Short Communication

Rapid Detection of Microbial Contamination in Intravenous Fluids by ATP-Based Monitoring System

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SUMMARY: In this study, we investigated four clinical cases of microbial contamination of in-use intravenous infusion fluid, detected by measuring “Adenosine triphosphate (ATP) + adenosine monophosphate (AMP)” (“ATP+AMP”) levels. High “ATP+AMP” values correlate with microbial contamination, and by utilizing these values as indicator for microbial contamination possibility, we were able to rapidly detect the contamination and recommend replacement of catheters and administration sets. In three out of four cases, changing the infusion fluid led to improvement in the condition of the patients. “ATP+AMP” levels can be used to confirm microbial contamination of in-use intravenous infusion fluids, as it is fast (several minutes) and convenient to measure them.

Microbial contamination of in-use intravenous infusion fluids often leads to serious health consequences, such as bacteremia and septic shock (1–5). However, microbial contamination of in-use infusion fluids is often ignored as it is not readily detectable by naked eyes. In few cases, where contamination of the infusion fluid is suspected, timely identification is not possible because culturing the microbes in the infusion fluids may require days. Previously, we reported a case where the measured ATP+AMP levels substantiated contamination of infusion fluids with *Candida parapsilosis* (6). In this study, we describe four clinical cases where measuring the amounts of “ATP+AMP” levels facilitated rapid detection of microbial contaminants in the infusion fluid.

We report four cases from November 2015 to August 2019, where the patients were consulted to determine the possibility of microbial contamination in their intravenous fluids. First three cases involved patients with signs of infection according to the attending physician. Cases 1 and 2 were from the Yamaguchi University Hospital, while cases 3 and 4 were from two different city hospitals within Yamaguchi. For the measurement of the “ATP+AMP” levels (relative

light units [RLU]), sampling was performed using the LuciPac Pen-AQUA (Kikkoman Biochemifa Co., Tokyo, Japan) and RLU was measured using the Lumitester PD-20 (Kikkoman Biochemifa Co.).

To investigate microbial contamination of the infusion fluids, the collected in-use infusion fluid samples were diluted with normal sterile saline. About 1 mL of undiluted or diluted samples were transferred using pipettes to monitor the growth of bacteria on trypticase soy agar plates containing blood and that of fungi on Sabouraud dextrose agar plates. The plates were streaked using a glass spreader or “hockey stick” and incubated at 35°C for 24–72 h. Thereafter, the number of colonies were counted and the strains were identified by Gram staining, morphological examination, oxidation fermentation test, cytochrome-oxidase test, and API system (bio-Mérieux SA, Marcy-l’Etoile, France).

CASE#1: Contamination of in-use peripheral venous infusion fluid containing glucose, electrolyte, and amino acids (Beefreed®) with *Serratia marcescens*

We received a consultation request from an attending physician for a patient who experienced an unexpected septic shock caused by *Serratia marcescens*. The bacterium was cultured from the blood of the patient. Since the patient was not expected to be susceptible to bloodstream infection, we investigated the environmental sources that could have caused the sepsis. The RLU of the fluid in the infusion bag was recorded to be three and no microbes were detected in the infusion fluid bag. However, the “ATP+AMP” levels of the fluid in the infusion tube were 60,932 RLU; moreover, the fluid contained 1.0×10^8 colony forming

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Fig. 1. (Color online) Microbial count and “ATP+AMP” (RLU) in cases of infusion fluid contamination.

units (CFU)/mL of *Serratia marcescens* (Fig. 1).

CASE#2: Contamination of in-use peripheral venous infusion fluid containing glucose, electrolyte, and amino acids (Beefreed®) with *Bacillus cereus*

We received a consultation request from an attending nurse who observed that the fluid in the infusion tube was cloudy. Therefore, we assessed the infusion fluid in the bag containing glucose, electrolytes, and amino acids, and measured the “ATP+AMP” levels to be 2 RLU, indicating that it did not contain any microbes; however, we recorded 1,780 RLU for the fluid in the infusion tube and confirmed the presence of 1.0 × 10⁷

CFU/mL of *Bacillus cereus* (Fig. 1). The bacterium was also cultured from the blood of the patient.

CASE#3: Contamination of in-use peripheral venous infusion fluid with *Serratia marcescens*

A pharmacist reported a case where a patient experienced septic shock caused by bloodstream infection with *Serratia marcescens*. The bacterium was also cultured from the blood of the patient; therefore, we investigated the infusion fluid (dextrose-acetated Ringer's solution and physiological saline containing morphine hydrochloride). The RLU of the fluid in the infusion bag was 0 and we did not detect any microbes

in the fluid present inside the infusion bag. However, the “ATP+AMP” level of the infusion fluid in the tube was 9,729 RLU and 9.6×10^6 CFU/mL of *Serratia marcescens* was subsequently detected (Fig. 1).

CASE#4: Contamination of in-use central venous hyperalimentation solution (IVH; Elneopa® - NF No.2) with *Candida tropicalis*

A nurse reported a case in which browning of inline filter was observed. The causative microorganism was not detected inside the high-calorie infusion fluid bag or in the fluid present in the main route from the inline filter to the patient. Although the measured RLU was between 1–65, 4.2×10^6 CFU/mL of *C. tropicalis* was detected in the fluid in the main route from the inline filter to the high calorie infusion bag, with an RLU of 1,826 (Fig. 1).

The infusion fluids, especially those containing glucose, electrolytes, and amino acids, are susceptible to contamination by gram-negative bacteria, such as *Serratia marcescens* and fungi, such as *Candida* spp. (7–9). As documented in this report, contaminating microbes may rapidly proliferate in injectable solutions to reach at least 4.2×10^6 CFU/mL. The measurement of “ATP+AMP” levels for rapid detection of microbial contamination (1.0×10^2 – 1.0×10^4 CFU/mL) has previously been evaluated in *in vitro* studies (10–13). We observed clinically that RLU higher than 1,780 and 4.2×10^6 – 1.0×10^8 CFU/mL are observed in our measurement system for cases of infusion fluids contamination with bacteria and fungi, respectively. Conversely, our assay exhibits low or minimal “ATP+AMP” background (normal range 0–10 RLU) for a variety of infusion fluids. This kit is able to measure the levels of “ATP+AMP” within a few minutes. Therefore, “ATP+AMP” measurement may be applicable and useful for rapid real-time detection of microbial contamination of infusion fluids in clinical settings. In cases 1–3 detailed in this study, the patients recovered from the infection after stopping the infusion and replacement with new catheter and administration set.

Conflict of interest None to declare.

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