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Animal Health Perspectives

Not all antimicrobial susceptibility tests are created equal

Practical considerations and clinical implications of different antimicrobial susceptibility testing methods

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WHAT'S INSIDE

The prudent use of antimicrobials for animal health and animal agriculture is a hot topic nowadays – we are facing an unprecedented challenge to human and animal health due to increasing antimicrobial resistance in bacteria. Old antimicrobials are not working the way they used to and there is minimal new inventory in the supply pipeline. The good news –the antimicrobials we have can still go a long way. All it takes is re-thinking some of our practices and learning a few rules of antimicrobial stewardship.

The golden rule of antimicrobial stewardship is to use the right drug at the right dose for the right amount of time. Antimicrobial susceptibility testing is critical for covering two out of three: What drug can we use and at what concentration will it work? That is where it can become quite complicated very quickly. There is more than one antimicrobial susceptibility test available – let us take a closer look and try to see the differences.

There is an old good (and cheap) disk diffusion method known as Kirby-Bauer. It is the most commonly offered antimicrobial susceptibility testing method – all it takes is an agar plate and a set of filter paper disks infused with different antimicrobials. When stuck to the agar, antimicrobial within the filter paper diffuses through the gel. The concentration of the diffused antimicrobial decreases as it gets further from the paper disk. Naturally, the tested bacteria will not grow close to the disc if it is sensitive to the antibiotic. By measuring the diameter of the growth inhibition we can gauge the degree of sensitivity (Figure 1). Laboratory clients receive a qualitative interpretation – bacteria are classified as "Sensitive"(S), "Intermediate" (I), or "Resistant"(R).

Figure 1.

FIGURE 1. Kirby-Bauer (agar diffusion) antimicrobial susceptibility test

This is a relatively low-tech and crude testing method (did I mention it is cheap?). It is quite difficult to standardize and compare the results between labs or even batches and next to impossible to know the exact drug concentration in the gel where bacteria stops growing. Most importantly, it is an experiment where bacteria are required to grow on a surface of agar – something dramatically different from a clinical case where bacteria live and grow in bodily tissue and fluids.

Next in line is a slightly more sophisticated gel diffusion method developed by BioMeriuex called eTest. It addresses the antimicrobial concentration issue to some extent by putting a gradient drug concentration on a testing strip (Figure 2); however, there is still this big question on how comparable the surface growth on agar is to real-life infection conditions.

Figure 2.

FIGURE 2. Etest antimicrobial susceptibility testing method (bioMerieux)

Then there is a plethora of proprietary automated methodologies (for example Vitek 2 from BioMerieux (Figure 3)). Naturally, each vendor claims "Trust me, we got it right!" however, these come with a premium price and not necessarily with full disclosure of all the technical details. These test results may be reported qualitatively (S, I, R) or sometimes a minimum inhibitory concentration (MIC) can be estimated.

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Finally, there is a method considered a "gold standard" of antimicrobial susceptibility testing (at least by Canadian and American AMR surveillance programs) – a serial microdilution technique (Figure 4).

For this test, a broth (or in some cases agar) is prepared with a known concentration of antimicrobial. The microorganism in question is inoculated into a series of tubes (or microplate wells) where the antimicrobial concentration increases twofold in each subsequent step. After a prescribed incubation period, observations are made as to which antimicrobial concentrations the tested microorganism can grow in. The lowest concentration that prevents the tested organism from growth is called the Minimum Inhibitory Concentration (MIC). It is quite a bit more work, but it replicates much closer the real-world encounter of the tested bug and drug in the patient's organism. The test result comes as a true MIC number (i.e. 4 ug/ml). When paired with information about the achievable drug concentration at the site of infection (the pharmacokinetic/pharmacodynamics relationship), the in vitro/in vivo correlation can be translated into effective dosing and improved clinical outcomes. In other words, the use of MIC's in the choice of drug and determination of dose regimen is as close as we can get to predicting whether the antibiotic in question will be effective against the tested microorganism in a specific clinical situation.

Prairie Diagnostic Services (PDS) has secured targeted funding under the Growing Forward 2 (GF2) program to provide a better level of antimicrobial susceptibility testing support to our veterinary practice and research clients. Historically, PDS has been offering only Kirby-Bauer antimicrobial susceptibility testing with qualitative interpretation (S, I, R); however, more and more of our clients are realizing that it is not sufficient – knowledge of true MIC is required to answer many clinical and research questions. Starting July, 2017, PDS now offers Serial Microdilution (Sensititre) antimicrobial susceptibility testing as a regular diagnostic service. There are two comprehensive antibiotic panels offered at this time – food animal (Figure 5) and companion animal (Figure 6).

This GF2 funding has also enabled PDS to enhance the professional support for antimicrobial susceptibility testing service – please contact Dr. Musangu Ngeleka (musangu.ngeleka@pds.usask.ca – diagnostic microbiology), Kazal Ghosh (kazal.ghosh@pds.usask.ca - antimicrobial resistance research), and Dr. Anatoliy Trokhymchuk (anatoliy.trokhymchuk@pds.usask.ca – data analysis and epidemiology) for assistance with your clinical and research needs and questions.

FIGURE 3. Vitek 2 antimicrobial susceptibility testing card (bioMerieux)

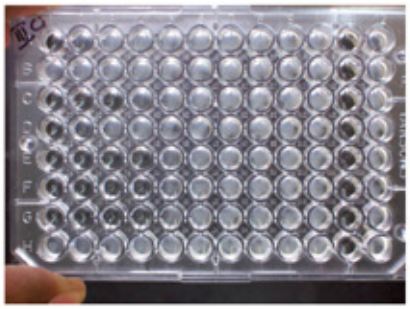

FIGURE 4. Serial microdilution antimicrobial susceptibility testing

FIGURE 5. Food animal serial microdilution antimicrobial susceptibility testing panel

TREK DIAGNOSTIC SYSTEMS

SENSITITRE CUSTOM PLATE FORMAT

Plate Code: BOPO6F

	1	2	3	4	5	6	7	8	9	10	11	12
A	TIO 8	TIA 32	CTET 8	OXY 8	PEN 8	AMP 16	DANO 1	SXT 2/38	TYLT 4	TUL 4	CLI 16	SDM 256
B	TIO 4	TIA 16	CTET 4	OXY 4	PEN 4	AMP 8	DANO 0.5	SPE 64	TYLT 2	TUL 2	CLI 8	ENRO 2
C	TIO 2	TIA 8	CTET 2	OXY 2	PEN 2	AMP 4	DANO 0.25	SPE 32	TYLT 1	TUL 1	CLI 4	ENRO 1
D	TIO 1	TIA 4	CTET 1	OXY 1	PEN 1	AMP 2	DANO 0.12	SPE 16	TYLT 0.5	TIL 64	CLI 2	ENRO 0.5
E	TIO 0.5	TIA 2	CTET 0.5	OXY 0.5	PEN 0.5	AMP 1	NEO 32	SPE 8	TUL 64	TIL 32	CLI 1	ENRO 0.25
F	TIO 0.25	TIA 1	TIA 0.5	GEN 16	PEN 0.25	AMP 0.5	NEO 16	TYLT 32	TUL 32	TIL 16	CLI 0.5	ENRO 0.12
G	GEN 8	GEN 4	GEN 2	GEN 1	PEN 0.12	AMP 0.25	NEO 8	TYLT 16	TUL 16	TIL 8	CLI 0.25	POS
H	FFN 8	FFN 4	FFN 2	FFN 1	FFN 0.5	FFN 0.25	NEO 4	TYLT 8	TUL 8	TIL 4	POS	POS

ANTIMICROBICS

TIO Ceftiofur
TIA Tiamulin
CTET Chlortetracycline
GEN Gentamicin
FFN Florfenicol
OXY Oxytetracycline
PEN Penicillin
AMP Ampicillin
DANO Danofloxacin
SDM Sulphadimethoxine
NEO Neomycin
SXT Trimethoprim / sulfamethoxazole
SPE Spectinomycin
TYLT Tylosin tartrate
TUL Tulathromycin
TIL Tilmicosin
CLI Clindamycin
ENRO Enrofloxacin

SENSITITRE CUSTOM PLATE FORMAT

TREK DIAGNOSTIC SYSTEMS

Plate Code: COMPAN1F

	1	2	3	4	5	6	7	8	9	10	11	12
A	AMP 0.25	AMP 0.5	AMP 1	AMP 2	AMP 4	AMP 8	AMP 16	OXA+ 0.25	OXA+ 0.5	OXA+ 1	OXA+ 2	OXA+ 4
B	AUG2 4/2	AUG2 8/4	AUG2 16/8	AUG2 32/16	AMI 4	AMI 8	AMI 16	AMI 32	FOX 2	FOX 4	FOX 8	FOX 16
C	TIC 8	TIC 16	TIC 32	TIC 64	POD 2	POD 4	POD 8	POD 16	TIM2 8/2	TIM2 16/2	TIM2 32/2	TIM2 64/2
D	SXT 0.5/9.5	SXT 1/19	SXT 2/38	FOV 0.25	FOV 0.5	FOV 1	FOV 2	FOV 4	FOV 8	FAZ 4	FAZ 8	FAZ 16
E	GEN 1	GEN 2	GEN 4	GEN 8	IMI 1	IMI 2	IMI 4	IMI 8	CLI 0.5	CLI 1	CLI 2	CLI 4
F	PEN 0.06	PEN 0.12	PEN 0.25	PEN 0.5	PEN 1	PEN 2	PEN 4	PEN 8	DOX 2	DOX 4	DOX 8	POS
G	XNL 0.25	XNL 0.5	XNL 1	XNL 2	XNL 4	MAR 0.25	MAR 0.5	MAR 1	MAR 2	RIF 1	RIF 2	POS
H	ENRO 0.25	ENRO 0.5	ENRO 1	ENRO 2	ERY 0.5	ERY 1	ERY 2	ERY 4	CHL 4	CHL 8	CHL 16	POS

ANTIMICROBICS

AMP Ampicillin
AUG2 Amoxicillin / clavulanic acid 2:1 ratio
TIC Ticarcillin
SXT Trimethoprim / sulfamethoxazole
GEN Gentamicin
PEN Penicillin
XNL Ceftiofur
ENRO Enrofloxacin
FOV Cefovecin
AMI Amikacin
POD Cefpodoxime
IMI Imipenem
ERY Erythromycin
MAR Marbofloxacin
OXA+ Oxacillin+2%NaCl
FOX Cefoxitin
TIM2 Ticarcillin / clavulanic acid constant 2
CLI Clindamycin
DOX Doxycycline
CHL Chloramphenicol
FAZ Cefazolin
RIF Rifampin
POS Positive Control

FIGURE 6. Companion animal serial microdilution antimicrobial susceptibility testing panel

Lymphoid neoplasms: Planning for diagnostics "beyond the scope"

By: Ryan Dickinson, Clinical Pathologist, Dept of Veterinary Pathology, WCVM

As we all know, cytopathologic evaluation of smears prepared from fine needle aspiration (FNA) of solid masses, lymph nodes and organs is a powerful diagnostic tool that can lead to relatively quick, useful and clinically relevant answer such as a definitive diagnosis or a workable differential diagnoses list. One of the more common cytopathologic interpretations made by clinical pathologists is the diagnosis of a lymphoid neoplasm by evaluating smears from lymph nodes or various other affected organs, or from evaluation of a peripheral blood smear that contains circulating neoplastic cells. In many instances the cytologic picture is classic and unmistakable, leading to a rapid and definitive diagnosis. Less commonly there may not be classic features of a lymphoid neoplasm for a variety of reasons, including a mixed lymphoid population, limited yield of intact cells, limited yield of morphologically atypical cells, etc. In such cases, a clinical pathologist's evaluation of smears may not lead to a definitive diagnosis, though there may be sufficient concern based on the findings to warrant further investigation. There are then several options to consider depending on how well we are prepared to pursue with that "next diagnostic step".

In some cases, such as with limited yield of intact cells, a clinical pathologist may simply request that an additional FNA from the lesion of concern. In other instances, such as with a mixed population of lymphoid cells, the request may instead be for a biopsy of a lymph node or other tissue where architectural evaluation +/- immunohistochemical staining may further aid in the diagnosis. Other techniques that are becoming more frequently used in recent years include the use of polymerase chain reaction (PCR) to detect clonality lymphocyte antigen receptor rearrangement from the genetic material in a lymphocyte population (otherwise referred to as "PARR"), or flow cytometric evaluation of surface markers on cells within peripheral blood or body cavity fluids. In each of these scenarios, the attending clinician would often need to collect an additional sample (whether it is a "fresher" sample or a different type of sample) from the patient's lesion to submit for the appropriate testing method(s). This may or may not be feasible, depending on the given situation. Perhaps the patient lives quite a distance from the veterinary clinic and cannot visit frequently, precluding the ability to easily obtain additional sample(s). Perhaps it is a fractious patient that creates a situation where it is difficult or dangerous to collect a quality specimen. Or that sampling requires anesthesia and specialized equipment and personnel. Or perhaps the patient is now deceased and a fresh sample is simply no longer available. In any of these situations overlap with a clinician's initial suspicion that a lymphoid tumour is the underlying problem, there are some pre-emptive options.

When glass slides containing FNA smears are received by our laboratory, the typical expectation is that these smears were intended for cytopathologic evaluation only and the default is to stain the smears with modified-Wright's stain and then place a permanent glass coverslip on the smears to facilitate microscopic evaluation and subsequent archiving. This process works out very well for the grand majority of cases. However, there are instances where cytopathologic evaluation by the pathologist did not lead to a definitive diagnosis (possibly for some of the reasons listed above, though that is not a comprehensive list) and additional steps are required to make a diagnosis. A biopsy, if required, would obviously involve collection of a new and different sample. On the other hand, if

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PARR was a consideration because the goal was to highlight a clonal population in a low-cellular sample or mixed cellular sample, then additional sample collection may not be required, depending on how many slides were initially received and/or what the default decision was regarding how to process the slides. PARR can be performed on cells washed off of stained or unstained glass slides that contain the sample material, however once the smears have been coverslipped, they could no longer be used for this technique.

In a recent PDS clinical pathology quarterly meeting, staff and pathologists discussed this topic and posed the question: "How would this be best handled?" If a clinician suspects that a lesion may be lymphosarcoma, they could routinely set aside and label 3-5 unstained smears from the rest that are destined to be stained and evaluated at the laboratory. These 3-5 unstained smears could be kept and stored by the submitting clinician or they could be labeled as "extras" that could be used for additional diagnostics such as PARR and included concurrently with the unstained smears that were intended for cytopathologic evaluation. In this case, if the initial cytopathologic diagnosis was unequivocal for lymphosarcoma, then the additional slides would not be needed. If, however, the initial cytopathologic diagnosis was not definitive, the additional unstained slides would be immediately available should the clinician wish to proceed with PARR (currently a "send-out" test). This suggested option may not be necessary for every submission, however it could be a method used if procuring a specimen was, or is anticipated to become, problematic in the future. The great thing about air dried, unstained, protected smears is that there is typically no real urgency to run the additional tests from a sample quality standpoint… once the smears are prepared and protected in a slide container, there can be several days of delay before the genetic material begins to disintegrate.

Flow cytometry is a different story. With flow cytometry samples for peripheral blood or body cavity fluid samples, the volume of sample may not always be the limiting factor as there is often abundant sample to work with (though in some cases, limited sample volume can serve as an obstacle). In contrast to air-dried smears, cells within fluid samples can deteroriate much more rapidly, leading to altered surface expression of certain markers that are detected by antibody-antigen binding. Thus, time is the limited factor in most cases of "flow" evaluation, particularly since transport time must be taken into account. For this reason, it is important for clinicians to be aware that they may be contacted by the pathologist for authorization to pursue with flow cytometry, should it be indicated. If the clinician has already considered this as a potential diagnostic tool that might be performed and has already discussed with their client about the process and obtained their consent, then this could significantly reduce the time from when the specimen is received by PDS and when it is shipped to the laboratory that performs flow cytometry, which is sometimes in the United States.

NEW FACE AT PDS

PDS is very pleased to announce that Dr. Steve Mills joined the roster of PDS clinical pathologists in July for a one year term. Steve will be working remotely from his home in Edmonton, Alberta—a new working situation for everyone. Steve grew up in Calgary, Alberta and attained his Bachelor of Commerce and subsequent Bachelor of Science from the University of Calgary before starting a Master of Science degree. Just six months later however, Steve pulled up stakes and headed to

Saskatoon to start veterinary school at the Western College of Veterinary Medicine. Steven finished his DVM degree in 2011, and then pursued a residency in Veterinary Clinical Pathology. When Steve graduated in 2014, he moved to the Edmonton area to practice small animal medicine, but was unable to challenge the board exam until 2016, when he attained his ACVP diplomate status. Steve and his wife Ginevra have three young children Adrien, Everett, and Eleanor. He is absolutely thrilled to be practicing clinical pathology again full time. Steve can be reached via e-mail (steve.mills@pds.usask.ca) or cell phone (306.220.4788).

ACHIEVEMENTS

Dr. Rambod Movasseghi (PDS Anatomic Pathologist) became a diplomate of the American College of Veterinary Pathologists (ACVP) in August 2017. More on the unique difficulties Rambod experienced on the road to achieving board certification will follow in a future issue of the newsletter… so stay tuned.

READERS' FEEDBACK

The **Animal Health Perspectives** editorial team (Dr. Moira Kerr, Brian Zwaan and Kathryn Tonita) invite readers' comment on material published in the newsletter or questions on material submitted by contributors.

Submit your comments or concerns to Dr. Moira Kerr (email: moira.kerr@pds.usask.ca) and they will be forwarded appropriately.