Service contract for the provision of EU networking and support for public health reference laboratory functions for antimicrobial resistance in *Salmonella* species and *Campylobacter* species in human samples

SC 2019 74 09

MIC determination by gradient test

April 2022

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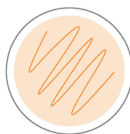

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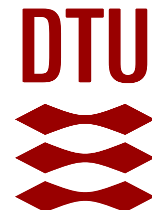

Health and Digital Executive Agency

Third EU Health Programme

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1. Introduction

This protocol has been prepared for the purpose of presenting and describing the laboratory activities covered by the FWD-AMR-RefLabCap Training Course hosted at DTU Food, Denmark in May 2022.

MIC determination by gradient strip is one of several phenotypic assays which can be used to determine the antimicrobial resistance profile of a bacterial organism. Gradient strip estimate in vitro antimicrobial susceptibility.

An agar plate is inoculated with a standardized inoculum of the bacteria and a gradient strip is placed on the inoculated plate. The gradient strip contains a standardized, known amount of an antimicrobial agent which diffuses into the agar when in contact with the agar surface. The plate is incubated under standardized conditions following the manufacturer's guidelines. During incubation, the antimicrobial agent diffuses into the agar and inhibits growth of the inoculated bacteria, thereby producing an inhibition zone.
After incubation, the MIC value is determinated and results can be interpretated using EUCAST guidelines.

Highly standardized methods are essential for all types of antimicrobial susceptibility testing. The test results are highly sensitive to variations in inoculum density, media formulation, agar thickness and moisture, correct range of the gradient strip, correct storage of the gradient strip, incubation time and how you read and interpretate the obtained MIC values.

Publications Office

2. Equipment

- Nephelometer
- McFarland standard 0.5
- Vortex mixer
- Sterile forceps
- Loops
- Sterile cotton swabs
- 0,85% sterile saline
- MH or MH-F agar plates (where MH is for *Salmonella* (Mueller Hinton agar) and MH-F is for *Campylobacter* (Mueller Hinton agar for fastidious organisms, i.e. Mueller Hinton supplemented with 5% lysed horse blood and 20 mg/L β-NAD))
- Gradient strips

3. Procedure day 1

- Allow your gradient strip to reach room temperature
- Make sure your MH or MH-F agar plates are not too wet from condensation (if they are, they can be dried 15 minutes in an incubator. This is especially important for MH-F plates)
- Standardize the inoculum: From a pure overnight culture, pick material from at least 3-4 colonies. Suspend in 5 ml saline in a tube of the same type as the one for the McFarland 0.5 standard. Mix. Adjust to McFarland 0.5 using a nephelometer. Calibrate the nephelometer before use using the McFarland 0.5 standard and gently invert your test suspension a couple of times by turning the tube upside-down before measuring. If necessary, adjust turbidity of inoculum to match the standard by adding either more colony material or more saline to the inoculum.
- Place a cotton swab in your 0.5 McFarland suspension
- Remove excess fluid from the cotton swab by pressing the swab against the inside of the tube above the inoculum level (this applies to Gram negatives, only)
- Cover the agar plate with streaks in three different directions to ensure an even growth or use a plate rotator
- Apply your gradient test using sterile forceps
- Turn your plates upside down and incubate them at 35° +/- 1° for 18 +/- 2 h for *Salmonella* and 41° in a microaerophile environment for 24 h for *Campylobacter*.

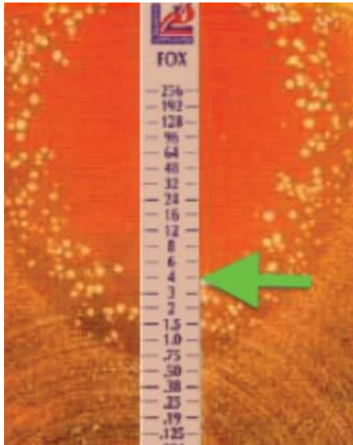
Observe the 15-15-15 minutes rule, meaning that from the time you make your suspension you should inoculate your agar plate within 15 minutes. From the time you have inoculated your agar plate you should apply the gradient strip no more than 15 minutes after. From the time you applied your gradient strip to the agar plate, your plate should be in the incubator within 15 minutes.

4. Procedure day 2

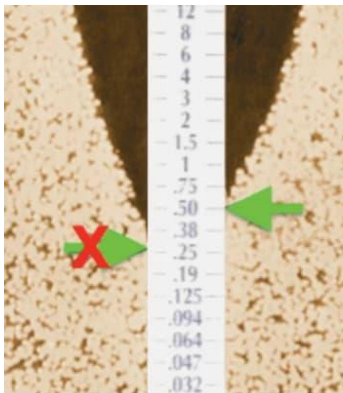
- Take your plates out of the incubator
- Examinate your plates. A confluent lawn of growth is the result of a correctly inoculated and satisfactorily streaked plate. If individual colonies can be seen, the inoculum is too light for the result to be read and the test must be repeated.
- Gradient strip test plates are read from above with the lid removed and reflected light
- Determine the MIC based on where the ellipse intersects the scale. If this is in-between two values, round up to the higher value.
- If the intersect differs on either side of the strip read the MIC as the higher value

5. Reading examples

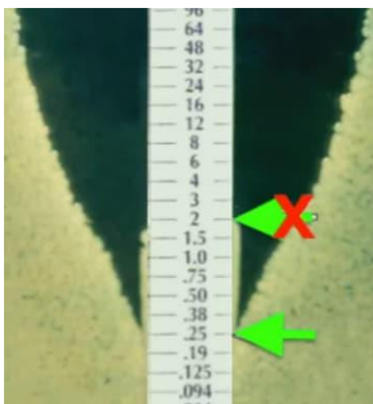
If single colonies are seen within the ellipse these should be taken into account. Therefore the MIC should be read above the colonies.



If uneven growth is observed, read the upper MIC value.
If the values on each side of the gradient strip differ more than +/- 1 two-fold dilution, repeat the test.



Ignore the growth along the strip



6. Quality control

To ensure that you obtain reliable results when performing antimicrobial susceptibility testing, quality control (QC) is crucial.

Traceability is key when performing quality control. Ensure that you perform check of new batches of media etc. and ensure that your document and track your QC results allowing you to trace back if you need to troubleshoot, for example if you observe that your QC strain is one step out of range you can trace back to check if this might have started when you started to use a new batch of something.

If you do not perform a particular type of test routinely, ensure that you consider which QC-measures are relevant, for example including a QC strain in parallel to your test strains. If you obtain results from the QC strain that are within the acceptance range, you have an indication that your test strain results are reliable.

Annually, in January, EUCAST update their breakpoint tables and QC tables. This might include updated ranges or the addition of breakpoints and ranges for new antimicrobial agents. Keep updated on the newest version when interpretating your results by looking into the most recent QC table. The current QC table is V.12 and can be found here
EUCAST: Quality Control

Storage of gradient strips should always follow the manufacturer's instructions. After opening, gradient strips should be stored in sealed containers with a moisture-indicating dessicant and protected from light. Perform frequent quality control when testing to make sure that the gradient strip has not lost potency during storage.

7. Media preparation

For media preparation, commercial media can be used if they follow EUCAST recommendations.

For EUCAST media preparation guide see EUCAST: Media preparation

8. Appendix 1

The following appendix is not something that will be shown in the laboratory work during the workshop but demonstrates ESBL E-test.

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Etest® ESBL

CEFOTAXIME/CEFOTAXIME + CLAVULANIC ACID

CEFTAZIDIME/CEFTAZIDIME + CLAVULANIC ACID

For *in vitro* confirmation of ESBL

INTENDED USE

Etest® ESBL Cefotaxime/cefotaxime + clavulanic acid (CT/CTL) strip and Ceftazidime/ceftazidime + clavulanic acid (TZ/TZL) strips are designed to confirm the presence of clavulanic acid inhibitable ESBL (Extended Spectrum Beta-Lactamase) enzymes in *Escherichia coli*, *Klebsiella pneumoniae* and *K. oxytoca*. The suspected presence of ESBL in strains with phenotypic susceptibility patterns where MIC values of aztreonam, cefotaxime, ceftazidime, ceftriaxone or cefpodoxime are ≥1 µg/mL, can be confirmed using both Etest CT/CTL and TZ/TZL strips.

SUMMARY

ESBLs are plasmid-mediated enzymes that have evolved through point mutations of key amino acids in parent TEM and SHV enzymes. Although ESBLs vary in their ß-lactam substrate affinity and enzyme kinetics, they essentially inactivate all penicillins and cephalosporins. They are generally inhibited by ß-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam but inhibitor resistant TEM (IRT) ESBL enzymes have been reported. Currently, ESBLs are not active against cefamycins and carbapenems. Multiple ESBL enzymes (1 to 5) and chromosomally or plasmid encoded AmpC ß-lactamases may be simultaneously produced in the same clinical isolate. Plasmids encoding for ESBL may also carry resistance genes for trimethoprim/sulfamethoxazole and aminoglycosides. Chromosomal fluoroquinolone resistance is commonly found in ESBL producing strains.

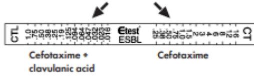
ESBLs have been selected for after many years of extensive use of expanded spectrum cephalosporins (ESC) such as ceftazidime. Although *K. pneumoniae* and *E. coli* are the main pathogens producing ESBLs, more recently *K. oxytoca*, *Enterobacter cloacae*, *E. aerogenes*, *Serratia marcesens*, *Citrobacter diversus*, *Providencia stuartii*, *Proteus mirabilis*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Acinetobacter* spp. have also been reported to produce ESBL. ESBLs are often produced by nosocomial pathogens found in intensive care units, oncology, burn and neonatal wards, nursing homes and in infections associated with indwelling devices. Several ESBL outbreaks have occurred in the USA, South America and Western Europe and prevalence is increasing worldwide.

Besides being associated with high morbidity and mortality, few options remain for treatment of infections involving ESBLs. The efficacy of ß-lactam therapy, including the use of ESC, is compromised while co-resistance to trimethoprim/sulfamethoxazole, aminoglycosides and fluoroquinolones has been reported. Therefore, accurate *in vitro* detection of ESBLs is essential to guide therapy selection and allow for efficient infection control interventions to be rapidly implemented. Current susceptibility test methods are not useful for ESBL detection because MIC values and zone sizes of ESBL producers overlap those of susceptible strains. CLSI recommends ESBL screen criteria for *K. pneumoniae*, *K. oxytoca* and *E. coli* of MIC ≥ 8 µg/mL to cefpodoxime, MIC ≥ 2 µg/mL to ceftazidime, cefotaxime, ceftriaxone or aztreonam, or zone diameters of ≤ 17 mm for cefpodoxime, ≤ 22 mm for ceftazidime, ≤ 27 mm for aztreonam or cefotaxime or ≤ 25 mm for ceftriaxone. The CLSI MIC confirmatory criterion for ESBL is a reduction of ≥ 3 log_2 dilutions for either cefotaxime or ceftazidime in the presence of 4 µg/mL clavulanic acid.

PRINCIPLES OF USE

The Etest ESBL CT/CTL and TZ/TZL strips (Figure 1) consist of a thin, inert and non-porous plastic carrier (5 x 60 mm). One side of the strip is calibrated with MIC reading scales in µg/mL while the reverse surface carries two predefined exponential gradients. CT codes for the cefotaxime (0.25-16 µg/mL) gradient and CTL the cefotaxime (0.016-1 µg/mL) plus 4 µg/mL clavulanic acid. TZ codes for the ceftazidime (0.5-32 µg/mL) gradient and TZL the ceftazidime (0.064-4 µg/mL) plus 4 µg/mL clavulanic acid. The test is set up according to standard Etest procedures for Gram negative aerobes, however, an inhibition ellipse may be observed at each end of the strip (Figure 4).

Testing must be done with at least **both CT/CTL and TZ/TZL strips**. The presence of ESBL is confirmed by the appearance of a phantom zone or deformation of the CT or TZ ellipse (**READING AND INTERPRETATION**, Figures 5 and 6) or when either the MIC of CT or TZ is reduced by ≥ 3 log_2 dilutions in the presence of clavulanic acid.



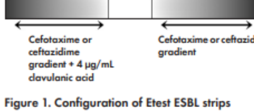


Figure 1. Configuration of Etest ESBL strips

REAGENTS

- 100 or 30 reagent units of either Etest CT/CTL or TZ/TZL
- 1 package insert

STORAGE

All unopened packages and unused Etest ESBL strips must be stored at -20 °C or the temperature denoted on the package until the given expiry date. Unused strips must be stored in an airtight storage container with colour indicating desiccant. The batch number and expiry date should be clearly marked on the package and/or storage container.

Protect Etest ESBL strips from moisture, heat and direct exposure to strong light at all times.

Prevent moisture from penetrating into or forming within the package or storage container. Etest ESBL strips must be kept dry.

HANDLING

Before using Etest ESBL strips from an unopened package, visually inspect to ensure the package is intact. Do not use the strips if the package has been damaged.

When removed from the -20 °C freezer, allow the package or storage container to equilibrate to room temperature for about 30 minutes. Moisture condensing on the outer surface must evaporate completely before opening the package.

Open the package according to the instructions. When handling Etest ESBL strips manually, grip only the strip at the area Etest ESBL. **Do not touch the surface of the strip with the antibiotic gradient i.e. the side opposite the scale.** Strips can be placed in an applicator try until ready to use (Figure 2). The vacuum pen Nema C88™ (AB BIODISK) can be used to efficiently apply the strips to the agar surface.

PRECAUTIONS AND WARNINGS

- Etest ESBL is intended for *in vitro* diagnostic use only.
- Although the procedure is straightforward, proper use of the system requires the judgement of skilled personnel trained in microbiology and antimicrobial susceptibility testing.
- Etest ESBL should be used strictly in accordance with the procedures described herein.
- Aseptic procedures should be used at all times when handling bacterial specimens and established precautions against microbiological hazards strictly adhered to. Agar plates should be sterilised after use, before discarding.
- Occasionally, static electricity can cause two or more strips to stick together. Make sure that you separate the strips and apply only one at a time onto the agar surface.
- Due to the instantaneous release of antibiotic, Etest ESBL strips cannot be moved once in contact with the agar surface.
- Please consult Etest references and technical guides (www.abbiodisk.com), and read the package insert thoroughly before using Etest ESBL for the first time.

PROCEDURE

Materials required but not provided:

- Mueller Hinton agar plates (depth of 4 ± 0.5 mm)
- Sterile saline (0.85% NaCl)
- Sterile loops, swabs (not too tightly spun), test tubes, pipettes and scissors
- Forceps or Etest manual applicator or Biotools™ (Retro C80™, Nema C88, AB BIODISK)
- 0.5 McFarland turbidity standard
- Incubator (35 ± 2 °C)
- Quality control organisms
- Storage container with desiccant
- Additional technical information from www.abbiodisk.com/ Etest Technical Manual

Agar medium

Ensure that the agar depth is 4.0 ± 0.5 mm, pH 7.3 ± 0.1 and results fulfil specifications (**QUALITY CONTROL**, Table 2).

Inoculum preparation

Emulsify several well-isolated colonies from an overnight agar plate in saline to achieve a turbidity equivalent to a 0.5 McFarland standard. When the inoculum is correct, a confluent or almost confluent lawn of growth will be obtained after incubation. Perform regular colony counts to **verify that your procedure gives the correct inoculum density in terms of CFU/mL.**

Note:

As the ESBL amount is inoculum dependent, too heavy or too light an inoculum may affect results. Excess enzyme may quench the clavulanic acid component in the test and potentially reduce the MIC ratio of CT/CTL or TZ/TZL and give a false negative result. On the contrary, too little enzyme may give a lower MIC for CT or TZ, and reduce the CT/CTL and TZ/TZL ratio.

Inoculation

Dip a sterile, non-toxic swab into the inoculum suspension. Remove excess fluid by pressing the swab against the inside wall of the test tube. Swab the entire agar surface three times, rotating the plate approximately 60 degrees each time to ensure an even distribution of inoculum. Alternatively, use Retro C80 (rota-plater) to efficiently streak the inoculum over the agar surface. Allow excess moisture to be absorbed for about 15 minutes so that the **surface is completely dry** before applying Etest ESBL strips.

Application

Check that the inoculated agar surface is **completely dry** before applying Etest ESBL strips. Open the package and handle the strips as described under **HANDLING**. Etest ESBL strips can be applied to the inoculated agar surface with a pair of forceps, a manual Etest applicator, or Nema C88 (Figure 3). Always place the strip on the agar with the MIC scale facing upward i.e. towards the opening of the plate, and the antibiotic gradient on the agar surface. If incorrectly placed upside down, no ellipse will form because the antibiotic cannot diffuse across the non-porous plastic strip.

Ensure the whole length of the strip is in complete contact with the agar surface. If necessary, remove air pockets by pressing gently on the strip with forceps, always moving from the lowest concentration upwards. Small bubbles under the strip will not affect results. Once applied, the strip cannot be moved as the antibiotic is instantaneously released into the agar.

Incubation

Incubate the agar plates in an inverted position at 35 ± 2 °C for 16-20 hours in ambient atmosphere.

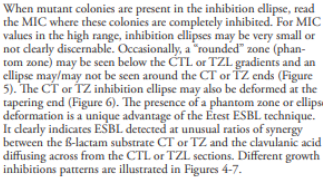


Figure 2. ESBL strips in an Etest Applicator tray.

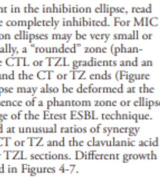


Figure 3. Applying Etest strips with a manual Etest Applicator

READING AND INTERPRETATION

Reading

When bacterial growth is visible, read the CT, CTL, TZ and TZL MIC values where the respective inhibition ellipses intersect the strips (Figure 4). Growth along the entire gradient i.e. no inhibition ellipse indicates that the MIC is greater than or equal to (≥) the highest value on the reading scale. An inhibition ellipse below the gradient indicates a MIC less than (<) the lowest value on the scale.

When mutant colonies are present in the inhibition ellipse, read the MIC where these colonies are completely inhibited. For MIC values in the high range, inhibition ellipses may be very small or not clearly discernable. Occasionally, a "rounded" zone (phantom zone) may be seen below the CTL or TZL gradients and an ellipse may/may not be seen around the CT or TZ ends (Figure 5). The CT or TZ inhibition ellipse may also be deformed at the tapering end (Figure 6). The presence of a phantom zone or ellipse deformation is a unique advantage of the Etest ESBL technique. It clearly indicates ESBL detected at unusual ratios of synergy between the ß-lactam substrate CT or TZ and the clavulanic acid diffusing across from the CTL or TZL sections. Different growth inhibitions patterns are illustrated in Figures 4-7.

Different growth-inhibition patterns:

Figure 4. Clear cut ESBL positive: MIC CT/CTL =1.5/0.047 =32

Figure 5. A "rounded" phantom inhibition zone below CT indicative of ESBL.

Figure 6. Deformation of the TZ inhibition ellipse indicative of ESBL.

Figure 7. When MIC values are above the test ranges, result is Non-Determinable (ND).

Interpretation

Table 1: Guidelines for interpretation of Etest ESBL

ESBL	MIC Ratio	Reporting
Positive	CT ≥0.5 and CT/CTL ≥8 OR TZ ≥1 and TZ/TZL ≥8 OR "Phantom" zone or deformation of the CT or TZ ellipse.	ESBL producer and resistant to all penicillins, cephalosporins and aztreonam (CLSI M100-S series).
Negative	CT <0.5 or CT/CTL <8 AND TZ <1 or TZ/TZL <8	ESBL non-producer and report actual MICs of relevant drugs as determined by a MIC method.
Non-determinable (ND)	CT >16 and CTL >1 AND TZ >32 and TZL >4 OR When one strip is ESBL negative and the other ND.	ESBL non-determinable and report actual MICs of all relevant drugs as determined by a MIC method. If ESBL is suspected, confirm results with genotyping.

Examples of how to interpret MIC ratios:

CT/CTL 8/0.125 = 64 = ESBL +
TZ/TZL >32/<0.064 = >500 = ESBL +
CT/CTL 1/<0.016 = >62 = ESBL +
CT/CTL 4/>1 = <4 = ESBL -
TZ/TZL 0.5/0.25 = 2 = ESBL -
CT/CTL 0.25/0.19 = 1.3 = ESBL -
TZ/TZL 1/4[1] = 0.25 = ESBL -
TZ/TZL >32/>4 = out of range = ND [2]
CT/CTL ESBL negative and TZ/TZL ND = ND [3]

Notes:

1) When MICs of CTL or TZL are higher than CT or TZ respectively, it may reflect the induction of ß-lactamase production by clavulanic acid.
2) When both MIC values are above the test ranges, the result is ND = Non-determinable. This may suggest the presence of IRT (inhibitor resistant TEM) or AmpC enzymes or that MIC values are outside the test device range.
3) When one result is ESBL negative and the other ND, the interpretation for the strain should be ND.

QUALITY CONTROL

Quality control strains should be tested by the method as outlined under **PROCEDURE** to check the quality of the reagents and the ability of the test to confirm ESBL production. The expected MIC values and interpretation for control strains are provided in Table 2. *E. coli* ATCC® 35218 produces TEM-1 ß-lactamase (non-ESBL) and serves as a negative control for the test. *K. pneumoniae* ATCC 700603, an ESBL positive genotype, provides on-scale MIC values and serves as a positive control, either by MIC ratio criteria or the deformed ellipse phenomenon. Careful attention should be given to maintenance and storage of *K. pneumoniae* ATCC 700603 as spontaneous loss of the plasmid encoding the ESBL has been documented and may give QC results outside the acceptable limits. When CTL or TZL MIC values are above specifications, this may imply either degradation of the clavulanic acid or excessively high inoculum. Check the storage and handling of strips and repeat the test using the correct inoculum.

Table 2. Quality control specifications for Etest ESBL CT/CTL and TZ/TZL strips.

Strain	MIC (µg/mL) Cefotaxime (CT)	Cefotaxime + clavulanic acid (CTL)	ESBL Interpretation[1]
E. coli ATCC 35218	≤0.25[2]	0.016 - 0.064	Negative
K. pneumoniae ATCC 700603	1 - 4[3]	0.125 - 1	Positive
Strain	**Ceftazidime (TZ)**	**Ceftazidime + clavulanic acid (TZL)**	**ESBL Interpretation[1]**
E. coli ATCC 35218	≤0.5[2]	≤0.064[2]	Negative
K. pneumoniae ATCC 700603	8 - ≥32	0.125 - 0.5	Positive

Notes:
1) See **READING AND INTERPRETATION**.
2) MIC value below the strip range.
3) MIC ratio may be ≤ 8 but deformation of the CT ellipse is indicative of ESBL.

PERFORMANCE CHARACTERISTICS

Several *in vitro* studies have compared the performance of Etest ESBL CT/CTL and TZ/TZL strips to ESBL genotype characterisation and/or the CLSI agar dilution method and confirmatory criteria for ESBL. In an FDA criteria study (Bolmström *et al.*), Etest detected ESBL enzymes produced by a total of 73 genotypically characterised strains in studies performed at different independent sites. Comparison of Etest performance to the CLSI method based on 479 strains comprising clinical and challenge organisms tested at three independent sites (328 positive ESBL phenotypes and 151 negative controls) is summarised in Table 3.

Table 3. Etest ESBL CT/CTL and TZ/TZL performance compared to CLSI reference method.

Agreement N (%)	CLSI ESBL+	CLSI ESBL-
Etest ESBL +	324 (99)	0
Etest ESBL -	0	144 (95)
Etest ND	4 (1)	7 (5)

LIMITATIONS
1. Inhibitor resistant TEM (IRT) enzymes cannot be detected by Etest ESBL strips.
2. An ESBL negative result with elevated MICs to CT/TL and TZ/TZL may be due to an IRT, AmpC or an ESBL masked by the concurrent presence of these enzymes and/or other resistance mechanisms.
3. Strains showing non-determinable (ND) results should be further investigated by genotyping.
4. Performance of Etest ESBL is based on the use of at least both TZ/TZL and CT/CTL strips simultaneously. The use of only one Etest ESBL strip to confirm the presence of ESBL is not valid.

REFERENCES AND BIBLIOGRAPHY

1. Cormican M.G. *et al.* (1996). Detection of extended spectrum ß-lactamases (ESBL) producing strains by the Etest ESBL screen. *Journal of Clinical Microbiology (JCM)*. **34**(8): 1880-1884.
2. Gales A.C. *et al.* (1997). Antimicrobial susceptibility of *Klebsiella pneumoniae* producing extended spectrum ß-lactamase (ESBL) isolated in hospitals in Brazil. *Brazilian Journal of Infectious Disease*. 1(4): 196-203.
3. Bolmström A. *et al.* (1999). Validation of new Etest ESBL strips for the detection and confirmation of strains producing extended spectrum ß-lactamases (ESBL): a trial using FDA criteria for susceptibility testing devices. Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), poster 898.
4. Nas Y. *et al.* (1999). Detection of extended spectrum ß-lactamases in *E. coli* and *K. pneumoniae*. *JCM*. **11**(2): 103-106.
5. CLSI M7-A7, January 2006. *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically.*
6. CLSI M100-S series, latest edition.

WARRANTY AND DISCLAIMER

EXPRESS LIMITED WARRANTY AND DISCLAIMER

AB BIODISK expressly warrants that Etest ESBL CT/CTL, TZ/TZL and PM/PML will detect clavulanic acid inhibitable Extended-Spectrum Beta-Lactamases (ESBLs), if the procedures, precautions and limitations indicated in this package insert are strictly complied with. If the Etest ESBL strip does not do so, AB BIODISK shall refund the cost of the product or replace the defective test strips.

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Etest® ESBL

For *In Vitro* Confirmation of ESBL

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