Antimicrobial Susceptibility Testing

How to interpret your VITEK antimicrobial susceptibility test report

Antimicrobial susceptibility testing in the UCDVH diagnostic laboratory is now carried out using a VITEK machine. The advantages of this system compared to the disc diffusion method previously performed in the laboratory are:

- The bacteria are tested against a much wider range of antimicrobial agents (previously the standard number tested was 6 compared to approximately 20 currently).
- The Minimum Inhibitory Concentration (MIC) data generated allows the clinician to select the most effective agent where a number of antimicrobial agents show activity against the bacterial pathogen isolated.

When you receive your report by email, there will be two attachments: the usual Filemaker report that you have always got, which contains basic information on the organisms isolated and their antimicrobial susceptibilities and another 2-page VITEK file giving additional susceptibility and background information.

1. How do I interpret the antimicrobial susceptibility results in the VITEK report?

- An example report for a dog is given below; it usually consists of 2 pages, the first page containing some notes and a second page containing the results. A detailed explanation of the contents of each section is presented

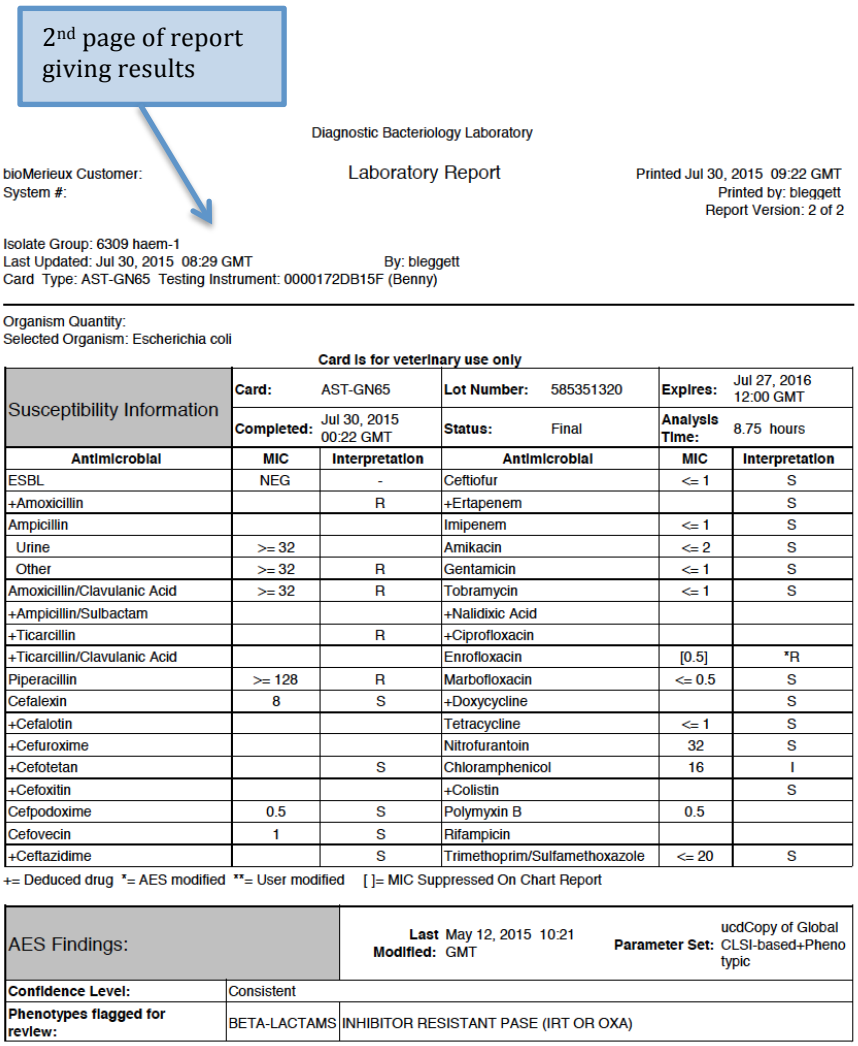
Page I of the report gives notes for the laboratory staff on interpretation of the results and does not usually concern the client. In this example the user is alerted to the fact that the clinical breakpoints (see page 3 of this document for an explanation of breakpoints) determining susceptibility to gentamicin are different in dogs and horses from those used in other species. In dogs and horses, *E. coli* with an MIC of ≥8 µg/ml for gentamicin is deemed to be resistant (see table 2) whereas *E. coli* isolated from other animal species are deemed to be resistant at the higher concentration of ≥16 µg/ml.

In cases where the notes are of importance for the client, laboratory staff will take this information into account and modify the report on page 2 for you to reflect the recommendations in the comments box.

Selected Organism: Escherichia coli

Comments:	**Gentamicin BP for Dog + Horse only, other sample BP <=4, 8,>=16 **Ampicillin BP for Dogs only, other GNB BP <=8, 16,>=32 **Cefalotin BP for Dogs only, other BP <=8, 16, >=32 **Cefazolin BP for Dogs + Horse only, GNB BP <=8, 16 >=32 **Enrofloxacin BP of <=0.25, 0.5-1, >=2 Bovine-Mannhaemia, for Chickien-E. coli, Bovine/Chicken-Pasteurella

Identification Information			
Selected Organism	Escherichia coli		
	Entered: Jul 30, 2015 08:29 GMT	**By:**	bleggett
Analysis Messages: The following antibiotic(s) are not claimed: Rifampicin,			



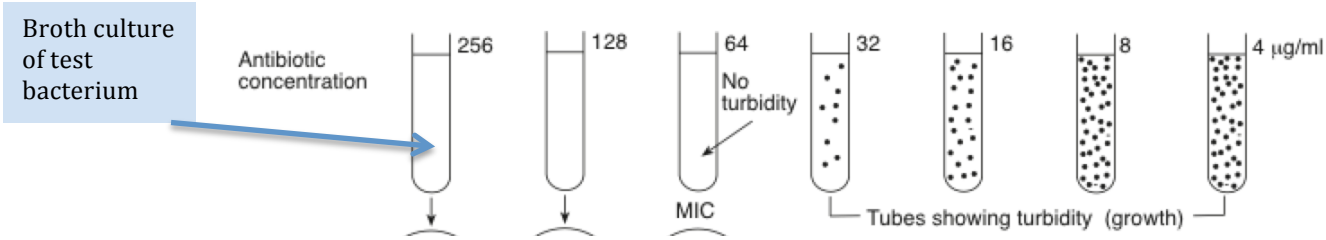
Page 2 of the results gives the list of antibiotics tested; these will vary depending on whether the organism is Gram-negative as in this example, Gram-positive or a *Streptococcus* (although streptococci are Gram-positive, a dedicated card is used for testing this group of organisms). The MIC of the organism tested against each antimicrobial is listed, together with the interpretation, R (resistant), I (intermediately susceptible) or S (susceptible).

In relation to clinical outcomes organisms categorised as susceptible should respond to therapy while resistant organisms should not

Some organisms will not have a result for some of the antimicrobials listed. This may be because testing is not relevant for that particular organism/antimicrobial combination. Or, the interpretation 'R' may be listed without any accompanying MIC. This is usually because the organism is intrinsically resistant to that particular agent (see Table 1 for list of intrinsic resistance attributes of common animal pathogens).

Utilisation of Minimum Inhibitory Concentration Values.

2. **What is the minimum inhibitory concentration?**
   The minimum inhibitory concentration (MIC) of an organism is the lowest concentration of an antimicrobial that will inhibit growth of that organism.



The MIC of the organism in this example is 64µg/ml of the antibiotic in question.

3. **What is the relationship between MIC and clinical resistance?**
   MIC alone does not determine the effectiveness of an antibiotic in a clinical case
   Clinical breakpoints are calculated to determine if an isolate is clinically susceptible, intermediate or resistant and are based on:
   - MIC distribution in a bacterial population; the MIC90 is the concentration that will inhibit growth of 90% of a particular species of organism (Pharmacodynamic criteria)
   - Achievable drug concentration in plasma or tissue; Cmax (Pharmacokinetic criteria)

   Clinical breakpoints are set by organisations such as the Clinical Laboratory Standards Institute (CLSI)(USA) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Clinical breakpoints for some agents commonly used in animals are listed in Tables 2 to 4.

4. **How do I select the antimicrobial agent most likely to be effective based on the MIC data provided?**
   The actual MIC given on the report can be compared to the clinical breakpoint (set by CLSI or EUCAST as explained above) for each agent
   If the MIC of the test organism is greater or equal to the clinical breakpoint for a particular antimicrobial, the organism is deemed to be clinically resistant to that agent. If the MIC of the organism is less than the clinical breakpoint for a particular antimicrobial, the organism is deemed to be clinically susceptible to that agent.
   If there is more than one agent to which the organism is susceptible and the agents are licensed for use and available for the animal you wish to treat, you can use the MIC to help decide which is likely to be most effective antibiotic in the clinical case. The following example shows how MIC values can be used to give an indication of the relative potency of different antimicrobial agents to which an organism is susceptible:

	Escherichia coli	
	MIC (µg/ml) (from example report above)	MIC BP (µg/ml) (from Table 2)
Tetracycline/doxycycline	≤ 1	≥ 16
Cephalexin	= 8	≥ 64
Marbofloxacin	≤ 0.5	≥ 4

Although E. coli is susceptible to all three drugs in the above example, tetracycline or marbofloxacin are **more potent** against *E. coli* than cephalexin as both have a lower MIC against *E. coli* than cephalexin.

Furthermore, tetracycline may be more effective than marbofloxacin as there is a 16-fold difference between the MIC and the clinical breakpoint compared to an 8-fold difference for marbofloxacin. [Note: MICs are tested using doubling dilutions of the antimicrobial in question, i.e. 0.5, 1,2,4,8,16, and so on].

Although such comparisons are overly simplistic as they do not account for pharmacokinetics and other factors, they can be useful as a guide to antimicrobial choice.

Another factor which may be important in determining antibiotic choice is the route of excretion. For example, if an antibiotic is concentrated in the urine during excretion, it may be effective for treating urinary infections *in vivo* even though the *in vitro* result indicates intermediate susceptibility. This is because the drug accumulates in urine to levels well above those achieved in plasma.

5. How can I calculate the ideal dose of an antimicrobial using pharmacodynamic and pharmacokinetic data?

In some circumstances it may be advantageous to calculate the dosage of an antimicrobial agent rather than using the recommended dosage on the datasheet.

The dose can be calculated using the following formula

Dose = C_{max} x V_d/F (mg/kg.day)

Where C_{max} is the maximum concentration achieved (if C_{max} is not available, it can be calculated as MICx$2^{\text{dosing interval/half life}}$)

V_d is the volume distribution

F = systemic availability, the fraction of the drug that reaches the blood unchanged.

The above data are available on the data sheets of many of the newer antimicrobial agents but are less readily available for older agents. A summary of some of these data for selected agents is given in Tables 2 to 4 below on a species basis. Unfortunately, comprehensive data are not available in certain cases, in particular for farm animals.

Example calculation:

To calculate the dose of oral doxycycline for treatment of the *E. coli* infection in the above example:

Dose = C_{max} x V_d/F mg/kg/day

C_{max} 4.5mg/ml (from data sheet)
V_d = 1.5 (from Table 2)
F = 0.45 (from Table 2)

Dose = 4.5 x 1.5/0.45
= 15mg/kg/day

Alternatively, the following calculation could be used:

Dose = $\mathbf{MICx2^{dosing\ interval/half\ life}\ x\ V_d/F}$

MIC = 1 (from example report)
Dosing interval = once a day (from datasheet)
Half-life = 7.8 (from Table 2)
V_d = 1.5 (from Table 2)
F = 0.45 (from Table 2)
Dose = $1x2^{1/7.8}$ x 1.5/0.45
= 1 x 1.092 x (1.5/0.45)
= 3.64 mg/kg/day

The dosage calculated is different depending on which formula is used. This is likely to be because the values for the different parameters are mean values derived from many different experimental studies and references. The much lower value in the second example is because the actual MIC value of doxycycline against the *E. coli* isolate is used and in this case the MIC value is particularly low. Thus a lower dose rate is likely to be effective.

NB. The dosage given on the product data sheet is 10mg/kg/day. It must be remembered that any change in dosage from that given on the datasheet based on these calculations represents off-label use and must be justifiable as such.

6. What are time- versus concentration-dependent antimicrobials?

Once an antimicrobial has reached and bound to its site of action in the bacterium, the two major determinants of inactivation of the organism are the *concentration* and the *time* that the antimicrobial remains on the binding sites.

Time-dependent:

For some classes of antimicrobials time is more critical (beta-lactams, macrolides, clindamycin) and these are classified as 'time-dependent antimicrobials. For these antimicrobials efficacy is enhanced if the concentration in the body remains above the MIC for most (at least 50%) of the dosing interval. Increasing the dose may be beneficial but shortening the dose interval is usually more effective, especially if the drug has a short half-life.

Concentration dependent:

Antimicrobials for which concentration is more critical (fluoroquinolones and aminoglycosides) are classified as 'concentration-dependent' antimicrobials. The efficacy of these drugs is best predicted by the ratio of the maximum drug concentration

(C_{max}) to the MIC. This ratio should be at least between 8:1 and 10:1. These drugs can usually be administered at longer dosing intervals.

Some antimicrobial agents, such as the tetracyclines, have features of both time and concentration-dependent killing.

Reference:

Plumb's veterinary drug handbook / Donald C. Plumb
John Wiley & Sons, Inc. | 2015 | 8th edition.

Table 1. Intrinsic resistance of veterinary pathogens against selected veterinary antimicrobial agents.

Organism	Resistant to:
Enterobacteriaceae	Benzylpenicillin, macrolides, lincosamides, rifampicin, fusidic acid
Proteus spp.	Resistant to all of above plus tetracyclines and Polymixin B/colistin. *Proteus vulgaris* is also resistant to ampicillin and first /second generation cephalosporins
Acinetobacter baumannii	Benzylpenicillin, ampicillin, many cephalosporins, macrolides, lincosamides, rifampicin, trimethoprim, fusidic acid
Burkholderia cepacia	Benzylpenicillin, ampicillin, amoxicillin clavulanate, 1st generation cephalosporins, macrolides, lincosamides, rifampicin, ciprofloxacin, aminoglycosides, trimethoprim, Polymixin B/colistin, fusidic acid
Pseudomonas aeruginosa	Benzylpenicillin, ampicillin, amoxicillin clavulanate, cephalosporins, macrolides, lincosamides, rifampicin, kanamycin and neomycin, trimethoprim-sulphamethoxazole, fusidic acid, chloramphenicol
Campylobacter species	Lincosamides, trimethoprim
Staphylococci	Polymixin B/Colistin
Streptococci	Polymixin B/Colistin, low level resistance to aminoglycosides
Enterococci	Fusidic acid, Polymixin B/Colistin, cephalosporins, low level resistance to aminoglycosides, erythromycin, clindamycin, sulphonamides
Listeria monocytogenes	Cephalosporins

Table 2. MIC Breakpoints, Volume of distribution (V_d), Systemic availability (F) and half-life ($T_{½}$) of selected antimicrobial agents used in dogs and cats.

Animal species/Agent	MIC BP	V_d (L/kg)	F	$T_{½}$ (hrs)
Dogs and Cats				
Ampicillin Skin infections Other infections	 ≥0.5 (*S. pseudintermedius*) ≥1 (*E. coli*) ≥2 (*Bordetella*) ≥8 *(E. coli* - urine) ≥16 (Enterococci) ≥32 (Other Gram negs)	0.3 (dogs) 0.17 (cats)	0.5 (after oral admin)	0.75-1.33
Amoxicillin-clavulanic acid Staph Other Gram neg. organisms	≥8 ≥32	0.2	0.75 (after oral admin)	1.5 (dog) 1 – 2 (cat)
Cephalexin	≥64	NA*	0.75	1-2
Cephalothin Skin and soft tissue *E. coli* Other	 ≥8 ≥8 ≥32	NA	NA	NA
Cefovecin	≥8	0.12 (dog) 0.09 (cat)	1.0	133 (dog) 166 (cat)
Cefpodoxime	≥8	0.15	0.63	3-6
Clindamycin	≥4	0.9 (dog) 1.6-3 (cat)	0.73 (after oral admin, dog)	2-5 (after oral, dog) 10-13 (after sc inj, dog) 16 (cat, oral capsules)
Colistin	≥4	NA	NA	NA
Erythromycin	≥1 (Strep) ≥8 (other)	2(dog) 2.3(cat)		1-1.5
Enrofloxacin	≥4	3-4	0.8	4-5 (dog) 6 (cat)
Gentamicin	≥8 (*Enterobacteriaceae, Pseudomonas*) ≥16 (Staph, other Gram neg)	0.15-0.3	0.9 (i.m. or sc)	0.5-1.5
Marbofloxacin	≥4	1.2-1.9	0.94	9-12 (dog) 13(cat)
Doxycycline	≥16 ≥4 (Beta Strep)	1.5	0.9-1 (inj) 0.45 after oral admin	7.8 (dog) 5.8 (cat)
Trimethoprim-sulfamethoxazole	≥80 (4/76)	1.5	NA	2.5 (trimeth) 9.8 (sulfa)

*NA = not available

Table 3. MIC Breakpoints, Volume of distribution (V_d), Systemic availability (F) and half-life ($T_{½}$) of selected antimicrobial agents used in horses

Animal species/Agent	MIC BP	V_d (L/kg)	F	$T_{½}$ (hrs)
Horses				
Amikacin	≥64	NA*	NA	NA
Ampicillin	≥2 (*Bordetella*) ≥8 *(E. coli* - urine) ≥16 (Enterococci) ≥32 (Other Gram negs)	NA	NA	NA
Ceftiofur	≥8	NA	NA	NA
Erythromycin	≥1 (Strep) ≥8 (other)	2.3 (mare) 3.7-7.2 (foal)	NA	1-1.2
Enrofloxacin	≥4	1.25	0.6-0.8	5-10
Florfenicol	Not available (≥8 cattle and pigs resp dis, CLSI)	NA	NA	NA
Gentamicin	≥8 (*Enterobacteriaceae, Pseudomonas*) ≥16 (Staph, other Gram neg)	0.26-0.58	NA	1.8-3.2
Marbofloxacin	≥4	NA	NA	NA
Penicillin	≥2	NA	NA	<1
Tetracycline	≥16 ≥8 (Beta Strep)	NA	0.6-0.8	NA
Trimethoprim-sulfamethoxazole	≥80 (4/76)	0.6-1.5	0.74(sulfa) 0.46 (trim)	2.7 (sulfa) 1.9-3 (trim)

*NA = not available

Table 4. MIC Breakpoints, Volume of distribution (V_d), Systemic availability (F) and half-life ($T_{½}$) of selected antimicrobial agents used in cattle.

Animal species/Agent	MIC BP	V_d (L/kg)	F	$T_{½}$ (hrs)
Cattle				
Amikacin	≥64	NA	NA	NA
Ampicillin	≥8 (Streps) ≥16 (Enterococci) ≥32 (Gram negs)	0.16-0.5	NA	NA
Ceftiofur	≥8	0.3	1 (goats)	8-12
Erythromycin	≥1 (Strep) ≥8 (other)	0.8-1.6	0.4 (sc) 0.65 (i.m)	2.5
Enrofloxacin	≥2 (bovine resp. disease) ≥4 (other)	1.5	0.65-0.75 (sheep)	1.5-4.5 (sheep)
Florfenicol	≥8 (bovine resp. disease)	0.7	0.8	18
Gentamicin	≥16	NA	NA	2.2-2.7 (calves) 1.8 (cows)
Marbofloxacin	≥2 (bovine resp. disease) ≥4 (other)	NA	1	5-9 (calves) 4-7 (adult)
Penicillin	≥1 (bovine resp. disease)	NA	NA	<1
Tetracycline	≥8 (bovine resp. disease) ≥16 (other)	1-2.5	NA	NA
Trimethoprim-sulfamethoxazole	≥80 (4/76)	NA	NA	2.5 (sulfa) 1.5 (trim)

*NA = not available