Using High Throughput Johne's PCR (HT-J test) to detect or diagnose Johne's disease

Know your DNA

Mycobacterium avium subspecies paratuberculosis (*Map*) is a slow growing mycobacterial agent which causes Johne's disease (JD). Diagnosis of JD by culture is a long and complex procedure requiring multiple steps and tests to achieve a positive or negative result.

High Throughput Johne's PCR (HT-J test) detects DNA consistent with the causative agent of JD (*Map*) . It is very specific to *Map* and results are usually obtained within 2-4 weeks. The HT-J test has been approved for herd tests in Australia and New Zealand by the Subcommittee on Animal Health Laboratory Standards (SCAHLS).

The benefits of running a HT-J test are: it is far quicker than faecal culture which can take months, it does not require the slaughter of animals for histology or tissue culture and it is more sensitive than the ELISA blood test.

Diagnosis of JD by faecal culture is a long and complex procedure requiring multiple steps and analyses to achieve a positive or negative result.

There are 4 steps:

1. Preparation
2. Liquid media culture
3. DNA testing of cultured organisms by PCR
4. Acid-fast staining using the Ziehl Neelsen stain

We no longer perform solid media culture.

Species:
Cattle, Sheep, Alpacas and Goats

Specimen:
Faeces. Pooled or single samples.
If pooled in laboratory, pooling fees apply.

Container:
Yellow top pot

Pooling:
Bovine 5, Ovine 50, Caprine 50 and Alpaca 5. If pooled in the lab, a pooling fee applies.

How to use HT-J

The HT-J test is used to assess herd/flock infection status by using pooled faecal samples. The test can also be used on faeces sampled from individual animals, however, negative results are not definitive (at an individual animal level), as shedding can be absent or occur intermittently early in the course of infection. Other tests, such as ELISA and faecal culture are still required to confirm individual status.

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Diagnosis of JD by HT-J means the herd/flock is likely to be infected. Further testing by culture may be required.

When negative HT-J test results are found across the whole herd/flock with sampling from high risk animals (e.g. animals greater than 2 years of age) then this indicates that the herd/flock is not shedding JD bacteria (*Map*). JD results should always be reviewed in the context of the history of disease on the property.

It is worth keeping in mind that although the HT-J test gives a quick result, if positive results are reported, it will take approximately 3 months for a confirmed diagnosis, if further JD testing by faecal culture is required.

The four step process to confirm Johne's disease in HT-J positive/inconclusive samples:

Step	Test Procedure	Time Frame	Alternate Terminology	Purpose
1	Preparation	≈ 1 week	Decontamination phase	To prevent overgrowth by non *Map* organisms
2	Liquid media culture	≤ 12 weeks	• Liquid M7H9C	To accelerate growth of *Map*
3	PCR	≈ 2 week	• DNA testing • Strain typing	To detect DNA insertion sequences IS900 and IS1311 (in *Map* grown in liquid media) and determine strain type
4	ZN staining	Done in same week as PCR	• Acid fasting	To demonstrate acid fast staining of organisms grown in liquid media (other mycobacteria will also produce positive results)

Reference: Australian and New Zealand Standard Diagnostic Procedure, July 2015

Pricing (excluding GST)

Request	Pooled 4 week TAT	Pooled 2 week TAT	Single 4 week TAT	Single 2 week TAT
High Throughput Johne's (HT-J) PCR (1-30 samples)	$90.00	$164.40	$85.00	$142.40

To order the HT-J test please send required samples on ice packs in eskies to Gribbles Veterinary Pathology along with your submission form (available to download at **gribblesvets.com.au/veterinarians/ordering-a-test**). Please write High Throughput Johne's PCR in testing instructions.

Samples need to arrive in the laboratory within 48 hours of collection. Please keep this in mind when scheduling sample collection.

Paratuberculosis (Johne's disease) is a notifiable disease. Refer to **www.agriculture.gov.au/pests-diseases-weeds/animal/notifiable** for reporting obligations in your state or territory.

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