Enzootic Bovine Leukosis

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Part 1. Diagnostic Overview

Summary

Enzootic bovine leukosis (EBL) is a retroviral infection mainly affecting cattle. Cattle of all ages may be infected but most animals become infected soon after birth following the ingestion of milk from an infected cow. The causal virus may also be transmitted by any activity that transfers virusinfected lymphocytes between animals. Disease is seen in few adult cattle. Infection is most frequently diagnosed serologically. The diagnostic method most frequently used is the enzymelinked immunosorbent assay (ELISA), testing either serum or milk. ELISA kits available commercially. Most have high sensitivity, allowing pooled samples to be tested. The agar gel immunodiffusion test was regularly used in the past but is now infrequently used because of its lower sensitivity. Virus or nucleic acid detection procedures such as virus isolation or the polymerase chain reaction are rarely used for routine EBL diagnosis.

1. Aetiology

Enzootic bovine leukosis (EBL) is a lymphoproliferative disease of cattle that is caused by an exogenous retrovirus, bovine leukaemia virus (BLV). Although many calves are infected soon after birth, cattle may be infected at any age. In Australia and New Zealand, infection is restricted to cattle, though natural infection has also been described in buffaloes and sheep in other countries. The virus infects a subpopulation of B lymphocytes, and proviral DNA is integrated to a number of sites on the cellular DNA¹ The structural proteins of the virus include internal proteins (p24, p15, p12 and p10) and envelope glycoproteins (gp51 and gp30). Eight independent epitopes of gp 51 have been identified.

2. Clinical Signs

Infected cattle have a persistent humoral response to the viral structural proteins but most remain healthy for life. Some time after infection, about 30% of infected cattle have a persistent lymphocytosis, characterised by an increase in the number of circulating B lymphocytes. Less than 5% of infected cattle develop lymphomas and about 50% of these also have a lymphoblastic leukaemia². The development of lymphomas is a very infrequent response to BLV infection. Most cases occur in adult cattle 3–10 years of age and there may be multiple cases in some infected herds.

The clinical picture varies according to the organ system involved. The animal is often emaciated and may show signs suggestive of disorders of the respiratory, circulatory, digestive, reproductive, urinary or nervous system. Superficial lymph nodes are generally enlarged and rectal examination may reveal enlarged nodes in the pelvic cavity. In cattle with lymphoma, about 50% have a lymphoblastic leukaemia, 30% a lymphocytosis with an increased number of B cells and 20% have normal lymphocyte counts. About 30% of clinically normal, BLV-infected cattle have persistent lymphocytosis. Death invariably follows within 1–5 months of the onset of clinical signs.

3. Epidemiology

The virus is spread both within and between herds mainly by horizontal transmission. In utero transmission does occur but is generally of minor significance. Cattle become infected only by exposure to virus-infected lymphocytes in blood, secretions and excretions³ As infection can be transmitted by a very small volume of blood^{4,5} iatrogenic transmission may occur through the use of blood-contaminated needles⁶, instruments for tattooing⁷ or dehorning⁸ and rectal palpation using contaminated gloves⁹. Milk from infected cows often contains BLV-infected cells¹⁰ and feeding of infected milk to calves is a common cause of infection⁵. However, calves can be protected against infection if they are fed colostrum from an infected cow for not more than 24 hours. Embryos can be transferred from BLV-infected donors without risk of transmitting infection to either the recipients or the calves¹¹. Attempts to transmit infection to sheep using semen, urine, nasal secretions, skin scrapings and saliva have been unsuccessful¹². Whether insect vectors transmit BLV is unknown¹³.

4. Occurrence and Distribution

BLV has a worldwide distribution. In Australia, EBL was first diagnosed on haematological grounds in animals with either persistent lymphocytosis or lymphoblastic leukaemia¹⁴. Following isolation of the virus¹⁵ and the development of serological tests to detect antibodies to BLV¹⁶, the infection was found to be widespread in Queensland dairy cattle herds¹⁷. The Queensland Department of Primary Industries introduced an EBL accreditation scheme in 1983, which was successful in eradicating BLV infection from individual dairy herds. Since 1993, testing has been progressively adopted in dairy herds throughout Australia. The introduction of enzyme-linked immunosorbent assays (ELISAs) to test bulk samples of milk, as well as individual milk and serum samples, has accelerated the identification of infected dairy herds in all States. A national EBL eradication programme is advanced, with few infected herds now remaining nationwide. The current situation in individual states can be found at <u>www.aahc.com.au</u>. There has been no attempt to eradicate EBL from beef cattle herds where the infection is usually absent or the prevalence is extremely low. The exception is the beef cattle population in tropical Australia (North Queensland, Northern Territory and the far north of Western Australia) where the prevalence can be moderately high in some individual herds.

EBL has not been a major cause of financial loss in dairy herds, except in isolated herds where there was a high incidence of lymphoma cases. There is no evidence than BLV is transmissible to humans, and the virus is readily inactivated by pasteurisation of milk. However, loss of international markets and the effect of adverse publicity on the domestic market, have the potential to be economically disadvantageous for the dairy industry, hence the motivation for the national eradication programme.

In New Zealand, there is also a national control programme that is aimed at eradication of BLV infection from dairy herds. Approximately 100 dairy herds remain infected. Within individual infected herds the prevalence is low. Infection in beef cattle is uncommon.

5. Gross Pathology

At necropsy, lymph nodes and a wide range of tissues are found to be infiltrated by neoplastic cells. Organs most frequently involved are the abomasum, heart, spleen, intestine, liver, kidney, omasum, lung and uterus. Infiltration of skin and skeletal muscle is unusual but the latter is sometimes seen in association with infiltration of the vertebral canal.

Normal histological structure of lymph nodes is often lost. Lymphoblasts and mitotic figures are common. Masses of lymphoblasts accumulate in the red pulp of the spleen. Infiltration of excess lymphocytes and lymphoblasts in non-lymphoid tissues often causes distortion of normal tissue structure. In leukaemic animals, abnormal numbers of lymphocytes are seen in capillaries, particularly in the liver and lung.

6. Diagnostic Tests

BLV infection is commonly diagnosed by the detection of antibodies in serum or milk samples. Antibodies are detected in the serum of most animals within 2–8 weeks of infection. Maternally-derived antibodies may take up to 7 months to disappear. There is no known way of distinguishing passively transferred antibodies from those resulting from active infection. The serological methods currently used for detection of infected animals are the enzyme-linked immunosorbent assay (ELISA) and the agar gel immunodiffusion (AGID) test. The ELISAs have much higher sensitivity that the AGID and are more suitable for large scale testing. Consequently, the AGID test is infrequently used.

Antibody titres fluctuate and may occasionally fall to low levels, particularly in late gestation or early lactation. Antibody levels may fall below the limit of detection by AGID tests, resulting in a false negative result. The higher sensitivity of ELISAs has allowed infected animals to be detected consistently and enabling large scale testing to be carried out very economically. Both serum and milk samples can be tested by ELISA and it is possible to test a pooled sample either created from a number of individual samples or, at a herd level, bulk (tank) milk samples. The ELISAs are now the preferred serological test for detecting antibodies to BLV. All of the ELISAs in use in Australia are available as commercial kits. The performance and sensitivity of different ELISAs is described in Parts 2 & 3.

Virus isolation is not routinely used for diagnostic purposes. However, on rare occasions, it may be employed to investigate difficult cases involving animals that are seropositive or have inconclusive serological status. Virus isolation is also employed for the certification of biological materials for freedom from contamination with BLV. For virus isolation, both in vivo and in vitro screening methods are available but in vivo screening by sheep inoculation is the most sensitive method.

Sheep are very susceptible to experimental infection with BLV^{18} . Inoculation of sheep with whole blood or lymphocytes from suspect animals is the most sensitive assay for the detection of BLV. Antibodies to BLV can be detected in sheep within 2–10 weeks of inoculation. The AGID must be used to screen sheep for antibodies as most ELISAs are not suitable for this purpose. A competitive or blocking ELISA would permit testing of sheep sera but the test available has not been evaluated for screening of sheep sera. When biological materials are being tested for freedom from BLV

contamination, it may be necessary to screen a relatively large volume of specimen (20-50 mL) to ensure the detection of low levels of virus. This can be readily achieved by sheep inoculation but is not practical by in vitro methods.

Lymphocytes from infected animals produce BLV and viral antigens only after in vitro culture of cells. Virus can be detected in infected cultured lymphocytes by electron microscopy (EM) or by use of the polymerase chain reaction.

The polymerase chain reaction (PCR) has also recently been applied to the direct detection of BLV infection¹⁹. It is capable of identifying infected animals before they seroconvert and of differentiating infected calves from those that are uninfected but serologically positive due to the presence of maternally derived antibody. While it is not applicable to large-scale routine diagnostic testing, the PCR can be a useful adjunct to the sheep bioassay as a sensitive test for BLV infection.

7. References

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Enzootic Bovine Leukosis

Part 2: Diagnostic Test Methods

For EBL diagnosis or the certification of freedom from BLV infection, the tests in common use detect antibodies to BLV. The preferred tests for the detection of specific antibodies to BLV are enzyme-linked immunosorbent assays (ELISAs). ELISAs are markedly more sensitive than the agar gel immunodiffusion (AGID) test for testing serum samples to detect BLV antibodies. Due to the high sensitivity, both individual milk samples and either pooled milk or pooled serum samples may be tested. We actively discourage use of the AGID test because of its low sensitivity and the limited availability of reagents. This test is used only when an importing country specifies that the AGID test must be used.

1 Enzyme-Linked Immunosorbent Assays

1.1 Individual Animal Tests and Pooled Samples

Various ELISA techniques, both indirect and competitive, have been developed to test individual animals. There are commercial kits available for testing either serum or milk samples. Some kits have been developed to test both individual milk and serum samples with the same reagents, while other kits have been calibrated to test only milk or serum samples. All kits in use in Australia have has their minimum analytical sensitivity validated by the manufacturers against the European standard sera (E4 and P63²⁰). The kits evaluated for use in Australia and their relative performance characteristics are listed in Part 3 of this document.

Most kits have very similar sensitivity and specificity when testing samples from individual animals. These kits do, however, vary in their analytical sensitivity or detectability (ie the lowest level of antibody that can be detected). Consequently, when individual samples are pooled the number of individual samples that can be combined in the pool must not exceed the proven sensitivity of the test. These limits are described in Part 3 of the ANZSDP for Enzootic Bovine Leucosis²¹.

In general, the following conditions should apply to the use of commercial ELISA kits:

(i) All kits must be used strictly in accordance with the manufacturer's instructions;

(ii) Milk samples should be treated with a preservative (see section 3 below) and held at 4°C. Freezing of whole milk samples is not recommended.

(iii) When individual samples of milk or serum are being pooled, the volume of each sample added to the pool should be identical.

(iv) The number of individual milk or serum samples contributing to a pool should not exceed the established limits of detectability for the ELISA to be used (refer to the SCAHLS website²¹ for complete information). A conservative pool size is recommended.

(v) The pooled sample is tested in the same manner as an individual sample. However, the manufacturer's instructions should be checked closely because the test 'cut-off' limit may vary for pooled samples.

(vi) When either a positive or inconclusive result is obtained for a pooled sample, all samples contributing to the pool should be tested individually. The results for the individual samples take precedence over results for the pool even if all individual samples give negative results.

1.2 Bulk (Tank) Milk Samples

The use of ELISAs for EBL to test samples of pooled milk from a milk tank, combined with the testing of pools of individual samples, is a major reason for the success of the EBL eradication programme in Australia. Herds are monitored for infection with BLV by testing bulk milk samples at intervals, usually at least three times a year. The key criteria for the selection of the most appropriate ELISA for bulk milk monitoring for antibodies to BLV are:

(a) the herd size, and

(b) the level of sensitivity (detectability) of the preferred ELISA.

Note that the level of detectability indicated by the manufacturer is sometimes based on testing of a limited number of samples. Comprehensive evaluation and widespread field use of some of these kits in Australia and New Zealand have established conservative conditions for the testing of tank milk samples (refer to the SCAHLS website²¹ for complete information). If other kits or different sample sizes are to be used, extensive evaluation should be undertaken before routine testing is undertaken.

In all circumstances, where the size of the milking herd exceeds the level of detectability of the ELISA being used, the herd should be sub-sampled at an appropriate level. Each of the ELISAs for testing of bulk milk samples should be used strictly in accordance with the manufacturer's directions, especially the calculation of cut-off values, unless extensive evaluations have been taken to support a different cut-off level. Milk samples should be treated with a preservative (see section 3 below) and held at 4°C. Freezing of whole milk samples is not recommended. If samples are adequately preserved, storage at 4°C for several months is possible without adverse effect on antibody levels.

2 Agar Gel Immunodiffusion (AGID) Test

Before the widespread introduction of ELISAs, the AGID test was the method of choice for testing for antibodies to BLV. It is very specific and simple to perform but lacks sensitivity. The test can be used only on serum or plasma and is unsuitable for testing milk. Reagents for the AGID test are no longer produced in Australia due to the minimal need for this test. Use of the test is now actively discouraged but some importing countries may require animals to be tested with the AGID. Reagents are available from commercial sources in the USA and Europe (refer to the SCAHLS website²¹ for complete information).

The test method is as follows:

2.1 Preparation of Agarose Plates

Prepare a 0.9% (w/v) solution of agarose in 8.5% (w/v) sodium chloride solution and heat until the agarose is completely dissolved.

Dispense in petri dishes to give a depth of about 3 mm , for example, 15 mL of agarose in 85 mm diameter dishes.

Once the agarose has set, store the plates inverted in canisters at room temperature or in sealed bags at 4°C. Do not use plates that are more than 7 days old.

Cut seven-well patterns in each agarose plate and remove the plugs by suction. Each pattern consists of a central well and 6 wells in a hexagonal array around the central well. Each well is 6.5 mm in diameter (use a minimum of 5 mm) and the distance between wells is 3 mm. Plates should be used on the same day the patterns are cut.

2.2 Test Procedure

Place BLV antigen (50 uL) in the central well of each pattern on an agarose plate.

Place 50 uL of BLV antiserum in alternate peripheral wells of each pattern.

Place test sera (50 uL) in the remaining 3 peripheral wells of all patterns.

In at least one pattern per batch of plates, replace the test sera with negative, weak positive and positive reference sera.

Hold the AGID plates at room temperature in a closed, humidified chamber for 72 h.

Read the plates at 48 and 72h against a black background using a strong narrow beam of light to provide good illumination. A magnifying glass attached above the light source aids in reading the test.

Results are recorded as negative, suspect, weak positive or positive.

Negative: The reference line, which is formed between the antigen and the reference serum, continues into the well containing the test serum without bending towards the antigen well.

Positive: A line is formed between the test serum and the antigen and becomes continuous with the reference line.

Weak Positive: The reference line bends slightly towards or across the face of the well containing the test serum and may or may not form a complete line very close to the well containing the test serum.

Suspect: A sample that cannot conclusively be classified as either negative or a weak positive should be described as suspect.

Positive serum with more than one line: A positive serum may show a second precipitin line that indicates a reaction to another BLV structural protein antigen (usually p24).

Non-specific Lines: These precipitin lines are formed by antigen-antibody reactions other than BLV. These lines usually cross the BLV specific reference line. Both specific and non-specific lines can occur with the same test serum.

All sera that are read as suspect, weak positive or positive are retested to confirm the results. This is necessary because a positive reaction can be recorded for a negative serum if seepage of a positive serum occurs under the agarose or if splashing of a positive serum into a negative well occurs when reagents are being added.

3. Preservation of Milk Samples for Detection of Antibodies to Bovine Leukaemia Virus

3.1 Preservatives for milk samples, sample processing and storage

Generally, milk samples collected for some of the milk quality assays that are routinely performed at dairy factories are treated at the time of collection with a preservative. Specifically, preservatives used to treat milk samples for somatic cell counting are most suitable for samples collected for the detection of antibodies to BLV and other agents. The most commonly used preservative is bronopol, with a coloured dye added. In most circumstances, 2-3 mL of this preservative-treated milk is needed to facilitate removal or avoidance of the milk fat.

While some kits recommend complete removal of the milk fat, this is not essential. The sample to be tested must, however, be free of fat globules. This can be most readily be achieved by allowing samples to stand at 4°C overnight (or longer). Carefully remove the samples from refrigeration, taking care not to shake or invert them. After removing the lid from the container, carefully tilt the sample container. The fat layer will remain in a solid mass and will separate from the side of the sample container, giving access to the whey below. A sample can then be easily collected for testing.

3.2 Preparation of bronopol treated sample tubes

Preservative pellets are often used by dairy factories to prepare 30 mL sample tubes. One pellet is added to each tube. However, when it is necessary for a veterinary laboratory to prepare treated tubes, it is more usual to prepare 5 mL sample tubes. This is achieved as follows:

Prepare a solution by adding 25 g of bronopol (Myacide[®]) and 1 g of methylene blue to 1 L of distilled or purified water. (Sometimes bronopol is available as a 200 gm/L solution that can be diluted 1/8 to prepare the 25 g/L solution.)

Add 100 uL of the solution to each 5 mL tube and allow to dry in a fume cabinet.

Once the solution has dried completely, cap all tubes and store at room temperature.

For use, add 3-4 mL of milk to the tube and mix by inverting until the bronopol solution has completely dissolved.

Enzootic Bovine Leukosis

Part 3: Reagents and Test Kits for EBL Diagnosis in Australia and New Zealand

The list of suppliers provided below may not be exhaustive but includes all materials (complete kits and other reagents) that have been evaluated and found to be suitable for use under Australian or New Zealand conditions. These reagents and kits have only been approved for use in Australia or New Zealand for the purposes and within the limits described below. Other suppliers who have materials that may be used for the purposes described below are welcome to submit reagents and kits evaluation by contacting the Executive Officer, SCAHLS at www.scahls.org.au.

1. ELISA Kits for BLV Antibody

The kits that have been evaluated for use in Australia and New Zealand are manufactured by Bommeli Diagnostics (Switzerland), Institute Pourquier (Montpellier, France), Svanova Biotech AB (Uppsala, Sweden) and Synbiotics (Europe) (Lyon, France). The respective kits and their performance characteristics are as follows:

1.1 Tests for Individual Animals and Pooled Samples

The following kits have been used for the testing of either individual serum or milk samples, and in most cases, pools formed from the aggregation of equal volumes of a number of individual samples. Where limits of detectability have been specified for the testing of pooled samples, these limits should not be exceeded. If a kit is found to have different sensitivity, data must be submitted for SCAHLS approval before other pool sizes can be tested.

1.1.1 Institute Pourquier

a) Bovine Leucosis Serodiagnosis – Screening kit.

This test is an indirect ELISA that is suitable for testing of individual or pooled serum samples. A pool should not include more than 10 individual samples. The test is designed as a screening test as there are only test wells coated with viral antigens. Both envelope and capsid antigens are included. Positive results of the tests of both individual and pooled samples should be confirmed in a confirmatory test, such as the Bovine Leucosis Serodiagnosis – Verification kit (see 1.1.1 b, below).

b) Bovine Leucosis Serodiagnosis – Verification kit.

This kit is identical to the kit above, except that there are test wells coated with both viral antigens and cell culture control antigens.

c) Bovine Leucosis Serodiagnosis – Gp51 Serum Verification kit.

This test is an indirect ELISA where the envelope glycoprotein Gp51 is bound to the test wells by a specific monoclonal antibody. There are test wells coated with both viral antigens and cell culture control antigens. The test is suitable for testing of individual serum samples.

1.1.2 Svanova Biotech AB a) Svanovir EBL ELISA

This test is a dual well indirect ELISA and is suitable for testing of either individual or pooled serum or milk samples. The test is routinely used with alternate wells coated with viral antigens and uninfected cell culture control antigens. For testing of individual animals, a pool of either milk or serum should not include more than 10 individual samples. This test has also been used for testing of bulk milk samples (see below)

1.1.3 Synbiotics (Europe)

a) Lactelisa

This test is an indirect ELISA that is suitable for testing of individual or pooled milk samples. It is a bi-well test with alternate wells coated with viral antigens and an extract of uninfected cell cultures. The test has a proven detectability of 1/50 (that is, it will detect 1 positive in a pool of 50 samples). However, for practical reasons, pools with this test are usually restricted to 20 or 30 individual milk samples. The Synbiotics "Lactelisa T250" (see below) is an even more sensitive test and, while it can be used to test individual samples, its use in Australia has been restricted (for reasons of cost) to testing of bulk milk samples.

b) Serelisa BLV Ab Mono-blocking ELISA

This test is a blocking ELISA, using a monoclonal antibody specific to BLV. The test is used for both primary and confirmatory testing of individual and pooled serum samples. A pool should not include more than 10 individual samples.

c) Serelisa BLV Ab Mono-indirect ELISA

This test is a single well indirect ELISA that is used for screening of serum samples. It does not have a 'control' uninfected cell antigen. It is suitable for testing of individual or pooled serum samples. A pool should not include more than 10 individual samples.

d) Serelisa BLV Ab Bi-indirect ELISA

This test is similar to the Serelisa Mono-indirect ELISA but is used as either a primary or a confirmatory test for testing of serum samples. In contrast to the screening test, it is a bi-well test with the second well coated with an extract of uninfected cell cultures. It, too, is suitable for testing of individual or pooled serum or milk samples. A pool should not include more than 10 individual samples.

1.2 Tests for Bulk (Tank) Milk Samples

The indirect ELISAs used for screening of bulk milk tanks from dairy herds have a very high level of sensitivity and are able to detect a single infected animal in the herd, provided the herd size does not exceed the limits of detectability of the test. In all circumstances, where the size of the milking herd exceeds the level of detectability of the ELISA being used, the herd should be sub-sampled at an appropriately lower level.

1.2.1 Bommeli Diagnostics

a) Chekit Leucotest Milk Biphasic EIA

This is a bi-well indirect ELISA that has been designed to test both milk and serum samples. It has been evaluated in New Zealand to test bulk milk samples for herd monitoring. The test was shown to have quite high sensitivity but very poor specificity (D. Hayes, personal communication). As a result of this evaluation, although it cannot be recommended for routine 'stand-alone' use to monitor herds, it could play a role in an emergency to screen herds provided follow-up testing occurred, either with a more specific assay for tank milks or by testing individual animals. As this

test also has poor specificity when used for testing individual animals, confirmatory testing should use a different test.

1.2.2 Synbiotics (Europe)

a) Lactelisa

Until 2001, the main test used for bulk milk testing was the Synbiotics "Lactelisa" (see 1.1.3(a) above). This was the most sensitive of the assays that are designed to test milk samples until it was superseded by the Synbiotics "Lactelisa T250" (below). The standard Lactelisa is registered in Europe to detect 1 infected animal contributing to a bulk milk tank from a herd of 50 cows (that is, a test of a sample of the bulk milk will give a positive result if milk from a single infected animal is present). While it will always detect 1 positive in 50, depending on the strength of reactivity of the individual positive cow, it may detect a single positive animal in up to 180 animals.

b) Lactelisa 'T250'

The Synbiotics "Lactelisa 'T250'" has a level of detectability of about 5-fold higher than the standard Lactelisa. While the test has been registered to detect one positive animal in tank milk from 250 cows, rigorous evaluation in Australia has reduced this limit to 1 in 200 cows. Therefore, if the herd has more than 200 cows, sub-samples should be collected for testing. The upper limit of sensitivity for this test has been the detection of a single positive animal in a herd of 650.

In New Zealand, the test has been extensively evaluated to allow the screening of larger milk pools. To achieve this, an alternative test cut-off value has been set. However, these test results are not considered in isolation and also take into account factors such as the herd history, the status of individual animals in a herd and animal movements. These parameters are specific to the New Zealand dairy herd and cannot be directly transferred to the Australian situation. (D. Hayes, Personal communication)

1.2.3 Institute Pourquier and Svanova *a)LeukosisMilk Verification ELISA b)Svanovir EBL ELISA*

While the assay kits produced by the Institute Pourquier and Svanova have been used to test tank milk samples, these tests in their current format are not sufficiently sensitive to reliably detect 1 positive animal in a herd of 50 cows. The exact level of detectability of these tests under Australian field conditions has not been determined but is likely to be in the range of 1/30 to 1/40. If these kits are to be used for testing of tank milk samples, further evaluation is required.

2. AGID Test

Reagents (antigen and reference serum) are supplied in kit form by the following suppliers:

2.1 Institute Pourquier

AGID Leukosis

2.2 Synbiotics (Europe)

Leukose Bovine AGID

3. Suppliers of Commercial Reagents and Kits.

The commercial distributors of the above ELISA kits and reagents for the AGID test are:

Bommeli Diagnostics kits

Idexx Laboratories Pty Ltd Unit 20/38-46 South St., Rydalmere NSW 2116 Tel: 02 9898 7300 or 1800 655 978 Fax: 02 9310 2932

Idexx kits – AGID test

Idexx Laboratories Pty Ltd Unit 20/38-46 South St., Rydalmere NSW 2116 Tel: 02 9898 7300 or 1800 655 978 Fax: 02 9310 2932

Institute Pourquier – ELISAs and AGID

Laboratory Diagnostics Unit 8, 106 Canterbury Rd., Bankstown NSW 2200 Tel: 02 8707 4222 or 1800 023 623 Fax: 02 8707 4200

Svanovir - ELISA

Australian Laboratory Services PO Box 328, Sydney Markets NSW 2129 Tel: 02 9764 4055 or 1800 252 286 Fax: 02 9764 3533

Synbiotics kits – ELISAs and AGID

Laboratory Diagnostics Unit 8, 106 Canterbury Rd., Bankstown NSW 2200 Tel: 02 8707 4222 or 1800 023 623 Fax: 02 8707 4200

3.2 New Zealand:

Bommeli Diagnostics kits - ELISA

Idexx Laboratories Pty Ltd Unit 20/38-46 South St., Rydalmere NSW 2116 Australia Tel: 61 2 9898 7300 Fax: 61 2 9310 2932 EBL

Institute Pourquier – ELISAs

Diagnostic Bioserve PO Box 5246 Mt Maunganui, NZ. Tel: 64 7 542 2325 Fax: 64 7 542 2326

Synbiotics kits – ELISAs

Alpha-Scientific, Gribbles, 57 Sunshine Ave, Hamilton, NZ. Tel: 64 7 850 0777