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Comparison of sample preparation and bacterial enumeration methods for the detection of B. cereus 3A in artificially contaminated wipes preserved with benzalkonium chloride

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Cosmetic wipes are made of diverse materials that contain preservatives to prevent microbial contamination but, recently there have been recalls due to microbial contamination of wipes.

For preparing artificially preserved wipes, single dry wipes made from different materials were placed in sterile containers and were individually wetted with 5 or 10 ml of 0.002% BAK solution inoculated with B. cereus 3A spore suspensions at high (~6.7 log CFU/ml), medium (~5.7 log CFU/ml), and low (4.7 log CFU/ml) levels of contamination. Wipes wetted with uninoculated preserved solution were used as negative controls. After 14 days at room temperature, 5 replicate wipe samples of each B. cereus concentration level and the uninoculated negative controls were analyzed by following either BAM, by cutting 1 g out of a wipe, or following ISO or mISO, using the entire wipe.

For commercial wet wipes preserved with 0.002% BAK, entire wipes were aseptically individually placed in sterile containers and inoculated with 300 µl of B. cereus 3A spore suspensions at high (~6.7 log CFU/ml), medium (~5.7 log CFU/ml), and low (4.7 log CFU/ml) levels. Also, 1 g sample size was precut and then inoculated with 35 µl of B. cereus 3A spore suspensions at the same 3 levels as above. All samples were aged 14 days at room temperature prior to analysis.

All the test portions were enumerated using non-selective Modified Letheen Agar (MLA) plates as specified in BAM. In addition, B. cereus counts were determined manually using selective BACARA plates and by automation with the TEMPO® instrument using B. cereus cards. The control test portions, 5 for each method, were similarly analyzed and enumerated. The set of analyses was repeated three times.

There was evidence of two-way interaction between the types of wipes (artificial vs wet) and the analysis methods (P=0.0007). For artificially preserved wipes, the BAM sample preparation method allowed higher recovery than ISO ($p < 0.001$), and mISO ($p < 0.001$); the highest mean difference was 0.3 log CFU/g. On the other hand, BAM, ISO, and mISO were comparable ($p>0.05$) for the recovery of B. cereus from commercially preserved wet wipes.

The enumeration methods revealed that cell counts with TEMPO® were slightly higher or comparable to MLA and BACARA plates. MLA plate counts were comparable ($p>0.05$) to those from BACARA plates.

In conclusion, B. cereus in contaminated wet wipes can be recovered similarly from wipes with all the 3 methods. In addition to the enumeration plates, TEMPO® can be a useful tool for the count of B. cereus in contaminated wipes.

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