

Antimicrobial resistance diagnostics are used to measure the susceptibility of bacteria to a range of antibiotics. Resistance of bacteria against antibiotics plays an increasingly important role e. g. in livestock farming as well as in other fields of veterinary medicine. To be able to choose optimal treatment knowing the resistance level of the responsible bacterial pathogens is essential. In addition, the use of antibiotics in veterinary medicine is becoming increasingly regulated in Germany. Through the Ordinance on Veterinary Pharmacies (TÄHAV) 2018 e. g. there is an obligation to test for resistance in many cases.

At IVD GmbH, resistance testing is carried out using two different test methods:
agar diffusion method & micro dilution method (microbroth).

For the **agar diffusion method** at first agar plates are inoculated with the microorganism of interest. Then, small paper discs containing different antimicrobial compounds are placed on the inoculated agar. The antibiotic substances diffuse into the culture medium and create concentration gradients around the paper discs. Therefore, the growth of microbes sensitive to the tested antimicrobials is inhibited around the paper discs. The so called 'zones of inhibition' are measured and rated according to their respective sizes as 'sensitive', 'intermediate' or 'resistant'.

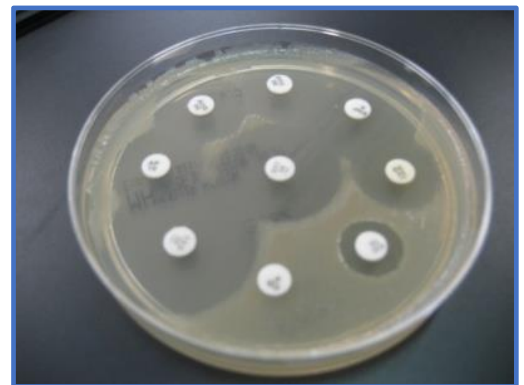


Figure 1: Agar diffusion test with *E. coli*

For **micro dilution method** 96 well plates are commonly used. Two-fold serial dilutions of antimicrobial compounds in liquid growth medium are created and inoculated with the microorganism in question. After incubation the minimum inhibitory concentration (MIC) of different antimicrobial compounds is determined. MIC is defined as the lowest concentration of an antimicrobial at which no bacterial growth can be detected in vitro. For our reports MICs are also rated as 'sensitive', 'intermediate' or 'resistant'. Additionally, reports include the MIC values themselves.

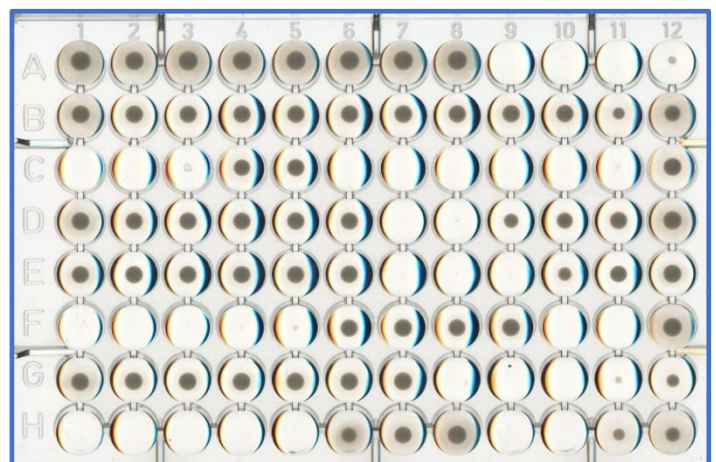


Figure 2: Microdilution method with *E. coli*

In principle, both methods can be used for most pathogens. There are exceptions, however, as some pathogens can only be tested with the microbroth dilution method:

- Anaerobic pathogens (e. g. *Clostridium perfringens*, *Fusobacterium species*, *Bacteroides species*)
- Slow growing bacteria (e. g. *Corynebacterium species*, *Schaalia hyovaginalis*, *Trueperella species*, *Helcococcus species*)

At IVD GmbH the resistance tests are carried out and interpreted in accordance with the **Clinical and Laboratory Standards Institute (CLSI)**. CLSI is an US-American organization that defines methods for conducting resistance testing and determining MIC values and zones of inhibition specifically for veterinary pathogens.

Important: Both methods involve combinations of pathogens and antibiotic active ingredients for which there are no limit values (sensitive - intermediate - resistant) defined by the CLSI.

The following antibiotic substances are currently being tested at IVD GmbH:

Antibiotic compound	Class of antibiotics	Agar diffusion method	Microdilution method
Amoxicillin/Clavulanic acid	Aminopenicillins/ β -Laktamase-inhibitors	x	x
Ampicillin	Aminopenicillins	x	x
Apramycin	Aminoglycosides	x	
Cefquinome	4th generation Cephalosporins	x	
Ceftiofur	3rd generation Cephalosporins	x	x
Clindamycin	Lincosamides	x	
Colistin	Polypeptide antibiotics/polymyxins	x	x
Enrofloxacin	Fluoroquinolones, gyrase inhibitors	x	x
Erythromycin	Makrolides		x
Florfenicol	Fluoroquinolones, gyrase inhibitors	x	x
Gamithromycin	Makrolides	x	x
Gentamicin	Aminoglycosides	x	x
Lincomycin/Neomycin	Lincosamide/Aminoglycosides	x	
Neomycin	Aminoglycosides	x	
Paromomycin	Aminoglycosides	x	
Penicillin	Penicillins	x	x
Spectinomycin	Aminoglycosides	x	
Tetracycline	Tetracyclines	x	x
Tiamulin	Pleuromutilins	x	x
Tildipirosin	Macrolides		x
Tilmicosin	Macrolides	x	x
Trimethoprim/Sulfonamide	Diaminopyrimidines/Sulfonamides	x	x
Tulathromycin	Macrolides	x	x
Tylosin	Macrolides	x	

What are the advantages and disadvantages of both methods?

The agar diffusion test is cheaper than the micro dilution method. However, there are less limit values defined by the CLSI for agar diffusion method (zones of inhibition) than for micro dilution method (MICs).

The micro dilution method is more expensive but for this test we include not only the interpretations (sensitive, intermediate, resistant) but also the test range and the MIC values in our reports. These are helpful clues if there are no defined limit values available for a certain antibiotic substance and you want to assess whether it could still be effective. The micro dilution method is generally considered to be more modern and recognized than the agar diffusion test

In general, IVD GmbH tests at least one representative from **each class of antibiotics**. Some of the interpretations can be transferred to other antibiotic substances in the same class of antibiotics.

Here are some examples:

- The interpretation of ampicillin can be transferred to amoxicillin.
- The interpretation of enrofloxacin can be transferred to danofloxacin/difloxacin/marbofloxacin.
- The interpretation of erythromycin can be transferred to spiramycin/tylosin.
- The interpretation of tetracycline can be transferred to chlortetracycline/oxytetracycline/doxycycline.

Additional active ingredients can be tested upon request.

The bacteriology team will be happy to answer any questions you may have about resistance testing:

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