

ATCC® MINIS SUPPORT VITEK® 2 QUALITY CONTROL TESTING

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ABSTRACT

This study will demonstrate the use of ATCC Minis as quality control strains for microbial identification on the VITEK 2 platform (bioMérieux), employing strains recommended for use with the Anaerobic and Corynebacteria (ANC), *Bacillus* (BCL), Corynebacteria (CBC), Gram-Positive (GP), Gram-Negative (GN), *Neisseria-Haemophilus* (NH), and Yeast (YST) Streamlined QC Sets as examples.

INTRODUCTION

Commercial firms and research laboratories look to ATCC to provide top-quality microbial strains needed to maintain outstanding quality control programs for instruments, reagents, media, etc. To help aid in the rapid identification of microbial strains, ATCC now provides the same trusted quality control strains in a convenient, single-use, "mini" format that saves precious time and resources. ATCC Minis are a six pack of glass-free cryovials containing ready-to-use strains in glycerol stock, complete with peel-off labels for fast and reliable recordkeeping. Further, similar to their lyophilized counterparts, ATCC Minis are backed by polyphasic testing to establish identity, viability, and purity, and are maintained using a seed stock system to minimize subculturing.

To provide convenient reference materials for fast, accurate microbial identification using the VITEK 2 system, ATCC has expanded its collection of quality control strains to include bacterial and fungal isolates in the ATCC Minis frozen format. In the following study, we demonstrate the application of ATCC Minis as quality control strains for microbial identification on the VITEK 2 platform using ANC, BCL, CBC, GP, GN, NH, and YST Streamlined QC Sets as examples.

MATERIALS AND METHODS

ATCC Minis (Table 1) were thawed and cultured according to the recommended propagation procedure described on the product sheet. Following growth in broth, strains were streaked for isolation on the recommended agar medium and incubated under the appropriate temperature and atmospheric conditions until growth was established. To create the sample inoculum, isolated colonies were selected using a sterile cotton swab and suspended into 3.0 mL of 0.45% saline solution within a 12 mm x 75 mm polystyrene test tube. Inoculums were adjusted accordingly with the assistance of a calibrated DensiCHECK™ Plus (bioMérieux) to reach the McFarland Standard density range as recommended in the VITEK 2 Systems Product Information manual.¹ The sample inoculum and associated VITEK 2 card were then inserted into a VITEK 2 Cassette and processed according to instructions found within the VITEK 2 Compact System and OBSERVA® Computer System (bioMérieux) manuals. For this study, microbial identity was confirmed using the VITEK 2 ANC, BCL, CBC, GP, GN, NH, and YST cards, which provide species-level identification of anaerobes, bacilli, corynebacteria, Gram-positive bacteria, Gram-negative bacteria, *Neisseria* and *Haemophilus* strains, and yeasts, respectively.

Table 1: ATCC Minis

ATCC® No. Species

VITEK® 2 ANC STREAMLINED QC SET

BAA-1296-MINI-PACK™ Bacteroides ovatus (ATCC® BAA-1296)

12464-MINI-PACK™ Clostridium septicum (ATCC® 12464™)

VITEK® 2 BCL STREAMLINED QC SET

51663-MINI-PACK™ Brevibacillus agri (ATCC® 51663)

VITEK® 2 CBC STREAMLINED QC SET

<u>43044-MINI-PACK</u>™ Corynebacterium urealyticum (ATCC® <u>43044</u>)

15829-MINI-PACK™ Microbacterium testaceum (ATCC® 15829)

VITEK® 2 GP STREAMLINED QC SET

700327-MINI-PACK™ Enterococcus casseliflavus (ATCC® 700327)

BAA-750-MINI-PACK™ Staphylococcus saprophyticus (ATCC® BAA-750)

VITEK® 2 GN STREAMLINED QC SET

700323-MINI-PACK™ Enterobacter hormaechei (ATCC® 700323)

17666-MINI-PACK™ Stenotrophomonas maltophilia (ATCC® 17666)

VITEK® 2 NH STREAMLINED QC SET

BAA-1152-MINI-PACK™ Eikenella corrodens (ATCC® BAA-1152)

VITEK® 2 YST STREAMLINED QC SET

14053-MINI-PACK™ Candida albicans (ATCC® 14053)

RESULTS AND DISCUSSION

As part of the microbial identification process, the VITEK 2 software compares the resulting biochemical reaction outcomes of organisms present in each sample to the expected set of results for organisms that have been previously identified by the database. These biochemical test outcomes are represented as a bionumber, a unique species identification pattern generated from the conversion of test results into positive or negative test probabilities. The identity percent probability is based on the similarity of the observed reactions compared to that of the expected reactions of an organism. Here, the possible range of percent probabilities for a single organism match is 85-99%, with values closer to 99% indicating a perfect match between the test reaction pattern and the unique reaction pattern of a known species.

From this analysis, each of the ATCC Minis were identified using the appropriate VITEK 2 cards at a percent probability ranging from 91-99%, reflecting confidence levels of low, good, very good, and excellent (Table 2). Further, the results obtained using the ATCC Minis quality control strains corresponded with that of their lyophilized counterparts, indicating that the frozen format does not affect the biochemical phenotype of the strains (data not shown).

BioMérieux selects quality control strains based on their biochemical characteristics. In some cases, these quality control strains exhibit expected biochemical results, but are unidentified or misidentified in the VITEK 2 system. As an example, the data for Brevibacillus agri ATCC No. 51633-MINI-PACK reflected an identity probability of 96% and a low confidence interval on the BCL card. This particular species belongs to a pseudoslashline taxa with *Brevibacillus brevis*. Thus, it is possible for the two species to exhibit the same biopattern, which results in low discrimination between *B. agri* and *B. brevis*.

In some instances, VITEK 2 cards may be limited in the number of individual species within a given genera that can be identified. For example, the CBC card is capable of identifying *Microbacterium* spp. and Microbacterium lacticum, but not *M. testaceum* specifically. Accordingly, though the analysis of ATCC No. 15829-MINI-PACK, which is a strain of *Microbacterium testaceum*, exhibited a percent probability of 91% and a good confidence interval, it was identified only at the genus level. Therefore, in this instance, ATCC No. 15829-MINI-PACK performed as expected and was correctly identified within the identification constraints of the CBC card.

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Table 2: VITEK 2 Test Results

ATCC® No.	VITEK 2 Card	VITEK 2 Lot Number	Analysis Time (h)	Percent Probability	Bionumber	Confidence Level	Species Confirmed
VITEK® 2 ANC STREAMLINED QC SET							
BAA-1296-MINI-PACK™	ANC	244336210	6.25	99%	5057557176770	Excellent	Bacteroides ovatus
12464-MINI-PACK™	ANC	244336210	6.25	99%	0000003042011	Excellent	Clostridium septicum
VITEK® 2 BCL STREAMLINED QC SET							
51663-MINI-PACK™	BCL	239340320	14.25	96%	0727145601200401	Low	Brevibacillus agri
VITEK® 2 CBC STREAMLINED QC SET							
43044-MINI-PACK™	CBC	246310810	8.25	99%	04000040000000	Excellent	Corynebacterium urealyticum
15829-MINI-PACK™	CBC	246348420	8.00	91%	67577433625760	Good	Microbacterium spp.
VITEK® 2 GP STREAMLINED QC SET							
700327-MINI-PACK™	GP	242342010	4.75	93%	524213665777731	Very good	Enterococcus casseliflavus
BAA-750-MINI-PACK™	GP	242351110	6.00	94%	030002056670131	Very good	Staphylococcus saprophyticus
VITEK® 2 GN STREAMLINED QC SET							
700323-MINI-PACK™	GN	241318040	5.00	98%	2623634553533010	Excellent	Enterobacter hormaechei
17666-MINI-PACK™	GN	241318040	5.00	99%	1022103101540020	Excellent	Stenotrophomonas maltophilia
VITEK® 2 NH STREAMLINED QC SET							
BAA-1152-MINI-PACK™	NH	245341220	6.00	97%	0622002400	Excellent	Eikenella corrodens
VITEK® 2 YST STREAMLINED QC SET							
14053-MINI-PACK™	YST	243321210	18.50	99%	6102546065327771	Excellent	Candida albicans

CONCLUSION

Overall, this study demonstrates that ATCC Minis exhibit results consistent with those expected for each respective VITEK 2 card and are identical to their lyophilized counterparts, making them ideal for use as quality control strains for the identification of unknown microbial strains using the VITEK 2 system.

REFERENCES

1 bioMérieux, Inc. VITEK® 2 Systems Product Information, 2010.











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