

Working with your Lab

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Introduction

As Vet Nurses, you often have the opportunity to determine the quality of lab samples and submissions coming from your clinic to your diagnostic lab. You can play a pivotal role in providing the attention to detail that distinguishes a merely acceptable sample from a “Wow, that Vet Nurse is a star!” sample.

THE BASICS: Sample Types and Tests

The main types of samples that are received in a veterinary diagnostic lab include blood, fluids, tissues, faeces, urine, milk and swabs. The type of sample your vet usually submits to the lab depends on what test he or she is requesting. Here is a general breakdown of what sample types are commonly submitted for each lab area (minimum amounts may differ for different types of tests):

Biochemistry, Endocrinology and Serology

Vacutainer Type	Minimum sample amount	Commonly requested tests
Serum (red top)	1ml	Sick animal panels, Progesterone, Bile Acids, antibody & antigen tests
Lithium heparin (green top)	1ml	Relaxin
Fluoride oxalate (grey top)	1ml	Glucose levels

The majority of Biochemistry, Endocrinology and Serology tests require serum samples. For smaller animals or other cases where a limited amount of blood is available, you may want to prioritise the tests requested. This way, if there is insufficient blood to complete all requests, the most important tests are performed first. The Relaxin pregnancy test is the only test in which heparin is the required vacutainer type.

Other sample types used in these lab areas are:

Other Sample Types	Minimum sample amount	Other requested tests
Liver	0.1g (per test)	Copper, Selenium, B12
Fresh Plant Material	20g	Pasture Nitrate Estimate
Faeces	1 Tbsp.	ELISA antigen testing
Rumen contents	50g	pH Level

Haematology and Cytology

Vacutainer Type	Minimum sample amount	Commonly requested tests
EDTA (purple top)	½ filled tube (applies to all tube sizes)	CBC assays, Coombs test, Crossmatch, Cyto analysis
Citrate (blue top)	1:9 dilution	Coagulation profiles, von Willebrand's, PT, TT

EDTA is the preferred anticoagulant for almost all routine Haematology tests, including CBC assays. Please note that, as EDTA tubes come in different sizes, differing amounts of blood are required for each size in order to provide the correct sample-to-anticoagulant ratio. Check the tube you are using for the recommended blood volume, and remember that allowing a vacutainer to fill based on the vacuum in the tube should also ensure the correct ratio. A similar process should be followed when obtaining a Citrate sample. These tubes contain a volume of anticoagulant equivalent to 10% of the total volume required – thus, the final sample should be a 1:9 dilution, so you will want to fill the tube to its fill line. Obtaining the correct dilution is especially important because the lab's reference ranges are determined by taking the dilution factor of a properly filled tube into account. For Coagulation profiles where a larger amount of blood is needed, it is recommended that you use the larger tubes. However, if you are only able to obtain a small amount of blood, use a smaller tube so that the dilution ratio is maintained.

Other sample types used in Haematology and Cytology are:

Other Sample Types	Minimum sample amount	Other requested tests
Blood smears	Submit on slide	CBCs, manual differentials, Smear evaluation and interpretation
Bone marrow	Submit on slide	Evaluation and interpretation done if certain abnormalities found on CBC results
Cerebrospinal fluid	1ml	CSF Interpretation, Pandy's Test

Microbiology, Parasitology and Urinalysis

Sample Types	Minimum sample amount	Commonly requested tests
Urine	0.5 - 5ml	Urinalysis panels, isolations and sensitivities
Swabs	Submit in transport medium	Isolations and sensitivities
Skin or Tissue samples	Pottle	Fungal cultures, KOH, Ectoparasites
Body fluids	Serum vacutainer or pottle	Isolations and sensitivities
Faeces	1 Tbsp.	Faecal egg counts, Enteric screens
Milk	2ml	Mastitis culture +/- sensitivity

Histology and Necropsy

Sample Types	Minimum sample amount	Commonly requested tests
Fixed tissue	Submit in 10% buffered formalin	Histology interpretation
Vas deferens	Submit in 10% buffered formalin	Verification of vas deferens tissue
Foetus	Submit fresh	Necropsy
Brain	Submit in 10% buffered formalin	TSE Surveillance
Spinal cord section	Submit fresh	TSE Surveillance
Body	Submit fresh	Necropsy



NZVP can help:

Remember, you can always ring us at the lab if you are unsure what type of sample is required for a particular test. It's easier to call us first than have to re-sample the animal – or worse, the entire herd!

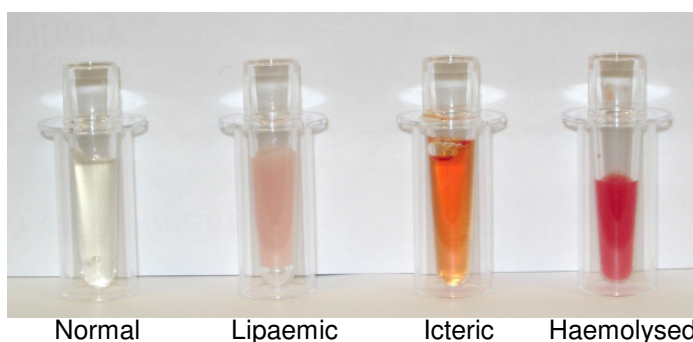
Further information about sample protocol, including a “Who’s Who of Vacutainers”, is available on our website at <http://www.nzvp.co.nz> under the “Information Sheets” tab.

THE SKILLS: Getting the Best Results from Your Samples

Factors that may affect Blood Test Results

Some blood samples that you submit to the lab may spin down to produce serum that doesn't look clear (i.e. normal), which can affect the validity of test results. Haemolysis, lipaemia and icterus (jaundice) are three interfering factors which may be visible in serum samples:

SERUM SAMPLES



Interference	Possible Causes	Affected Tests
LIPAEMIA	<ul style="list-style-type: none"> • Sampling immediately after a fatty meal (sample after a 6-12 hr. overnight fast instead) • Pancreatitis (marked lipaemia) • Diabetes mellitus, hypothyroidism, hyperadrenocorticism, nephritic syndrome (mild lipaemia) 	<ul style="list-style-type: none"> • Biochemistry tests that use spectrophotometric methods • Automated Haematology counts

HAEMOLYSIS	<ul style="list-style-type: none"> • Vigorous shaking of samples • Exposure to excessive temperatures • Poor phlebotomy due to overactive animals • Haemolytic anaemias • Use of fine gauge needles during venepuncture • Excessive pressure on syringe plunger during sample collection • Failure to separate serum or plasma prior to storage/couriering 	<ul style="list-style-type: none"> • Biochemistry tests that use spectrophotometric methods • Measurement of various erythrocyte parameters in Haematology
ICTERUS	<ul style="list-style-type: none"> • Liver disease • Obstruction of the bile duct 	<ul style="list-style-type: none"> • Biochemistry tests that use spectrophotometric methods

Tips for taking good blood samples:

- The larger the vein the sample is taken from, the less risk of haemolysis. Collect blood from the animal's jugular vein when possible.
- Transferring blood directly into the vacutainer tube is preferable to pushing it out through the needle – this causes unnecessary haemolysis.
- Preferred needle sizes for sampling companion animals are: Dog 20-22g and Cat 22-25g. The bigger the needle, the better – within reason, of course.
- When using a vacutainer with a preservative, just invert the sample gently to mix the blood – do not shake the tube!
- Store the samples in the fridge if you are not submitting them to the lab immediately. Be aware that extreme heat (and even extreme cold) can cause the cells to lyse (burst), so use a chilly pad when appropriate.

Blood Smears

Blood smears are an indispensable part of any haematology submission, particularly if there is going to be a significant delay between collection and submission of the sample. Taken as soon as possible after collection, a fresh blood smear preserves blood morphology so that potentially important changes can be recognised during interpretation. Sending in a fresh blood smear is especially helpful for:

- Suspected leukaemia – immature cells are easier to identify when fresh
- Anaemic animals – particularly cats, as Mycoplasma tends to fall off the red cells
- Cows, sheep, goats and alpacas – These animals always tend to be forgotten, and their white cells degenerate even faster than those of companion animals.
- Birds

Quick Tips on how to make a blood smear:

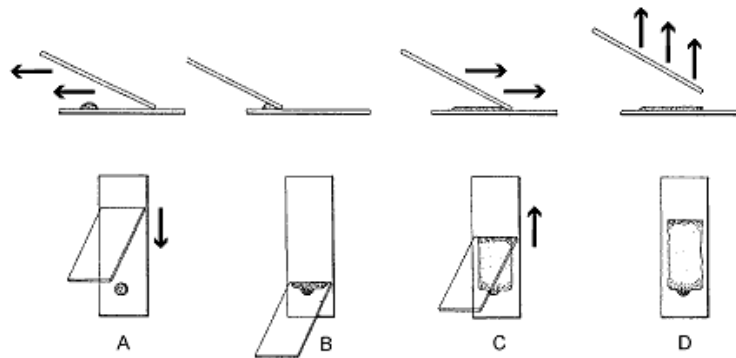
- 1) Ensure blood is well mixed.
- 2) Use a clean, dry microscope slide, preferably with a frosted end for ease of labelling.
- 3) Fill a capillary tube with blood and use this to place a drop of blood at the end of the slide.
 - *Not too small* or the smear will be too thin and there will be no white cells to count.
 - *Not too big* or you will either go all the way off the slide or end up with a large blob of blood and a very short smear.
- 4) Without delay, place the spreader on the slide at a 45° angle and move it back to make contact with the drop. The blood will spread out quickly. As this occurs—but

before it fully reaches the edge—spread the smear with a rapid, smooth movement. The smear should be 3-4 cm in length with a clear, feathery edge.

- 5) Label the slide in *pencil* (pen or marker may dissolve in the alcohol fixative) with the animal's name and let completely dry on a flat surface before placing in a slide container.

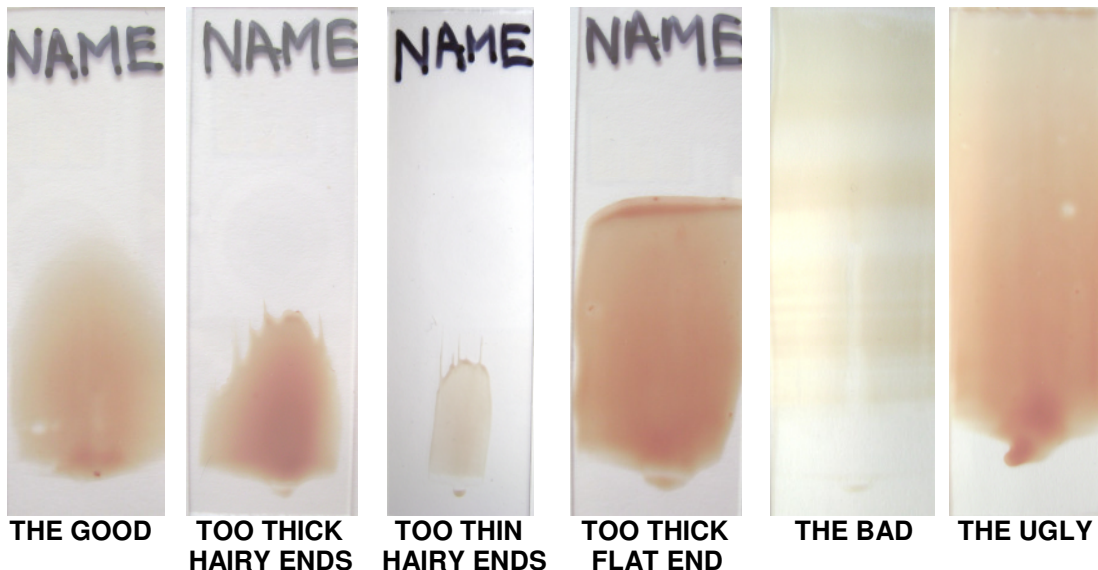
Hints for making a great smear:

- Using a special spreader slide with a flat, cut glass end can make all the difference to smear quality. These can be cleaned with a little saline between smears and re-used many times.
- To get a good smear, alter the angle of the spreader relative to the thickness of the sample – the greater the angle, the thicker the smear.
- Experiment with your speed – a good smear should look like the diagram below:



The Good, the Bad and the Ugly:

Below is an example of a good blood smear and some not-so-good blood smears. Note, the good blood smear has an even, smooth, rounded “tail” (or feathered edge) at the front. This rounded end or tail is here Haematology does most of the differential counting.



Some of the qualities that can make smears Bad and Ugly are:

- Dirty slides
- Water artefact
- Exposure to formalin
- EDTA artefact (from prolonged storage of blood in EDTA)

- White blood cell degeneration (from long delays between collection and slide preparation)
- A smear that is too thick or heavily contaminated with blood
- Poor smear preparation (which results in large numbers of smudged cells, bare nuclei or strands of nuclear protein)

Red cells are also finicky about their environment!

They dislike: Being breathed/blown on to dry them (would you like this?)
 Being put in the slide container while still wet
 Being held upside down or tipped on their sides while still wet
 The smell of formalin (they go “blue” in the face)
 Being put into a tube that is too big in relation to the volume of blood taken
 Big crowds (a too-thick smear)

Red cells do like *some* company (not too thin of a smear), and if you are in the field and conditions are unfavourable for making a smear, they will happily wait until you are back in the clinic. They are most content lying on a flat surface, and being allowed to completely dry before the slide is placed in the slide container.

Whew! Lots to process, and all these steps may sound intimidating, but remember -- as a general rule, any smear is better than no smear at all. So keep practicing -- your impressive skills at making quality blood smears will be invaluable to your clinic!

How many samples should be submitted?

It is not unusual for vets to submit only the types of samples they think might be relevant to a diagnosis for a particular case. However, it is to their advantage to provide the lab with as many types of samples as they can for each patient, e.g. faeces, blood *and* tissue. There is no increased cost for doing this, as the lab will prioritise the samples and stop testing when a diagnosis is found. This will also save on courier costs, as you will not need to send in multiple packages if the vet determines that additional testing should be carried out. And the bonus -- the more samples the vet sends in, the more opportunities you'll have to practice your sample prep and packaging.



NZVP can help:

If you are still unsure about making blood smears or just want more information, give our friendly Haematology team a call at the lab. They'll be happy to give you a crash course over the phone. By improving the overall quality of blood smears, it helps them give you the best possible test results.

THE PACKAGE: Sample Containment and Handling

Several basic rules apply when handling and packaging your samples to send to the lab:

- Use leakproof containers with tight-fitting lids.
- Use protective bubble wrap to line the packaging.
- Send glass slides in plastic slide mailers, or bubble-wrapped in a box or envelope.
- Use rubber bands to bunch tubes together rather than sellotape.
- When using sellotape, less is more.
- Faeces, urine, fresh tissues and other semisolid samples should be submitted in tightly sealed screw top pottles (not sellotaped).
- Large tissue samples, bodies and formalin-fixed samples require a little extra packaging to safeguard against leaks. Double-package these types of samples either

by putting the container in a sealable plastic bag for small samples and a bucket or box for larger samples. For large samples, consider fixing the tissue at the clinic for 2 days and then draining the sample and sending the tissue in a sealable, leakproof plastic bag.

- Package bodies with care and courtesy for the courier staff. For very wet specimens (e.g. aborted foetus), use plastic bins/buckets with tight-fitting lids. For small bodies, double-bagging and then placing in a cardboard box is usually sufficient.
- Include a chilly pad in hot weather or if there is a possibility that your sample will be delayed in transit.
- Put all samples and matching forms in the same parcel (one exception may be formalin-fixed samples – see below), and make sure the animal name or ID is written clearly on all the samples. Don't forget to include the submission form!
- Quarantine samples: Clearly label all samples with animal details/identification and ensure that these samples are double-bagged (details to follow).

NEVER SUBMIT:

- ANY samples in breakable (glass) jars or containers.
- ANY samples in syringes, especially syringes with the needle still attached! Syringes are leaky by nature and an inadvertent press on the base can send your sample shooting out of the syringe (or on the courier) – not a nice look. Transfer these samples into sterile serum (red top) vacutainers and submit them this way.
- Urine samples in transport medium containers (see photo at right). These are not leakproof and can be easily spilled. Extra precautions should be taken when handling urine samples, due to the potential zoonotic spread of leptospirosis. →
- Faecal samples in gloves (open or knotted), syringes, leaking bags, and definitely not in cardboard egg cartons (yes, this has happened)!
- Tissue samples or bodies in leaking cardboard boxes.
- Blood and cytology smears in the same package as samples fixed in formalin. Exposure to formalin or even formalin fumes can dramatically impact on cell preservation and staining quality of smears, often making interpretation impossible.



Examples of Good and Not-so-Good Packaging Techniques

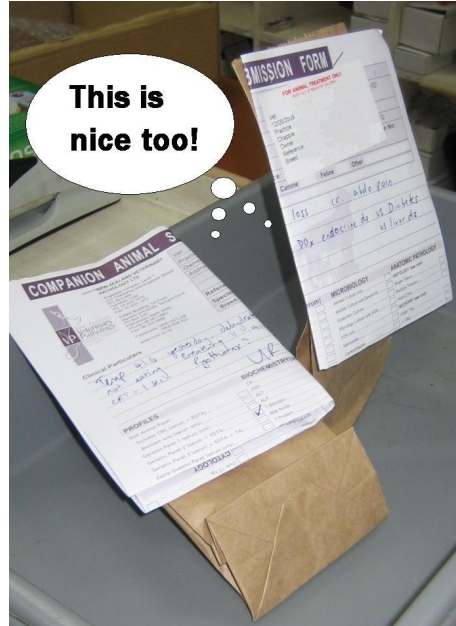
- 1) Blood Tubes: These are relatively easy to package as they are small and leakproof. We often receive blood tube submissions that have been enthusiastically wrapped and wrapped again in bubble wrap and sellotape:

Impressive effort, but largely unnecessary! →

Blood tubes can be sent in a small box or bag with a layer of bubble wrap loosely lining the inside, and that should be sufficient (see photos below). Slide cases can also be included here. There is no need to sellotape the box shut. The quicker the lab can unpack and process your samples, the faster you get your results.



Most labs carry a selection of suitable boxes and mailers that are available to clients:

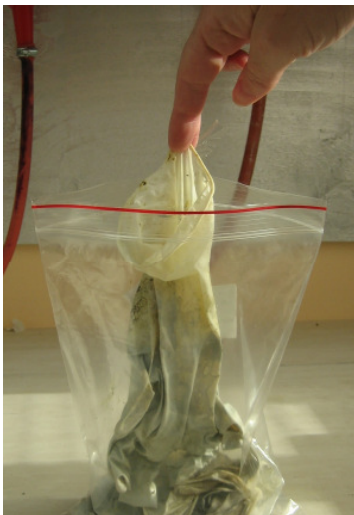
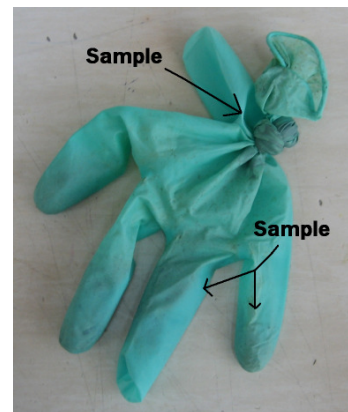


Another tip that will save your clinic on packaging costs: When sending in several blood tubes, don't be afraid to pack them in one box. As long as the box is bubble-wrap lined and each tube is properly labelled with its corresponding submission form filled out and included, you can fit quite a few tubes in one box.



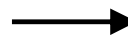
2) Faecal samples: You'd be amazed at how much of this goes in and out of a diagnostic lab, especially during calf scouring season!

One of the least-loved (and most time consuming) tasks for the Microbiology/Parasitology lab staff is having to squeeze samples out of glove fingers or knots, as illustrated by this submitted sample:



If your vets do find themselves in the field with only a glove to take a faecal sample, encourage them to make sure the sample goes in the actual glove and not the — and that there IS a knot!

Is this really apricot jam?



knot



Completely acceptable container for a faecal sample – just don't let the kids get a hold of it.



The best way to submit faecal samples is in labelled plastic pottles, as shown here:



- 3) Histology samples: Histology samples come in all shapes, sizes and conditions, making for interesting challenges when packaging samples for submission to the lab.

One of the most common types of situations we encounter is one that vets may not be aware of when initially packaging a sample to send to the lab. This especially applies to large pieces of tissue, and brains that are sent in for MAF's Transmissible spongiform encephalopathy (or TSE) Surveillance program. When a large animal's brain is initially placed in formalin, it is soft and pliant and fits easily into its container. However, by the time it reaches the lab, it is usually fixed and quite firm.

This causes a problem if the opening of the container is smaller than the fixed brain!



TSE Surveillance requires that the animal's brain stem be intact and in such a condition that the pathologist can accurately interpret the slides that are made from the trimmed-in tissue. If the brain won't come out of the container without being chopped up, it is not uncommon to see a Post Mortem Room Attendant sawing away at the container itself to get the sample out!

What is not ideal about the Histology containers pictured below?



Histology samples are best submitted in sealed, unbreakable, size-appropriate containers:



- 4) Necropsies: Packaging and sending bodies in for submission can be a delicate undertaking. Your client may want their pet returned to them after it is seen at the lab, and you also need to keep your courier's environment in mind. Transport delays, excessive temperatures and the fact that other packages are travelling with your submission can all be influencing factors.

One of the worst things both couriers and the lab staff can encounter is when a body is sent to the lab in a leaking, flimsy cardboard box. No one wants to lift a body that has been submitted in this type of box, and be left holding just the box!



Flimsy box, leaky bag and leaky chilly pads – a definite no-no

The ideal method for submitting extra-large tissue samples and bodies is to double-package the sample in a sealable plastic bag and then a bucket or box with a lid. Dry Cow buckets work especially well for wet samples (e.g. aborted fetuses):



NZVP's Specimen Reception department stocks a wide variety of packaging materials including plastic slide mailers, boxes & plastic pails, specimen & milk pottles, large plastic containers for histology specimens, bubble wrap, courier bags and tickets, vacutainers, urine tubes – everything you need to get your samples to the lab safely.



NZVP can help:

Our Specimen Reception department has a wide variety of packaging materials available including plastic slides mailers, boxes and plastic pails, specimen and milk pottles, large plastic containers for histology specimens, courier bags and tickets, vacutainers, urine tubes – the list goes on. If you'd like any information about couriers, lab products, or just want to know how to package a particular sample, don't hesitate to give us a call on 0800 VET LAB (0800 838 522).

THE DETAILS: Filling out Submission Forms

As you all know, vets are often rushing to get to their next consult or call, and may only have time to record the most basic information on a submission form. One of the things you can do to ensure that your sample is quickly and correctly routed at the lab is to double-check these forms before they are placed in their packaging for the courier. Adding that extra detail on your form can mean the difference between an expedited, accurate test result and a sample that is registered to the incorrect clinic or vet because of a missing piece of vital information (or not registered at all!).

The essential minimal information that your lab requires on all submission forms is:

- 1) Clinic name**
- 2) Submitting vet's name**
- 3) Owner's name**
- 4) Animal Name/ID**
- 5) Species**
- 6) Tests requested**

The lab must have this information in order to process your samples correctly.

Writing the Clinic name and Submitting vet's name on your submission form will ensure that your samples are registered to the correct clinic and the correct vet in the lab's software system. It may seem like a no-brainer, but if Specimen Reception doesn't have this information they may not be able to process your samples until you ring looking for results! The Owner's name field gives your lab an easy search method of quickly looking up your results in their system when you ring with a query. Ensure that the Animal Name/ID is written clearly on the submission form, and also that it matches the name on the samples you are submitting. The lab also requires that the Species of the patient be selected on the submission form. Many tests use species-specific reference ranges, and what may be a normal range for one species is considered abnormal for another. This type of information is vital so that you get a result that is accurate for your patient. Last but not least, don't forget to tick the boxes for Tests requested. Detailed submission forms are often received that contain Items 1-5 above, but there aren't any actual tests selected! There are generally additional test options to select on the back of the forms as well.

There are 4 other items which should also be recorded on your submission form, when applicable:

- 7) Age and breed**
- 8) Patient History**
- 9) No. at risk / No. affected / No. dead**
- 10) Quarantine samples**

Some tests use age-specific reference ranges, so including the animal's Age and breed will ensure that you are again receiving the most accurate result available. The same applies for having the submitting vet include as much Patient History on the submission form as

possible. In general, a pathologist's interpretation of your results will only be provided if a relevant history is included on the form, so encourage your vets to write down any pertinent history about the patient. Remember – depending on the case, the more specific your submission form detail is, the more specific the interpretation and diagnosis can be.

Item #9 above applies when your clinic is submitting samples for any Equine or Production Animal (Production Animal submissions can include cattle, sheep, deer, goats and camelids). The information your vet provides on the submission form about the No. at risk / No. affected / No. dead is used in the MAF Surveillance Program. A MAF Biosecurity-approved lab (MAFBNZ) uses these numbers to report surveillance information to MAF on a quarterly basis. Having this information assists in the lab's efforts to keep an eye out for exotic diseases, and provides a step up in its ability to contain and destroy potentially hazardous material received during the investigation of such diseases.

Related to the MAF affiliation is the final item above, Quarantine samples. This designation only applies when you are submitting samples from a quarantined animal. To comply with MAFBNZ protocol, remember to follow these procedures if you have such samples to submit:

- Advise the lab by telephone before you send the sample so that Specimen Reception will be expecting it and can trace it if it doesn't arrive.
- Clearly label all samples with animal details/identification.
- Write 'QUARANTINE SAMPLE' in large letters on the submission form.
- For safe transport, MAFBNZ requires that these samples are double-bagged. This will help prevent leaks in case of breakage.

A MAFBNZ-approved lab will have protocol in place that is followed to identify and track these samples. Once testing is completed, the samples are logged into the Biological Hazard register and stored separately in a special Transitional Area. After a month, samples are disposed of by a MAFBNZ-approved waste disposal company.

THE CONNECTION: The Diagnostic Lab and the Role of the Vet Nurse

Now you have the inside scoop on how to make the most of the relationship between your clinic and your diagnostic lab. Vet Nurses have a unique role in the clinic setting, in that they are skilled and trained in so many diverse areas: Surgical and clinical monitoring, animal behaviour, reception and sales, the collection and management of diagnostic samples, and countless other duties. By properly obtaining, recording and packaging your clinic's lab samples, you are facilitating quick and accurate results from the lab, which ultimately means that you are providing the best service possible to your clients.



NZVP can help:

If you require assistance selecting the right test options, please give our friendly lab staff a ring. We can post submission form pads to your clinic free of charge, and all of our submission forms (including TSE, Liver Analysis, Herd Bulk Milk Testing, and a host of others) are also available for download from our website, <http://www.nzvp.co.nz>.

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