

Fast-Track Communication

Nonfermenters under the Influence: Ethanol Disinfection of a Saline Dispenser Caused Misidentification by the Vitek 2 System Using the New GN Cards

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Since its introduction in 1997, the Vitek 2 system (bioMérieux, Marcy-l'Étoile, France) has established itself as a stable and accurate automated system for rapid bacterial identification. Bacterial suspensions and test cards containing a panel of substrates are both placed into the instrument, where the cards are automatically inoculated, sealed, incubated, and read to detect biochemical reactions. From the start, the ID-GNB card has been used for the identification of gram-negative bacilli. This card gives a final result in 3 hours but, as was shown by several studies, is better able to identify *Enterobacteriaceae* than nonfermenters, probably because of their slower metabolisms (2–4).

To improve overall identification results for gram-negative bacilli, the recently developed GN card is now being introduced worldwide. For this card, the Vitek 2 system uses colorimetric technology instead of fluorescence-based methods to detect biochemical reactions. After the Vitek instrument is adjusted by installation of new readers and a database update so that the card can be used, a greater diversity of species can be identified in both *Enterobacteriaceae* and nonfermenter groups (1).

When we started using the new GN card, we found that *Pseudomonas aeruginosa* and *Acinetobacter baumannii* were constantly misidentified as *Burkholderia cepacia* and *Klebsiella oxytoca*, respectively. This phenomenon did not occur while using ID-GNB cards, which were processed identically to GN cards.

A few random experiments focused our attention on the bottle-top dispenser used to transfer sterile saline (0.45% sodium chloride solution) to the test tubes used for suspension

preparation. The dispenser can be autoclaved for sterilization or decontamination according to the manufacturer's guidelines. For practical reasons however, we applied a local protocol in which at the end of the day the dispenser was rinsed with an 80% ethanol solution and left to soak in ethanol overnight. The next morning it was rinsed with sterile distilled water and suspension solution before use.

Because we suspected that residual ethanol in or on parts of the dispenser interfered with bacterial identification, we took samples from a bottle of saline solution to culture them for contaminants and to test them for the presence of ethanol. This bottle had been in use for 2 days, meaning that on two consecutive mornings it had been connected to the freshly rinsed dispenser. No contaminants could be demonstrated in the saline. However, ethanol was detected in a concentration of 0.4 g/liter (0.4 promille), which is consistent with 250 µl of 80% ethanol in a 500-ml saline bottle (5 to 6 drops). We subsequently inoculated GN cards with four different strains of *P. aeruginosa* and three different strains of *A. baumannii* from suspensions containing concentrations of ethanol ranging from 0 to 0.4 g/liter.

Table 1 shows the effect of ethanol on the final results. Very low quantities of ethanol were enough to cause misidentification, although for one strain of *P. aeruginosa* the reactions appeared unaffected.

We concluded that residual ethanol present on the outside of the filling tube attached to the main body of the bottle-top dispenser interfered with the reactions or the viability of the

TABLE 1. Results of testing bacterial suspensions containing ethanol with the Vitek 2 GN card

Strain	Identification result with the following concn of ethanol in saline solution (g/liter):				
	0	0.1	0.2	0.3	0.4
<i>P. aeruginosa</i> ATCC 25668	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	<i>B. cepacia</i>	<i>B. cepacia</i>	<i>B. cepacia</i>
<i>P. aeruginosa</i> ATCC 27853	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	<i>B. cepacia</i>	<i>B. cepacia</i>	<i>B. cepacia</i>
<i>P. aeruginosa</i> 1 ^a	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	<i>B. cepacia</i>	<i>B. cepacia</i>	<i>B. cepacia</i>
<i>P. aeruginosa</i> 2 ^a	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>	<i>P. aeruginosa</i>
<i>A. baumannii</i> ATCC 19606	<i>A. baumannii</i>	<i>K. oxytoca</i>	<i>K. oxytoca</i>	Unidentified	<i>K. oxytoca</i>
<i>A. baumannii</i> 1 ^a	<i>A. baumannii</i>	<i>K. oxytoca</i>	<i>K. oxytoca</i>	<i>K. oxytoca</i>	<i>K. oxytoca</i>
<i>A. baumannii</i> 2 ^a	<i>A. baumannii</i>	<i>K. oxytoca</i>	<i>K. oxytoca</i>	<i>K. oxytoca</i>	<i>K. oxytoca</i>

^a Strains from external quality assurance samples with different antimicrobial susceptibility patterns.

bacilli in the GN cards. The problem was solved after we changed to using disposable materials for saline dispensing and abandoned the practice of ethanol disinfection.

bioMérieux kindly provided the Vitek 2 GN cards.

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