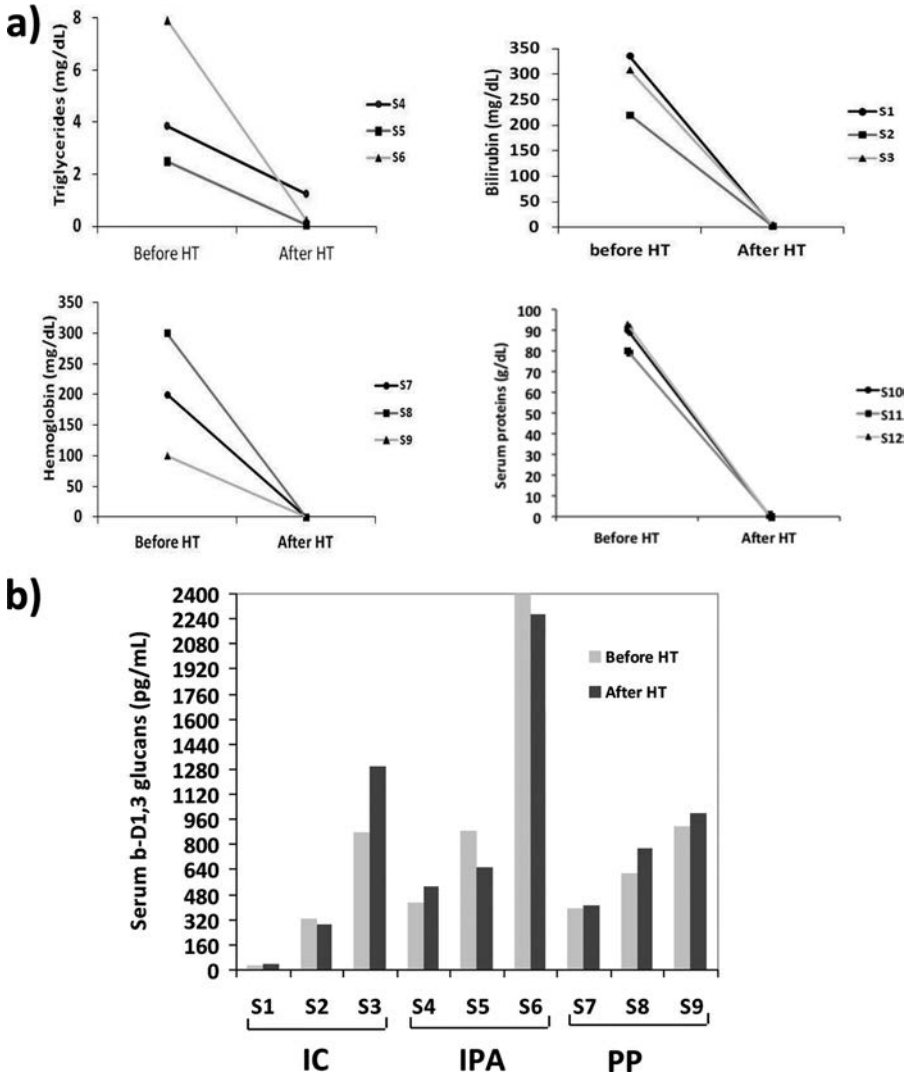

FIG 1 (a) Effect of heat treatment (HT) on sera (S) presenting high levels of substances described as interfering in the Fungitell assay. Levels of bilirubin (S1 to S3), triglycerides (S4 to S6), hemoglobin (S7 to S9), and proteins (S10 to S12) measured before and after HT are shown. (b) Effect of heat treatment (HT) on the level of serum (1,3)-β-D-glucans detected by using the Fungitell assay in sera from 3 patients with invasive candidiasis (IC [S1 to S3]), 3 patients with invasive pulmonary aspergillosis (IPA [S4 to S6]), and 3 patients with *Pneumocystis* pneumonia (PP [S7 to S9]).

detection of BG by development of a coagulate in contact with the mix of Fungitell reagent/pyrosol (unpublished observation). Since glucans are thermostable, we explored the effect of the classical heat dissociation procedure initially described for releasing mannans from serum complexes by boiling the serum at 100°C for 3 min in the presence of Na_2-EDTA followed by centrifugation at 10,000 × *g* (5, 6). Figure 1a shows that serum supernatants after heat treatment of 3 icteric, 3 lipemic, 3 hemolytic, and 3 hyperprotidic sera were depleted in bilirubin, triglycerides, hemoglobin, and proteins, respectively. We then assessed the impact of this procedure on the yield of detected glucanemia in 9 sera drawn from patients with proven invasive fungal infections caused by the 3 major fungal opportunistic pathogens for which BG detection is recommended. These consisted of 3 patients with *Candida albicans* invasive candidiasis, 3 patients with invasive pulmonary aspergillosis, and 3 patients with pneumocystosis. As shown in Fig. 1b, no significant variation was observed for BG concentrations determined without and with serum treatment. Altogether, our findings show that serum dissociation induced by heating with Na_2-EDTA is a simple and rapid procedure to overcome the interferences previously reported as limitations in the use of the BG detection test.

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REFERENCES

1. **Ostrosky-Zeichner L, Alexander BD, Kett DH, Vazquez J, Pappas PG, Saeki F, Ketchum PA, Wingard J, Schiff R, Tamura H, Finkelman MA, Rex JH.** 2005. Multicenter clinical evaluation of the (1→3) beta-D-glucan assay as an aid to diagnosis of fungal infections in humans. Clin. Infect. Dis. **41**:654–659.
2. **Mennink-Kersten MA, Warris A, Verweij PE.** 2006. 1,3-Beta-D-glucan in patients receiving intravenous amoxicillin-clavulanic acid. N. Engl. J. Med. **354**:2834–2835.
3. **Pickering JW, Sant HW, Bowles CA, Roberts WL, Woods GL.** 2005. Evaluation of a (1→3)-beta-D-glucan assay for diagnosis of invasive fungal infections. J. Clin. Microbiol. **43**:5957–5962.
4. **Mennink-Kersten MA, Ruegebrink D, Verweij PE.** 2008. Pseudomonas aeruginosa as a cause of 1,3-beta-D-glucan assay reactivity. Clin. Infect. Dis. **46**:1930–1931.
5. **Reiss E, Stockman L, Kuykendall RJ, Smith SJ.** 1982. Dissociation of mannan-serum complexes and detection of Candida albicans mannan by enzyme immunoassay variations. Clin. Chem. **28**:306–310.
6. **Sendid B, Tabouret M, Poirot JL, Mathieu D, Fruit J, Poulain D.** 1999. New enzyme immunoassays for sensitive detection of circulating Candida albicans mannan and antimannan antibodies: useful combined test for diagnosis of systemic candidiasis. J. Clin. Microbiol. **37**:1510–1517.