

Vetpath is a specialist veterinary laboratory dedicated to providing our clients with the finest laboratory diagnostic service. A team of veterinary pathologists and medical scientists with extensive experience in veterinary diagnostic pathology forms the core of the Vetpath team.

VN News

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What is PCR?

Vetpath has introduced a number of PCR tests in the last few years. But what exactly is PCR and why is this method useful?

The polymerase chain reaction (PCR) is a sophisticated molecular technique used to replicate and amplify a specific target nucleic sequence. Short strands of nucleic acids called primers are used to target the specific DNA sequence of interest. Then enzymes called polymerase and cycles of heat denaturing and annealing are used to form new DNA from free nucleotides. This 'chain reaction' occurs when the DNA formed in the test is subsequently used as a template for replication and exponentially produces millions of copies of the target DNA sequence (see figure). These DNA fragments can then be identified, verified as

true target material and in specific cases quantified. A similar PCR test has been developed to detect RNA (RT-PCR).

PCR testing is particularly useful for detecting the presence of infectious agents. A single PCR test can be developed for simultaneous detection of multiple organisms (termed multiplex PCR panels). PCR tests can also identify abnormal DNA sequences within the host genome, including genetic disorders such as ivermectin sensitivity or genetic mutations in lymphoid neoplasms.

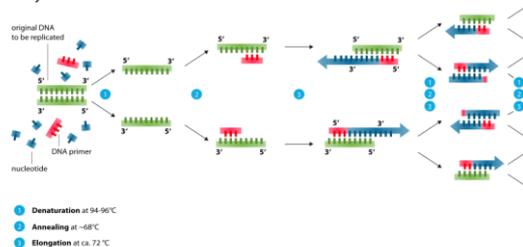
PCR is an extremely sensitive method of detecting infectious disease, with only a small amount of DNA being required for to successfully confirm the presence of an infectious agent. The specificity of PCR is also high as it detects pathogen DNA rather than an antibody response.

However, false negatives and positives can still occur. PCR may not identify an infectious

agent if it is not present in the sampled material, or if there is a technical problem with the assay such as poor DNA extraction or presence of inhibitory substances. A false positive result may occur with contamination of the sample either during sampling or the PCR reaction. Alternatively, the highly sensitive nature of PCR can result in identification of clinically insignificant pathogens.

Despite these potential limitations, the high sensitivity and specificity of PCR makes these tests extremely useful diagnostic tools.

Polymerase chain reaction - PCR



Therapeutic monitoring of levetiracetam levels

Levetiracetam (Keppra®) is a newer human anticonvulsant drug that is also being used in veterinary medicine. Therapeutic monitoring of levetiracetam is now available at Vetpath.

Levetiracetam's mechanism of action is unknown. It is not metabolized in the liver and is excreted unchanged in the urine. Levetiracetam has the shortest half-life of the anticonvulsant drugs (2 to 4 hours in dogs and 4 to 7 hours in cats), and there is likely significant individual variation.

The initial recommended dose is 20 mg/kg every 8 hours. The ideal blood level is 5ug/ml (trough) to 21 ug/ml (peak). The trough sample is collected at 6 – 8 hours after dosing and the peak sample is collected 2 hours after dosing. The initial concentration should be checked 2 weeks after beginning treatment, and again at 10 – 14 days after any dosage change.

The test is performed at a human laboratory and the estimated turn-around time for the test is 10 working days. A 3ml blood sample in a red top/serum tube is required. The serum must be

separated within 12 hours; careful consideration of sample travel time to Vetpath is recommended.

Other commonly monitored drugs in veterinary patients include:

Potassium Bromide: A single serum sample is required due to the long half-life of the drug. Although a trough sample is ideal (1 hour before next dose), a steady state is achieved after 2 – 3 months and therefore sampling at any time of day is acceptable.

Cyclosporine: The half-life of cyclosporine is relatively short and therefore peak and trough levels should ideally be checked. The trough sample is collected before the next dose is given and the peak sample is collected 2 – 4 hours after dosing. Alternatively, only the trough sample can be measured. EDTA anti-coagulated blood that is as fresh as possible should be submitted.

Phenobarbitone: A single serum sample collected at any time of the day is sufficient to monitor phenobarbitone. However, if a "break through" seizure occurs, collection of a trough and peak sample (4 – 5 hours after dosing) is recommended.

Thyroid hormones: Concentration of thyroid hormones may be achieved quickly; however it can take 4 – 6 weeks for physiologic equilibrium to occur. If dosing twice daily, the T4 levels should

be checked 4 – 6 hours after dosing on a serum sample. If dosing is once daily, a trough value should also be checked.



Sample storage

Correct sample storage is an important consideration in laboratory medicine. **Blood, urine and faeces** should always be stored in the refrigerator to preserve microscopic components and prevent bacterial overgrowth. **Swabs** can be stored at room temperature as the media will provide an adequate environment for infectious agents to survive. **Cytology and histopathology** should be stored at room temperature.



NATA Accredited
Laboratory Number 14776

Vetpath Laboratory Services

RECEPTION DIRECT +61 8 9259 3600
LOCAL COURIER PICK-UPS +61 8 9259 3666
AFTER HOURS MOBILE 0418 916 436
FACSIMILE +61 8 9259 3627
EMAIL enquiries@vetpath.com.au
WEBSITE www.vetpath.com.au

VETERINARY PATHOLOGISTS

Jenny Hill BVSc (Hons) Dip ACVP
John Jardine BVSc MMedVet (Path) Dip ACVP MRCVS
Jon Meyer BVSc DVSc Dip ACVP
Jason Stayt BSc BVSc Dip ACVP
Leanne Twomey BSc BVMS (Hons) PhD Dip ACVP