



PROTOCOL FOR RANDOM 60 COW SCREEN

Guidance for '60 cow random' milk sampling to generate an ATV in non-milk recording herds: Essentials

Why?

To get a realistic representation of the level of Johne's disease (MAP) in a herd without having to test every cow. In herds that don't routinely milk record, an Average Test Value (ATV) from 60 cows selected AT RANDOM is required for the annual NJMP declaration. If done correctly, it will provide a valuable measure of the level of the disease in that herd.

How to select a cross-section of 60 cows in a herd

Systematic random sampling is suitable for use with cows passing through a milking parlour. The sample collector simply samples cows passing at pre-determined intervals. The practical application is as follows:

- 1) Note total cows in milk on that day, *for example 285*.
Divide by 60 (the total samples required): $285 / 60 = 4.75$.
- 2) The result should always be **ROUNDED DOWN** to the integer, in this case to **4**.
That is the **interval** to use, so **every 4th cow** passing through the parlour.
- 3) Ask a colleague to pick a number between 1 and the interval (**4**). If they choose 3, then you start with the third cow to enter the parlour and then sample every 4th cow from then on.
- 4) *Carry on sampling every 4th cow until you have the 60 samples required.*

Collecting, labelling, packing and sending the samples

The MRO providing the testing service will provide practical instructions along with sample pots and appropriate labels.

Information to be provided with the samples

The MRO providing the testing service will provide a simple form:

For each sample:

- **Cow ID** (e.g. freeze brand – note that the official eartag ID **IS NOT** required). This information will remain confidential **for farmer and vet use only**. Cow sample IDs will be anonymised for third party use and **ONLY** the summary statistics (ATV and n(%) positive [30, 60, 100]) will be generated for reporting purposes.

For the whole batch of samples:

- **Herd ID** This information will remain confidential **for farmer and vet use only**. Herd IDs will be anonymised for third party use.

- Date milk samples collected
- Number of cows milked on the day
- Number of samples submitted

Guidance for '60 cow random' milk sampling to generate an ATV: Full details with background information

Background

The 'National Aspirational Standard' for the dairy sector

A 'National Aspirational Standard' has been set as part the National Johne's Management Plan (NJMP) Phase III. The aspiration is for the UK dairy sector to achieve a national **Johne's Control Index (JCI) of 5.5 by 2030**. The national JCI is defined as a simple mean (annual average) of the average test values (ATVs) of all the herds for which data are available. The more herds that can be included in the calculation of the national (industry-wide) JCI, the better it will reflect the situation of the UK dairy sector as a whole.

The requirement for individual herd ATV

Under the NJMP Phase III, obtaining an ATV on an annual basis is a requirement for all herds implementing the plan and is therefore also required for 'Red Tractor' certification. The annual ATV must be recorded before the annual declaration can be signed off.

Obtaining a herd ATV

How an ATV is obtained depends on whether a herd is already using the JD milk ELISA test and whether the herd is routinely milk-recording with a milk recording organisation (MRO).

1. Herds that already implement whole herd JD test

The necessary data to generate an annual ATV are already available for quarterly testing herds and herds that routinely carry out annual whole herd tests.

For **herds that do not carry out whole herd JD testing**, ATVs based on *random samples of cows* will be used. Under the NJMP Phase III, JD testing of a 60 cow random sample has been added as the *minimum requirement* to generate an ATV, which must be recorded in the annual declaration.

2. Herds that are routinely milk recording through an MRO

Herds that are routinely milk recording through a milk recording organisation (MRO) can request that the MRO randomly selects 60 individual cow milk samples from a routine milk recording collection and tests these with the JD milk ELISA test.

3. Herds that do not routinely milk record through a MRO

Herds that do not routinely milk record through a MRO will need to arrange for milk samples to be collected and sent to an MRO for testing. This will be under the direction of the farm's veterinarian who will be responsible for signing the annual NJMP declaration.

The value of an annual ATV

For the individual herd taking the trouble to implement a Johne's disease management plan, the ATV provides an annual indication of progress. The ATV is an important tool to be used in designing and refining the Johne's management plan.

1. Each year the herd ATV can be compared with ATV's of previous years. Simply, if the ATV is falling, the disease situation is improving, if not, then the management plan should be adjusted.
2. The herd ATV can also be compared against the 'National Aspirational Standard' (5.5). Attaining an ATV close to 5.5 can be considered as success, i.e. JD control has reached a satisfactory level.
3. The herd ATV can also be compared against other herds using an anonymised 'benchmark group' of herds, e.g. other herds within the same veterinary practice or a larger group derived from the National JD database.

In order for any of these three comparisons to be useful, the estimate of herd ATV must be an unbiased and accurate indicator of the true herd situation. *There is no advantage to the farmer in 'gaming the system' to get a low ATV result because this only disguises the true situation and hinders getting the disease under real control.* The following guidance is provided to help ensure that the samples are collected in a random way in order to provide an unbiased estimate of the herd's 'true' ATV.

Although not all the cows in the herd may be tested, it could still be useful to the farmer and their vet to know the individual test results for each cow tested. This would be particularly useful in the case of very high positive values, as such cows could then be removed or managed carefully to avoid disease transmission risk in the herd.

Another important use of the herd ATV is that it will contribute to calculation of the national JCI. In this respect it is important to stress that **herd IDs and cow sample IDs will be anonymised for third party use** and ONLY the summary statistics (ATV and n(%) positive [30, 60, 100]) will be generated for reporting purposes.

Why 60 random cows?

Why 60?

The recommendation is that the estimated ATV should be based on a **random** sample of cows in the herd. Relevant statistical considerations were explored using real data from the National JD database to arrive at a recommendation for the size of random sample to be used. It was found that a sample size of 60 would give a reasonably precise estimate of the herd ATV¹, with a 90% confidence interval of +/- about 1.5 ATV units.

Requirement for the sample to be 'random'

The cows from which milk is taken for the estimate of herd ATV must be representative of the whole herd. There are two requirements here:

1. Milk samples are needed from cow of all ages and at all stages of lactation, as present in the herd on the day of sampling.
2. Cows to be sampled should not be picked for any particular reason (e.g. "this is a good cow", "this cow might have Johne's", etc.). Such choices could be made unconsciously if the farmer is asked to pick cows for sampling. The result of such choices would be that the ATV estimate might not be an accurate reflection of the true herd ATV, or in statistical language the ATV estimate could be biased.

¹ A full discussion of the relevant statistical issues is provided in a separate technical document.

The purpose of using random sampling is to avoid any conscious or unconscious *bias* in the selection of the cows for sampling, removing a main source of *inaccuracy* in the ATV estimate. This is achieved by making the selection of cows the subject of a mechanical, mathematical process.

How to achieve a random sample in practice

Which cows to sample

Sample collection will be under the direction of the farm's veterinarian who will be responsible for signing the annual NJMP declaration. However, since the process of sample collection is likely to be carried out during a full milking time, it is unlikely that the vet would be present and collect the milk samples in person. This guidance is provided for both the vet and the farmer who will need to discuss the sampling process and agree on a strategy for the particular herd. Ultimately the vet will need to be satisfied that the correct number of milk samples have been collected in a random way in order to provide a true, unbiased estimate of the herd's ATV.

We do not require a complex statistically rigorous randomisation method as might be used in academic research. The only important requirement is that any hint of the sample collector making active choices of cows to sample is removed from the process. This can be done by introducing a simple mechanical, mathematical process to be followed in order to select cows for sampling. This gives the person taking the samples a simple process to follow that eliminates any need for conscious choice of cows.

A simple method is known as '*systematic random sampling*'. This method is suitable for use when cows are passing through a milking parlour, because the cows themselves will pass through in a reasonably random order. The sample collector simply collects milk from cows that pass through at pre-determined intervals.

The practical application is as follows:

- 1) Note of the number of cows to be milked, which should be all the cows in-milk on the day, **for example 285**.
- 2) Divide the number of cows by 60 (the total number of samples required 60): **$285 / 60 = 4.75$** .
- 3) The answer should always be **ROUNDED DOWN** to the integer, in this case to **4**.
- 4) The answer, **4**, is the interval between cows for collecting milk, i.e. milk should be collected from **every 4th cow** as they pass through the parlour.
- 5) The first cow to be sampled should be picked randomly. A simple way would be to ask another person to choose a number between 1 and 4 (in this case). If they choose 3, then you start with the third cow to enter the parlour and then sample every 4th cow from then on.
- 6) *Note that in this case the 60th sample will be collected from around the 240th cow to pass through. This is fine. Stop collecting when the required number of milk samples has been reached. The importance of always rounding down is to ensure that enough samples are obtained. If 4.75 had been rounded up to 5, and every fifth cow sampled, only 57 samples would have been collected when all 285 cows had gone through.*

Collecting, labelling, packing and sending the samples

The MRO providing the testing service will provide practical instructions along with sample pots and appropriate labels.

Information to be provided with the samples

The MRO providing the testing service will provide a simple form to be filled in and enclosed with the samples.

For each sample:

- **Cow ID** (e.g. freeze brand – note that the official eartag ID **IS NOT** required). This information will be confidential **for farmer and vet use only**. Cow sample IDs will be anonymised for third party use and **ONLY** the summary statistics (ATV and n(%) positive [30, 60, 100]) will be generated for reporting purposes.

For the whole batch of samples:

- **Herd ID** This information will be confidential **for farmer and vet use only**. Herd IDs will be anonymised for third party use.
- Date milk samples collected
- Number of cows milked on the day
- Number of samples submitted