Plasmalyte: No Longer a Culprit in Causing False-Positive Galactomannan Test Results

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False-positive galactomannan (GM) results have been reported in patients treated with gluconate-containing solutions, such as Plasmalyte. The GM optical density index was tested on 33 distinct batches of Plasmalyte and was found to be negative in all of the batches, confirming that Plasmalyte is no longer a cause of false-positive GM results.

Invasive aspergillosis is a serious infection associated with a high mortality rate in patients at risk, such as those receiving chemotherapy or undergoing allogeneic hematopoietic stem cell transplantation or those admitted in the intensive care unit (ICU). Galactomannan (GM), a fungal antigen produced by *Aspergillus* spp. during growth, is an important biomarker for early non-culture-based diagnosis of invasive aspergillosis in immunocompromised patients. Currently, positive serum testing on the GM enzyme immunoassay (Platelia *Aspergillus*; Bio-Rad, Marnes-La-Coquette, France) is one of the criteria for establishing the diagnosis of probable invasive aspergillosis in immunocompromised patients according to the European Organization for Research and Treatment of Cancer and Mycoses Study Group (EORTC/MSG) (1). For the last decade, GM testing has also been applied on bronchoalveolar lavage (BAL) fluid, especially in nonneutropenic ICU patients, as this has been shown to have good sensitivity and specificity in diagnosing invasive aspergillosis in patients without the classic immunocompromising risk factors (2).

An important limiting factor for GM testing is the association with false-positive test results, which have been reported in patient serum or BAL fluid, if the patient is treated with generic piperacillin-tazobactam and amoxicillin clavulanate (3, 4). These antibiotics are produced by fermentation in *Penicillium*, which generates GM recognized by the monoclonal antibody used in the Platelia assay.

Plasmalyte (Baxter, Lessines, Belgium) is a sterile, nonpyrogenic electrolyte solution that contains sodium chloride, potassium chloride, magnesium chloride, sodium acetate trihydrate, and bicarbonate ions with or without sodium gluconate. It is used for fluid resuscitation and hydration in severely ill patients. Fluid resuscitation in ICU patients has been a matter of debate during the last decade, with many studies investigating resuscitation efficacy in terms of hemodynamic and patient outcome versus potential adverse effects, such as kidney injury and the need for renal replacement therapy (5). Several studies suggested that balanced salt solutions, based on gluconate, acetate, or lactate should be used preferentially in order to avoid the side effects related to hyperchloremia and renal dysfunction associated with normal saline purely composed of chloride salts (5). Since 2012, Plasmalyte, a balanced electrolyte solution, has been the default resuscitation fluid in our hospital,

particularly in septic critically ill patients, as recommended by recent international consensus (6).

In Belgium, only gluconate-containing Plasmalyte solutions are available (Plasmalyte A Viaflo and Plasmalyte 148 plus Glucose 5% Viaflo, all containing 23 meq/liter gluconate). Low-molecular-weight organic acids, such as gluconate, are produced by a fermentation process involving *Aspergillus niger* and *Aspergillus terreus*. GM is released into the fermentation solution and is likely carried through the fractionation or filtration process for medical-grade gluconate (7, 8). Although Plasmalyte is sterile, GM may still persist in the gluconate-containing solution, depending on the type and extent of fractionation and filtration, potentially resulting in a false-positive serum and BAL fluid GM (7, 8).

When adding Plasmalyte as the preferred resuscitation fluid for ICU patients to our hospital's drug formulary, a surveillance program was set up in order to follow up the GM index in every new batch of Plasmalyte circulating in our hospital. In short, 1 ml of Plasmalyte out of one bag of every new batch entering the hospital was tested using a GM sandwich enzyme-linked immunosorbent assay according to the instructions of the manufacturer. Samples were considered positive if the GM optical density index (ODI) was ≥ 0.5 . Surprisingly, the GM index was negative (< 0.1) in the first three tested batches (13K 22T1A, 13I 24T1E, and 13J 31T1E). Thirty additional batches, all circulating from January 2013 to December 2014 in our hospital, were tested in order to confirm with 80% power that the probability for a false-positive test is $< 5\%$ (based on a binomial test for a single proportion). The GM index was negative (< 0.1) in all 30 tested batches.

These results are in contrast to what has been reported before

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in the literature. Hage et al. were the first to report that Plasmalyte was causing highly false-positive results (4.1 to 8.2) for GM in BAL fluid when using 100 ml Plasmalyte as the lavage fluid (9). The authors postulated that 1 liter of Plasmalyte contained 10 μ g of GM. They assumed that if this were administered intravenously to patients, serum GM would also have a false-positive result; one liter of Plasmalyte would result in a serum GM concentration of 2.5 ng/ml assuming distribution in a plasma volume of 3 to 4 liters (9). Subsequently, this was indeed confirmed in a case report by Surmont and Stockman that described a patient treated with intravenous Plasmalyte presenting with a false-positive serum GM ODI (1.85), which was ascribed to GM-containing Plasmalyte (ODI, 3.85) (10). After discontinuation, GM tests became negative. The authors confirmed the false positivity of serum GM by testing several patients treated with Plasmalyte but without any sign of fungal infection (10). In 2007, Racil et al. reported a study in which healthy volunteers receiving Plasmalyte demonstrated false-positive circulating GM that persisted for up to 24 h (11). This study confirmed again that, despite dilution in the intravascular compartment, GM levels are above the positivity threshold of the GM assay. Four different batches of Plasmalyte were tested, all resulting in highly false-positive GM values (ODI, 7.15 to 8.53) (11).

Petratiene et al. finally confirmed in 2011 that sodium gluconate was the component in Plasmalyte causing false-positive GM results by comparing GM kinetics in rabbits treated with Plasmalyte with and without gluconate (12).

All of the above-mentioned reports on false-positive GM results linked to Plasmalyte dated from 2011 or before. Our tests, showing negative GM indexes on 33 batches, were undertaken from the end of 2012 to 2015. Baxter, the manufacturer of Plasmalyte, confirmed that the number of providers delivering sodium gluconate as one of the Plasmalyte compounds was changed from two companies to a single provider in 2012 (A. Dasse, Baxter Belgium & Europe, personal communication), potentially explaining the change from highly false-positive GM results to the absence of GM in the batches produced after 2012. A quick internet search reveals, however, that there are numerous providers of sodium gluconate worldwide, which means that if the manufacturer were to change the Plasmalyte production process by working with an alternative provider in the future, the absence of GM is no longer guaranteed. Changes in the manufacturing process should, however, be managed in accordance with current International Conference on Harmonisation (ICH) guidelines and should be compliant with drug product marketing authorization.

As the change in the number of providers of sodium gluconate has only been adopted for Plasmalyte distributed in the European Union and as health care professionals are mostly not aware of changes in the manufacturing process, the absence of false positivity is restricted to the European Union and to the current manufacturing process. Therefore, in our opinion, GM testing should be incorporated as part of the quality control protocol at the level of the release of sodium gluconate by the provider or at the reception of sodium gluconate by the company that is using this compound in manufacturing processes. Ideally, the absence of GM should be listed in the sodium gluconate monograph in the European or U.S. Pharmacopeia, obliging providers in this way to optimize the filtration process and ultimately guaranteeing the absence of GM false positivity

at the bedside of the patient if drugs containing sodium gluconate are prescribed.

In conclusion, it seems that Plasmalyte can actually be used in a safe way as a resuscitation fluid in critically ill patients without generating false-positive serum or BAL fluid GM values. It is important that manufacturers of electrolyte solutions containing gluconate pursue methods to control for the absence of GM, as false-positive GM results have the potential to lead to useless antifungal treatment that can result in potential adverse events and additional costs.

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