BIOMÉRIEUX

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VITEK® 2 AST-ST03

℞ only IVD

Intended Use

The VITEK® 2 Streptococcus Susceptibility Card is intended for use with the VITEK® 2 Systems in clinical laboratories as an *in vitro* test to determine the susceptibility of *S. pneumoniae,* beta-hemolytic *Streptococcus*, and *Viridans Streptococcus* to antimicrobial agents when used as instructed.

Summary and Explanation

Susceptibility testing is indicated for any organism that contributes to an infectious process warranting antimicrobial chemotherapy. Susceptibility tests are most often indicated when the causative organism is thought to belong to a species capable of exhibiting resistance to commonly used agents. Isolated colonies of each type of organism that may play a pathogenic role are selected from an agar plate and tested for susceptibility. These tests are then examined and the Minimum Inhibitory Concentration (MIC) is determined. The MIC obtained using a dilution test may tell the physician the concentration of an antimicrobial agent needed at the site of infection to inhibit the infecting organism.

MICs have traditionally been determined using antimicrobial concentrations derived from serial twofold dilutions.[2] The MIC is then determined from the lowest concentration that exhibits inhibition of growth. An interpretive criterion (Susceptible, Intermediate, or Resistant) can then be assigned to MIC results to aid in the direction of therapy.

For some antimicrobials (e.g., high-level gentamicin, high-level streptomycin) a qualitative result is generated.

The standard and reference procedures are based on susceptibility tests requiring 16 to 24 hours of incubation for bacteria. Various manufacturers have now developed automated procedures designed to generate results more rapidly by using shortened incubation times. Laboratories worldwide use either variations of the standard reference procedure or a commercially available product to determine the MICs of infectious organisms.

AES (Advanced Expert System)

The AES (Advanced Expert System) is a software tool that provides information about the clinical isolate tested. AES determines the consistency level of the AST results, as well as alerts the user to unusual results. AES proposes phenotypes per each class of antimicrobial tested, and applies therapeutic corrections (TCs) based on the proposed phenotypes, and AES parameter set applied.

Since AES is proposing a phenotype based on each class of antimicrobials tested, results can vary depending on the card configuration. It is important to note that a proposal of a phenotype by AES is not considered confirmation of the presence of a particular resistance mechanism. Users are responsible for the results being released from their laboratory and have the ability to stop certain phenotypes for review (refer to VITEK® 2 Systems Software User Manual). AES can provide information about the isolate tested but does not replace the review of results by skilled laboratory personnel.

bioMérieux verifies all changes to the AES Knowledge Base (KB), and biological validation is performed for every AES KB update. Since AES phenotype proposals can vary according to the card configuration, it is recommended that the user perform review of results when updating from one version of software to the next, or when changing to a new card configuration, according to their internal procedures. This review will ensure that AES is providing expected results for their cards, or allow the user to make modifications in AES review settings if deemed appropriate.

Inducible Clindamycin Resistance Test (ICR)

A positive ICR test is indicative of inducible MLS_b resistance, which confers resistance to macrolides, lincosamides, and type B streptogramin. If the ICR test is positive and the clindamycin result is susceptible or intermediate, the clindamycin result will be forced resistant by the ICR test (in CLSI® mode or User-defined based on CLSI®). The following message will display on the laboratory report with a positive ICR test result: “This isolate is presumed to be resistant based on detection of inducible clindamycin resistance.”

Storage Conditions

Upon receipt, store VITEK® 2 AST cards unopened in their original package liner at 2°C to 8°C.

Principle of the Test

The AST card for VITEK® 2 Systems is an automated test methodology based on the MIC technique reported by MacLowry and Marsh and Gerlach.[15,16] The AST card is essentially a miniaturized and abbreviated version of the doubling dilution technique for MICs determined by the microdilution method.[1]

Each AST card contains a control well containing only microbiological culture medium. The remaining microwells contain premeasured amounts of specific antimicrobials combined with culture medium.

The organism suspension to be tested must be diluted to a standardized concentration in 0.45% saline before being used to rehydrate the antimicrobial medium within the card. The card is then filled, sealed, and placed into the instrument incubator/reader, either automatically (as with VITEK® 2 60 or VITEK® 2 XL) or manually (as with VITEK® 2 Compact). The instrument monitors the growth of each well in the card over a defined period of time (up to 24 hours for *Streptococcus*). At the completion of the incubation cycle, MIC values (or test results, as appropriate) are determined for each antimicrobial contained on the card.

Precautions

- For *In Vitro* Diagnostic Use Only.
- For US Only: Caution: US Federal Law restricts this device to sale by or on the order of a licensed practitioner.
- For professional use only.
- Suspensions outside of the appropriate ranges on the VITEK® 2 DENSICHEK®, the VITEK® 2 DENSICHEK® Plus, or the VITEK® DENSICHEK® may compromise card performance.
- The safety and efficacy of antimicrobial drugs, for which antimicrobial susceptibility is tested by this AST device, may or may not have been established in adequate and well-controlled clinical trials for treating clinical infections due to microorganisms outside of those found in the indications and usage in the drug label. The clinical significance of susceptibility information in those instances is unknown. The approved labeling for specific antimicrobial drugs provides the uses for which the antimicrobial drug is approved.
- Do not use the card after the expiration date shown on the package liner.
- Store the card unopened in the package liner. Do not use the card if the protective package liner is damaged or if no desiccant is present.
- Allow the card to come to room temperature before opening the package liner.
- Do not use powdered gloves. Powder may interfere with the optics.
- Use of culture media other than the recommended types must be validated by the customer laboratory for acceptable performance.
- The card performs as intended only when used in conjunction with VITEK® 2 Systems, following the instructions contained in the Instructions for Use.
- It is highly recommended that Good Laboratory Practices (e.g., FDA, CLSI, ISO, etc.) also be followed, according to local guidelines or requirements.
- **Do not use glass test tubes**. Use clear plastic (polystyrene) tubes only. Variation exists among test tubes of standard diameter. Carefully place the tube into the cassette. If resistance is encountered, discard and try another tube that does not require pressure to insert.
- Prior to inoculation, inspect cards for tape tears or damage to the tape and discard any that are suspect. Check the saline level in the tubes after the cassette has been processed to ensure proper filling of card.
  - VITEK® 2 60 or VITEK® 2 XL: Eject improperly filled cards.
  - VITEK® 2 Compact: Do not load improperly filled cards.
- Give special consideration to specimen source and patient therapy regimen. AST cards may contain some antimicrobials that are not proven to be effective for treatment of infections due to all organisms that may be tested. For interpreting and reporting of antimicrobial results that have been shown to be active against organism groups both *in vitro* and in clinical infections, refer to the individual pharmaceutical antimicrobial labeling or local therapy guidelines.

- Interpretation of test results requires the judgment and skill of a person knowledgeable in AST. Additional testing may be required.[17]

Warning: All patient specimens, microbial cultures, and inoculated VITEK® 2 cards, along with associated materials, are potentially infectious and should be treated with universal precautions [18,20]

Reagents

When used with VITEK® 2 instrumentation, the AST card is a complete system for routine susceptibility testing. Each AST card contains selected antimicrobials in varying concentrations, dried with a microbiological culture medium.

Table 1: Contents of the Card

Antimicrobic	Code	Concentration §	Calling Range ≤	Calling Range ≥	FDA Indications for Use
Ampicillin[NS2]	am01n	0.5, 1, 4, 8	0.25	16	**grpBetaHS, **grpVIR
Benzylpenicillin[NS2]	p01n	0.06, 0.12, 0.5, 2	0.06	8	**grpC/G, *S. pyogenes*, *S. agalactiae* , *S. pneumoniae* , **grpVIR
Cefotaxime	ctx01n	0.25, 0.5, 1, 2	0.12	8	*S. pneumoniae* , **SpyA, *Streptococcus* spp.
Ceftriaxone[NS2]	cro01n	0.12, 0.25, 1, 4	0.12	8	**grpVIR, *S. agalactiae* , *S. pneumoniae* , *S. pyogenes*
Chloramphenicol	c01n	1, 4, 8	1	16	**N/A
Clindamycin[c]	cm01n	CM 0.12, CM 0.25, CM 0.5, CM/E 0.5/0.1	0.25	1	**SP(Pen(S)) , *S. pyogenes*, *S. agalactiae* , *S. mitis*, *S. oralis*
Erythromycin	e01n	1, 2, 4, 16	0.12	8	*S. pneumoniae* , *S. pyogenes*, **grpVIR
Gentamicin	gm01n	512, 1024	64	512	**N/A
Inducible Clindamycin Resistance	icr01n	CM 0.5, CM/E 0.25/0.5	NEG	POS	*S. agalactiae* , *S. pyogenes*
Levofloxacin	lev01n	1, 2, 4, 16	0.25	16	**SP+MDRSP, *S. pyogenes*, **grpC/F, **grpG, *S. agalactiae* , **grpVIR, *S. milleri*
Linezolid[NS c]	lnz01n	2, 4	2	8	**SP+MDRSP, *S. agalactiae*[NS], *S. pyogenes*
Moxifloxacin	mxf01n	0.5, 1, 4, 8	0.06	4	*S. anginosus* , *S. constellatus*, **SP +MDRSP, *S. pyogenes*, *S. agalactiae* , **grpVIR
Rifampicin	ra01n	0.015, 0.03, 0.12, 0.5	0.06	4	**N/A

Antimicrobic	Code	Concentration §	Calling Range ≤	Calling Range ≥	FDA Indications for Use
Teicoplanin	tec01n	0.5, 1, 4	0.12	4	**N/A
Tetracycline	te01n	0.12, 0.5, 1, 4	0.25	16	*S. pneumoniae* , *S. pyogenes*, **grpVIR
Tigecycline[NS c]	tgc01n	0.12, 0.25, 0.5	0.06	1	**SP(Pen(S)) , **grpSang , *S. agalactiae* , *S. pyogenes*
Trimethoprim/ Sulfamethoxazole[c]	sxt01n	8/152, 16/304, 64/1216	10 (0.5/9.5)	320 (16/304)	*S. pneumoniae*
Vancomycin[NS]	va01n	0.5, 1, 2, 4	0.12	8	**grpVIR, *S. pyogenes*, *S. agalactiae*

Numerical values are expressed in µg/mL.

§ Equivalent standard method concentration by efficacy.

NEG = Negative

POS = Positive

[c] = Category agreement was established at the time of FDA clearance. Essential agreement was not established since test contains less than five discrete dilutions.

[NS] = The current absence of resistant isolates precludes defining any results other than susceptible. Isolates yielding MIC results suggestive of Nonsusceptible category should be submitted to a reference laboratory for further testing.

[NS2] = Beta-hemolytic Streptococci: The current absence of resistant isolates precludes defining any results other than susceptible. Isolates yielding MIC results suggestive of Nonsusceptible category should be submitted to a reference laboratory for further testing.

**N/A = No specific FDA Indications for Use available

**grpBetaHS = Beta-hemolytic group *Streptococcus* species

**grpVIR = Viridans group Streptococcus

**grpC/G = Beta-hemolytic *Streptococcus* groups C and G

**SpyA = *S. pyogenes* (Group A beta-hemolytic streptococci)

**SP(Pen(S)) = *S. pneumoniae* (penicillin-susceptible strains)

**grpC/F = Beta-hemolytic *Streptococcus* groups C and F

**grpG = Beta-hemolytic *Streptococcus* group G

**grpSang = *S. anginosus* grp. (includes *S. anginosus*, *S.intermedius*, and *S. constellatus*)

**SP+MDRSP = *S. pneumoniae* (including multi-drug resistant strains (MDRSP))

Instrument

The VITEK® 2 instruments are a family of *in vitro* diagnostic devices intended to rapidly assess the antimicrobial susceptibility of bacterial and yeast pathogens to available antimicrobial agents. For detailed information on the use and operation of these devices, refer to the appropriate Instrument User Manual.

Specimen Preparation

Table 2: Culture Requirements Table

VITEK® 2 Card	Media	Age of Culture	Incubation Conditions	McFarland Standards	Dilution for AST	Age of Suspension Before Loading Instrument
AST Streptococcus	TSAB CBA	18 to 24 hours	35°C to 37°C 5% to 10% CO_2	0.50 to 0.63	280 µL in 3.0 mL 0.45% saline	VITEK® 2 Compact: ≤ 30 minutes VITEK® 2: ≤ 1 hour
GP and AST ST Pair	TSAB[1] CBA[1]	18 to 24 hours	35°C to 37°C 5% to 10% CO_2	0.50 to 0.63	280 µL in 3.0 mL 0.45% saline	≤ 30 minutes

[1] These media were used in the identification product database developments and will give optimal performance.

Culture Requirements Table — Media Abbreviations

CBA = Columbia Sheep Blood Agar

TSAB = Trypticase Soy Agar with 5% Sheep Blood

Test Procedure

Warning: Failure to properly follow instructions and recommendations provided in this section for performing laboratory tasks may cause erroneous or delayed results.

Materials

Materials provided are:

- VITEK® 2 DENSICHEK® kit, the VITEK® 2 DENSICHEK® Plus Kit, or the VITEK® DENSICHEK® Kit
- VITEK® 2 DENSICHEK® Standards kit, the VITEK® 2 DENSICHEK® Plus Standards Kit, or the VITEK® DENSICHEK® McFarland References Kit
- VITEK® 2 Cassette
- Adjustable volume saline dispenser
- 12 mm x 75 mm clear plastic (polystyrene) disposable test tubes
- VITEK® 2 60 or VITEK® 2 XL only: VITEK® 2 Pipettor/Diluter Accessory Kit (containing instrument pipette tips and saline hookup) and 0.45% saline bag

Materials required, but not provided are:

- Sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0)
- Loops, sterile sticks or swabs
- Appropriate agar medium (See the Culture Requirements Table.)
- QC isolates
- VITEK® 2 AST Cards
- Micropipettors to deliver 280 µL
- Disposable pipette tips

Optional Accessories:

- Pre-dispensed saline test tubes (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0)
- Test tube caps

- Vortex

Test Card Setup Procedure

The following procedure contains general information applicable to all susceptibility products. (See the Culture Requirements Table for product-specific information.)

Note: Prepare the inoculum from a pure culture, according to good laboratory practices. In case of mixed cultures, a re-isolation step is required. It is recommended that a purity check plate be done to ensure that a pure culture was used for testing. To enhance and support good laboratory practices, bioMérieux recommends creating a purity plate using the transfer tube/card straw after the card has been filled in the VITEK® 2 system. Please note that underlying growth or other colony types on a purity plate may not be easily visible.

Note: Please refer to the user manual for your specific brand of dispensette to ensure the maintenance instructions are followed. The only recommended cleaning procedure for dispensettes is via autoclave. The use of chemicals or cleaning agents (like bleach or soap) can negatively impact the functionality of the dispensette as well as results. bioMérieux recommends autoclaving on a routine basis, at a minimum when a new bottle of saline is started.

Note: To enhance and support good laboratory practices, bioMérieux recommends to check for low-level saline contamination on a routine basis, by dispensing 1 mL of saline into a tubed broth media (for example Tryptic Soy Broth, BHI, and Thioglycolate) and incubate at 35-37° Centigrade for 2-3 days. Check every day for growth. If the above process is not possible, discard the open bottle of saline and use a new bottle. Autoclaving the dispensette is necessary when starting a new bottle of saline and should be performed on a routine basis. Undetected contamination of the saline can lead to the reporting of inappropriate results.

1. Do one of the following:
   - Select isolated colonies from a primary plate if culture requirements are met.
   - Subculture the organism to be tested to appropriate agar medium and incubate accordingly.
2. Aseptically transfer 3.0 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) into a clear plastic (polystyrene) test tube (12 mm x 75 mm).
3. Using sterile technique, prepare a homogenous organism suspension with a density equivalent to the appropriate McFarland standard or McFarland Reference using a compatible benchtop densitometer (see the Culture Requirements Table).
   Note: The age of the suspension before loading the instrument for AST testing must be less than one hour when using VITEK® 2 60 or VITEK® 2 XL, and less than 30 minutes when using VITEK® 2 Compact.
4. Choose one of the following:
   - **For an automatic dilution (VITEK® 2 60 or VITEK® 2 XL only):** Place the suspension tube prepared in step 3 into the cassette with or without an identification card. In the next cassette slot, place an empty tube and an AST card. The instrument will automatically dilute the bacterial suspension to prepare an inoculum suitable for the susceptibility card.
   - **For a manual dilution (VITEK® 2 Compact, VITEK® 2 60 or VITEK® 2 XL):** In a second tube containing 3.0 mL of saline, transfer 280 µL of the suspension prepared in step 3. Then place this tube in the cassette with a susceptibility card. The tube with the initial bacterial suspension can also be used for inoculation of an identification card.

   Note: Check the saline level in the tubes after filling. When it is evident by the saline level in the tube that a card has been improperly filled, do not load the card if using VITEK® 2 Compact; **or**, eject the card if using VITEK® 2 60 or VITEK® 2 XL.

   Note: Refer to the appropriate Instrument User Manual for detailed information regarding data entry, processing, etc.
5. Follow your local inspecting agency's guidelines for disposal of hazardous waste.

Quality Control

Quality Control organisms should be processed according to the Test Card Setup Procedure.

Note: If a QC strain appears in the QC Table with no expected results, it is not applicable to use that strain for quality control testing of that antimicrobial.

Table 3: Quality Control

CLSI® Quality Control Organisms VITEK® 2 Results				
Antimicrobic	Code	*S. pneumoniae* ATCC® 49619™	*S. aureus* ATCC® 29213™	*S. aureus* ATCC® BAA-977™
Ampicillin[NS2]	am01n	≤ 0.25	-	-
Benzylpenicillin[NS2]	p01n	0.25 - 1	-	-
Cefotaxime	ctx01n	≤ 0.12*	-	-
Ceftriaxone[NS2]	cro01n	≤ 0.12**	-	-
Chloramphenicol	c01n	2 - 8	-	-
Clindamycin	cm01n	≤ 0.25	-	-
Erythromycin	e01n	≤ 0.12	-	-
Gentamicin	gm01n	≤ 64	-	-
Inducible Clindamycin Resistance	icr01n	-	NEG	POS
Levofloxacin	lev01n	0.5 - 2	-	-
Linezolid[NS]	lnz01n	≤ 2	-	-
Moxifloxacin	mxf01n	≤ 0.06 - 0.25	-	-
Rifampicin	ra01n	≤ 0.06	-	-
Teicoplanin	tec01n	≤ 0.12	-	-
Tetracycline	te01n	≤ 0.25 - 0.5	-	-
Tigecycline[NS]	tgc01n	≤ 0.06 - 0.12	-	-
Trimethoprim/ Sulfamethoxazole	sxt01n	≤ 10 (0.5/9.5) - 20 (1/19)	-	-
Vancomycin[NS]	va01n	≤ 0.12 - 0.5	-	-

Numerical values are expressed in µg/mL.

NEG = Negative

POS = Positive

[NS] = The current absence of resistant isolates precludes defining any results other than susceptible. Isolates yielding MIC results suggestive of Nonsusceptible category should be submitted to a reference laboratory for further testing.

[NS2] = Beta-hemolytic Streptococci: The current absence of resistant isolates precludes defining any results other than susceptible. Isolates yielding MIC results suggestive of Nonsusceptible category should be submitted to a reference laboratory for further testing.

*Broth Microdilution for FDA expected QC range is 0.06 - 0.25 and CLSI is 0.03 - 0.12 µg/mL

**Broth Microdilution for FDA and CLSI expected QC range = 0.03 - 0.12 µg/mL

Certification Statement

This is to certify that bioMérieux complies with ISO 13485 and FDA Quality System Regulation (QSR) requirements for design, development, and manufacture of antimicrobial susceptibility systems.

Frequency of QC Testing

Refer to *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically,* CLSI® and/or your local guidelines.[2]

Preparation of QC Organisms

1. Rehydrate the organism according to the manufacturer's instructions.
2. Subculture to Trypticase Soy Agar with 5% sheep blood (TSAB).

3. Incubate at 35°C for 24 hours.
   Note: Gram-positive organisms may require an atmosphere of CO_2. (Refer to the Culture Requirements Table).
4. Check for purity.
5. Subculture to a TSAB plate.
6. Incubate at 35°C for 18-24 hours for Gram-positives, and 8-24 hours for Gram-negatives.

Short-Term Storage Conditions

1. Streak to a TSAB plate or slant.
2. Incubate for 24 hours.
3. Refrigerate at 2°C to 8°C for up to two weeks.
4. Subculture once as described above and use for QC.

Long-Term Storage Conditions

1. Make a heavy suspension in Tryptic Soy Broth (TSB) with 15% glycerol.
2. Freeze at -70°C.
3. Subculture to TSAB twice before running QC.
   Note: Avoid repeated thawing and refreezing by either freezing in single-use aliquots or removing a small portion of frozen organism preparation with a sterile applicator stick.

Results

Susceptibility Analytical Techniques

The system evaluates each organism's growth pattern in the presence of the antimicrobial in relation to the growth control well. Several parameters based on the growth characteristics are used to determine the MIC or qualitative result (for example, ICR POS/NEG). The MIC result must be linked to an organism identification to determine a category interpretation. Accurate identification is critical, especially with certain organism/antimicrobial combinations (e.g., *S. pneumoniae*/ benzylpenicillin).

In cases where the identification of an organism is in question, confirmatory testing is necessary to ensure correct interpretation of susceptibility results.

A category interpretation will be reported along with a MIC, according to the interpretations defined by the Food and Drug Administration (FDA), CLSI®, Comité de l'Antibiogramme de la Société Française de Microbiologie (CASFM), European Committee for Antimicrobial Susceptibility Testing (EUCAST), or to an adaptation of the global settings according to other local guidelines.

Note: When FDA and CLSI® breakpoints differ, VITEK® 2 Systems AST tests are cleared for use with FDA breakpoints applied.

Note: As some category interpretation definitions differ between US FDA, CLSI, and EUCAST, refer to the respective publications, websites, and/or the VITEK® 2 Software User Manual (Chapter: Maintaining the Workstation), for more detailed information.

Combination Antimicrobials

The MICs for the combination antimicrobials are listed on the laboratory and patient reports as the first concentration (e.g., ampicillin/sulbactam ≤ 8/4 µg/mL is reported as ≤ 8 µg/mL.). The actual concentrations for each value in the antimicrobial calling range are as follows:

- trimethoprim/sulfamethoxazole: **Note exception** — This drug is listed on the laboratory and patient reports as the sum of the two antimicrobial concentrations: 10 µg/mL = 0.5/9.5, 20 µg/mL = 1/19, 40 µg/mL = 2/38, 80 µg/mL = 4/76, 160 µg/mL = 8/152, 320 µg/mL = 16/304

Antimicrobial Deduction

Antimicrobials that have been deduced will only report an interpretive result and will be noted with a **+**.

Clinical Efficacy and Indications for Use

AST cards may contain some antimicrobials that are not proven to be effective for treatment of infections caused by all organisms that may be tested. For interpreting and reporting of antimicrobial results that have shown to be active against

organism groups both *in vitro* and in clinical infections, refer to the individual pharmaceutical antimicrobial labeling or the local therapy guidelines.

Indications for use specific to the FDA are found in each VITEK® 2 AST card package insert in the column labeled, "FDA Indications for Use". This list contains the antimicrobial/organism combinations that have been cleared by the FDA for testing and reporting on the VITEK® 2 System. FDA clearance has been granted according to the FDA approved pharmaceutical labeling and the VITEK® 2 AST clinical trial data. To report only organisms listed in the FDA Indications for Use section of the package insert, enable bioART Indications for Use suppression rules.

Limitations

A VITEK® 2 AST card cannot be used with a direct clinical specimen or sample or other sources containing mixed flora. Any change or modification in the procedure may affect the results.

A result for an antibiotic/organism combination, which may have a limitation, may be suppressed from reporting. This can be accomplished through the use of bioART rules in the VITEK® 2 Systems software. Refer to the software user manual for instructions.

Perform an alternative method of testing prior to reporting of results for the following antibiotic/organism combination(s):

- Erythromycin (e01n): *Streptococcus mitis, S. oralis, S. parasanguinis*

The ability of the AST card to detect resistance with the following combination(s) is unknown because resistant strains were not available at the time of comparative testing:

- Benzylpenicillin (p01n): *S. pneumoniae* (based on the breakpoints for pneumonia)
- Linezolid (lnz01n): *S. pneumoniae, S. agalactiae, S. pyogenes*
- Tigecycline (tgc01n): *S. pneumoniae* (penicillin-susceptible strains), *S. anginosus, S. intermedius, S. constellatus, S. agalactiae, S. pyogenes*
- Vancomycin (va01n): *Streptococcus agalactiae, Streptococcus pyogenes, Viridans* group *Streptococcus*

The ability of the AST card to detect resistance with this combination is unknown because resistant strains were not available at the time of comparative testing:

- Linezolid (lnz01n): (When applying CA-SFM breakpoints <2 S, 4 I, >8 R) *Streptococcus viridans* group

EUCAST Limitations

It is recommended that the existing bioART suppression rules be enabled, or new rules created and enabled, for these limitations if the EUCAST breakpoints are applied.

Perform an alternate method of testing prior to reporting of results for the following antibiotic/organism combination(s):

- Rifampicin (ra01n): *Beta hemolytic Streptococci*

Perform an alternate method of testing prior to reporting resistant results for the following antibiotic/organism combination(s).

- Ceftriaxone (cro01n): *Streptococcus anginosus*, *Streptococcus constellatus*, *Streptococcus intermedius*

The ability of the AST card to detect resistance with the following combination(s) is unknown because resistant strains were either not available or an insufficient number were encountered at the time of comparative testing:

- Tigecycline (tgc01n): *Beta hemolytic Streptococci*

Expected Values

Expected results for susceptibility tests will vary based on location and institution. VITEK® 2 Systems were tested at several geographically diverse locations to ensure that trends that occurred by location were integrated into the performance characteristics of the system. Organism resistance patterns will differ by institution; therefore, expected values will be directly related to the population of organisms at each site.

Performance Characteristics

The performance characteristics of the antimicrobial agents included in VITEK® 2 AST cards were established using the manual and autodilution modes (on a VITEK® 2 System) at multiple clinical laboratories. The VITEK® 2 AST card results were compared to results from a CLSI® reference method. Essential agreement (EA) represents VITEK® 2 results which agree exactly or are within a ± twofold dilution (± two doubling dilutions for antifungal) of the reference result.

Category agreement (CA) occurs when the VITEK® 2 and the reference interpretative results agree (Susceptible, Intermediate, and Resistant). There are instances when the category agreement for an antimicrobial falls below the essential agreement. This can occur when a significant number of MICs cluster around a category breakpoint during clinical trial testing, resulting in interpretative errors. For a description of interpretive errors, refer to the footnotes below the table that follows (Performance Characteristics). When the majority of the errors are of the minor type, a high corresponding essential agreement percentage demonstrates that the antimicrobial retains an acceptable overall performance.

There are instances when the performance is based solely on category agreement (CA) because at the time performance was established, less than five discrete twofold dilutions were evaluated. A minimum of five dilutions is necessary to calculate essential agreement (EA) based on ± one twofold dilution. These instances are designated by a "c" footnote in the Contents of the Card table. The following performance tables have values for CA only when EA was not established at the time of FDA clearance.

The reproducibility of the VITEK® 2 system was established by testing a set of on-scale organisms.*

*Data on file at bioMérieux, Inc

Table 4: Performance Characteristics for Streptococcus Species Antimicrobial Susceptibility Testing

Antimicrobial	Antimicrobial Code	Antibiotic Version	Bp[1]	Comment[2]	Essential Agreement				Category Agreement				% Reproducibility
					% Error				% Error				
					% EA	VME	ME	mE	% CA	VME	ME	mE	
Ampicillin	AM	am01n	CLSI	#, E	99.1	0.0	0.0	0.5	97.0	0.0	0.0	3.0	99.6
			CA-SFM	E, *S. pneumoniae* (pneumonia)	96.6	1.1	1.2	-	94.9	6.5	4.6	-	100
Benzylpenicillin (Penicillin)	P	p01n	CLSI[2]	#, E (Include *S. pneumoniae* with pneumonia breakpoint)	99.1	0.0	0.0	0.6	96.8	0.0	0.0	3.2	99.6
				#, E, *S. pneumoniae* (pneumonia)	97.7	0.0	0.0	1.1	92.3	0.0	0.0	7.7	
				#, E *S. pneumoniae* (meningitis)	97.7	0.6	0.5	N/A	97.7	1.2	3.2	N/A	
			Global	E, *S. pneumoniae* (oral)	97.7	0.0	0.0	2.0	89.7	0.0	0.0	10.3	
Cefotaxime	CTX	ctx01n	CLSI	#, E *Streptococcus* species	99.0	0.0	0.2	0.2	96.5	0.0	0.2	3.3	100
				#, E *S. pneumoniae* (non-meningitis)[4]	98.6	0.0	0.3	0.9	89.7	0.0	0.3	10.0	

Antimicrobial	Antimicrobial Code	Antibiotic Version	Bp[1]	Comment[2]	Essential Agreement				Category Agreement				% Reproducibility
					% Error				% Error				
					% EA	VME	ME	mE	% CA	VME	ME	mE	
Ceftriaxone	CRO	cro01n	FDA, CLSI	#, E *Streptococcus* species	98.9	0.0	0.2	0.1	97.7	0.0	0.2	2.1	100
				S. pneumoniae (meningitis)[3]	97.7	0.0	0.0	1.4	90.9	0.0	0.0	9.1	
				S. pneumoniae (non-meningitis)[3]	97.7	0.0	1.0	0.0	93.7	0.0	1.0	5.4	
				Beta hemolytic streptococci[3]	100	0.0	0.0	0.0	100	0.0	0.0	0.0	
				Viridans group streptococci[3]	97.8	0.0	0.0	0.5	96.3	0.0	0.0	3.7	
Chloramphenicol	C	c01n	Global	E *Streptococcus pneumoniae*	96.9	2.9	0.0	-	99.7	2.9	0.0	-	100
				Beta Hemolytic Streptococci	99.0	0.0	0.0	0.8	99.0	0.0	0.0	1.0	
				Viridans group Streptococci	98.3	0.0	0.0	0.0	100	0.0	0.0	0.0	
			CASFM	E Viridans group Streptococci	98.3	0.0	0.0	-	100	0.0	0.0	-	
Clindamycin	CM	cm01n	CLSI[2]	#, E	N/A	N/A	N/A	N/A	97.2	1.1	1.1	1.7	100
Erythromycin	E	e01n	FDA, CLSI	#, E	97.9	0.8	0.2	0.5	98.7	0.8	0.2	0.9	96.7
Inducible Clindamycin Resistance	ICR	icr01n	CLSI	#, E	N/A	N/A	N/A	N/A	99.2	1.5	0.7	N/A	100
Levofloxacin	LEV	lev01n	CLSI (FDA)	#, E	99.0	0.0	0.0	0.3	97.9	0.0	0.0	2.1	100
			CA-SFM	E, Viridans group *Streptococci*	98.6	0.0	0.0	0.2	87.3	0.0	0.0	12.7	
Linezolid	LNZ	lnz01n	CLSI	#, E	-	-	-	-	99.8	0.0	0.2	N/A	100
			CA-SFM	E, Viridans group *Streptococci*	99.3	0.0	0.0	0.7	99.0	0.0	0.0	1.0	
Moxifloxacin	MXF	mxf01n	FDA,CLSI	#, E	93.5	0.0	0.0	0.0	99.5	0.0	0.0	0.5	100
			CA-SFM	E, Viridans group *Streptococci*	94.5	0.0	0.4	-	99.7	0.0	0.4	-	
Rifampicin	RA	ra01n	Global	E *Streptococcus pneumoniae*	99.3	0.0	0.0	0.3	99.7	0.0	0.0	0.3	100
Tetracycline	TE	te01n	FDA	#, E	96.8	0.7	0.2	0.7	97.1	0.7	0.2	2.5	100
			CLSI	E	96.8	1.9	0.2	0.3	96.4	1.9	0.2	2.7	
			CA-SFM	E, Viridans group *Streptococci*	92.9	1.4	0.7	2.8	92.2	1.4	0.7	6.8	

Antimicrobial	Antimicrobial Code	Antibiotic Version	Bp[1]	Comment[2]	Essential Agreement				Category Agreement				% Reproducibility
					% Error				% Error				
					% EA	VME	ME	mE	% CA	VME	ME	mE	
Tigecycline	TGC	tgc01n	FDA	#, E	-	-	-	-	99.8	0.0	0.2	N/A	100
			CASFM	E Viridans group Streptococci	98.0	0.0	0.0	0.7	97.3	0.0	0.0	2.7	
Trimethoprim / Sulfamethoxazole	SXT	sxt01n	FDA, CLSI	#, E	-	-	-	-	96.1	0.0	0.0	3.9	100
Vancomycin	VA	va01n	FDA, CLSI	#, E	95.8	0.0	0.0	0.0	100	0.0	0.0	0.0	100

[1] Abbreviations — Bp = breakpoint committee; EA = essential agreement; CA = category agreement; VME = Very Major Error (susceptible result with resistant reference result); ME = Major Error (resistant result with susceptible reference result); mE = minor Error (susceptible or resistant result with an intermediate reference result, or an intermediate result with a susceptible or resistant reference result).

[2] FDA breakpoints are used in the CLSI Interpretation Standard (breakpoint committee) in the VITEK® 2 Systems software.

[3] The analysis was done using the FDA/CLSI breakpoints of:

S. pneumoniae (meningitis): S ≤ 0.5, I = 1, R ≥ 2

S. pneumoniae (non-meningitis): S ≤ 1, I = 2, R ≥ 4

Beta hemolytic streptococci: S ≤ 0.5

Viridans group streptococci: S ≤ 1, I = 2, R ≥ 4

[4] The analysis was done using the CLSI breakpoints of: S ≤ 1, I = 2, R ≥ 4

Key:

= US Food and Drug Administration 510(k) cleared

CLSI® = Clinical and Laboratory Standards Institute

CA-SFM = Comité de l'Antibiogramme de la Société Française de Microbiologie

E = External performance data

I = Internal performance data

– = Not available

N/A = Not applicable

❶ ❷ = Symbol identifies the performance characteristics for a specific antimicrobial version.

Ref. = Reference method for clinical performance study.

Table 5: EUCAST Performance Characteristics for Streptococcus Species Antimicrobial Susceptibility Testing

Antimicrobial	Antimicrobial Code	Antibiotic Version	Comment[1]	Essential Agreement				Category Agreement			
				% Error				% Error			
				%EA[2]	VME	ME	mE	%CA	VME	ME	mE
Ampicillin	AM	am01n	*S. pneumoniae*	96.9	0.0	0.0	2.6	92.9	0.0	0.0	7.1
			Viridans spp.	97.3	0.0	0.3	2.2	93.1	0.0	0.3	6.6
Benzylpenicillin (Penicillin)	P	p01n	*S. pneumoniae* (meningitis)	97.7	0.6	0.5	-	97.7	1.2	3.2	-
			S. pneumoniae (pneumonia)	97.7	0.0	0.0	1.7	90.0	0.0	0.0	10.0
			Beta Hemolytic Streptococci	100	0.0	0.0	-	100	0.0	0.0	-
			Viridans spp.	98.5	0.0	0.0	1.3	93.6	0.0	0.0	6.4
Cefotaxime	CTX	ctx01n	Beta Hemolytic Streptococci	100	0.0	0.0	0.0	100	0.0	0.0	0.0
			S. pneumoniae	98.6	0.0	0.0	1.4	94.3	0.0	0.0	5.7

Antimicrobial	Antimicrobial Code	Antibiotic Version	Comment[1]	Essential Agreement				Category Agreement			
				% Error				% Error			
				%EA[2]	VME	ME	mE	%CA	VME	ME	mE
Ceftriaxone	CRO	cro01n	Beta Hemolytic Streptococci	100	0.0	0.0	0.0	100	0.0	0.0	0.0
			S. pneumoniae	97.7	0.0	0.0	1.4	94.6	0.0	0.0	5.4
Chloramphenicol	C	c01n	*Streptococcus pneumoniae*	96.6	0.0	0.0	-	99.7	3.1	0.0	-
			Beta Hemolytic Streptococci	99.0	0.0	0.0	-	100	0.0	0.0	-
Clindamycin	CM	cm01n	*S. pneumoniae*, Beta Hemolytic Streptococci	98.5	0.6	0.6	0.0	98.8	1.2	1.2	0.0
Erythromycin	E	e01n	N/A	95.8	0.9	0.2	1.0	98.0	0.9	0.2	1.5
Gentamicin	GM	gm01n	Streptococci	100	0.0	0.0	-	99.9	0.0	0.1	-
Levofloxacin	LEV	lev01n	Streptococcus β-hemolytic group (A,B,C,G)	99.2	0.0	0.5	N/A	97.0	0.0	3.0	N/A
			S. pneumoniae	99.3	0.0	0.0	0.0	100	0.0	0.0	0.0
Linezolid	LNZ	lnz01n	*S. pneumoniae*	100	0.0	0.0	-	100	0.0	0.0	-
			Beta Hemolytic Streptococci	100	0.0	0.0	0.0	100	0.0	0.0	0.0
Moxifloxacin	MXF	mxf01n	*Streptococcus pneumoniae*	97.3	0.0	0.0	-	100	0.0	0.0	-
			Streptococcus β-hemolytic group (A,B,C,G)	90.9	0.0	2.5	N/A	97.3	0.0	2.7	N/A
Rifampicin	RA	ra01n	*Streptococcus pneumoniae*	100	0.0	0.0	0.0	99.3	0.0	0.0	0.7
			Beta Hemolytic Streptococci	96.5	0.0	0.0	3.5	65.4	0.0	0.0	34.6
Teicoplanin	TEC	tec01n	*Streptococcus pneumoniae*	100	0.0	0.0	-	100	0.0	0.0	-
			Beta Hemolytic Streptococci	99.2	0.0	0.0	-	100	0.0	0.0	-
			Viridans group Streptococci	99.0	0.0	0.0	-	100	0.0	0.0	-
Tetracycline	TE	te01n	*S. pneumoniae*, Beta Hemolytic Streptococci	98.3	0.2	0.2	0.5	97.7	0.2	0.2	2.2
Tigecycline	TGC	tgc01n	Beta Hemolytic Streptococci	98.9	0.0	0.0	0.4	99.6	0.0	0.0	0.4
Trimethoprim / Sulfamethoxazole	SXT	sxt01n	*S. pneumoniae*	99.2	0.0	0.0	0.8	97.5	0.0	0.0	2.5
			Beta Hemolytic Streptococci[2]	94.8	12.5	0.1	4.1	95.2	12.5	0.1	4.5
Vancomycin	VA	va01n	Beta-hemolytic Streptococcus, S. viridans group, *S. pneumoniae*	96.5	0.0	0.0	-	100	0.0	0.0	-

[1] Abbreviations — Bp = breakpoint committee; EA = essential agreement; CA = category agreement; VME = Very Major Error (susceptible result with resistant reference result); ME = Major Error (resistant result with susceptible reference result); mE = minor Error (susceptible or resistant result with an intermediate reference result, or an intermediate result with a susceptible or resistant reference result).

[2] For all gram-positive Beta-lactams The performance characteristics as listed only includes clinical isolate data.

Key:

= EUCAST = European Committee on Antimicrobial Susceptibility

N/A = Not applicable

List of Claims

Note: If the organism is not in the VITEK® 2 susceptibility database, results will not be reported.

Note: Organisms listed with an asterisk (*) indicate an AES claimed organism. No asterisk is displayed for a group; however, when an individual species (with an asterisk) is contained within a group, it is expertised.

Streptococcus Organisms Claimed for AST-ST (keyID)

- *Staphylococcus aureus* ATCC® 29213™
- *Staphylococcus aureus* ATCC® BAA-977™
- *Streptococcus agalactiae**
- *Streptococcus alactolyticus**
- *Streptococcus anginosus**
- *Streptococcus canis**
- *Streptococcus constellatus**
- *Streptococcus constellatus* ssp. *constellatus**
- *Streptococcus constellatus* ssp. *pharyngis**
- *Streptococcus cristatus**
- *Streptococcus downei**
- *Streptococcus dysgalactiae* ssp. *dysgalactiae**
- *Streptococcus dysgalactiae* ssp. *equisimilis**
- *Streptococcus equi* ssp. *equi**
- *Streptococcus equi* ssp. *zooepidemicus**
- *Streptococcus equinus**
- *Streptococcus gallolyticus* ssp. *gallolyticus**
- *Streptococcus gallolyticus* ssp. *pasteurianus**
- *Streptococcus gordonii**
- *Streptococcus* Group A*
- *Streptococcus* Group B*
- *Streptococcus* Group C*
- *Streptococcus* Group G*
- *Streptococcus infantarius* ssp. *coli* (*S. lutetiensis)**
- *Streptococcus infantarius* ssp. *infantarius**
- *Streptococcus intermedius**
- *Streptococcus mitis**
- *Streptococcus mitis/Streptococcus oralis**
- *Streptococcus mutans**
- *Streptococcus oralis**
- *Streptococcus parasanguinis**
- *Streptococcus pneumoniae**
- *Streptococcus pneumoniae* ATCC® 49619™
- *Streptococcus pyogenes**
- *Streptococcus salivarius* ssp. *salivarius**
- *Streptococcus salivarius* ssp. *thermophilus**
- *Streptococcus sanguinis**
- *Streptococcus sobrinus**
- *Streptococcus suis**
- *Streptococcus suis I**
- *Streptococcus suis II**
- *Streptococcus uberis**

- *Streptococcus vestibularis**
- *Streptococcus viridans* group except *S. pneumoniae**

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Barcodes

User MUST enter the following barcodes into "Flex Panel Entry" program before first use of this Susceptibility Card.

01 A340E00J--FM
02 BAST-ST03016
03 C--01020304N
04 D0D07080E09Z
05 E0A050G0H0IE
06 F0B0J060C00Y

If you have a compatible barcode reader for 2D barcodes (e.g., 1400g Honeywell 2D barcode reader), scan the following 2D barcode instead of the individual barcodes.

Index of Symbols

Symbol	Meaning
REF	Catalog number
IVD	In Vitro Diagnostic Medical Device
	Legal Manufacturer
	Temperature limitation
	Use by date
LOT	Batch code
	Consult Instructions for Use

Symbol	Meaning
	Date of manufacture
	Contains sufficient for <n> tests
	Authorized representative in the European Community
	For US Only : Caution : US Federal Law restricts this device to sale by or on the order of a licensed practitioner
	Importer

Instructions for use provided in the kit or downloadable from http://www.biomerieux.com.

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All hazardous waste must be disposed of by following your local inspecting agency's guidelines.

Revision History

Change type categories

N/A	Not applicable (First publication)
Correction	Correction of documentation anomalies
Technical change	Addition, revision and/or removal of information related to the product
Administrative	Implementation of non-technical changes noticeable to the user

Note: *Minor typographical, grammar, and formatting changes are not included in the revision history.*

Release Date	Part Number	Change Type	Change Summary
2021-08	046698-03	Technical change	• Reagents • Test Procedure • Results • Limitations • Performance Characteristics • Index of Symbols

Release Date	Part Number	Change Type	Change Summary
2019-05	046698-02	Technical change	• Updated with EUCAST limitations
2016-12	046698-01	Administrative	• Formatting changes do not affect the fit, form, or function of the product
		Technical change	• Combined product package insert content with VITEK® 2 Product Information Manual AST content • Updated Limited Warranty section • Updated with RX only information • Updated with EUCAST limitations

For users in the European Union (Regulation (EU) 2017/746) and in countries with similar requirements: Should a serious incident occur during the use of this device or as a result of its use, please report it to the manufacturer and/or their authorized representative as well as to your national authority.

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