

Import Risk Analysis:
Cooked Duck Meat
From Australia

15 September 2006

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Import risk analysis: Cooked duck (Anas platyrhynchos) meat from Australia

**Biosecurity New Zealand
Ministry of Agriculture and Forestry
Wellington
New Zealand**



15 September 2006

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Biosecurity New Zealand

*Import risk analysis: Cooked duck (*Anas platyrhynchos*) meat from Australia*

15 September 2006

Approved for general release

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GLOSSARY OF TERMS

AQIS: Australian Quarantine and Inspection Service.

Compartment: one or more establishments under a common biosecurity management system containing an animal subpopulation with a distinct health status with respect to a specific disease.

Duck: In this document the term is used only for *Anas platyrhynchos*, including domestic ducks and the wild Mallard duck. Specifically it does not include the domesticated Muscovy duck *Cairina moschata*.

Endemic (disease): Present or established in a country.

Exotic (disease): Foreign to, or absent from, a country.

Likelihood: This term is used to indicate the probability of an event occurring and does not refer to the possibility of an event occurring.

MAF: New Zealand Ministry of Agriculture and Forestry.

OIE: World Organisation for Animal Health

Risk analysis: The process composed of hazard identification, risk assessment, risk management and risk communication.

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1 EXECUTIVE SUMMARY

This document is a qualitative analysis of the risks posed by the importation into New Zealand of six cooked duck meat products (specified in appendix 2) produced by the Australian company Luv-A-Duck Pty Ltd.

The risk analysis framework is based on that recommended by OIE in the *Terrestrial Animal Health Code*, and in particular it follows MAF's publication *Import Risk Analysis – Animals and Animal Products* (Murray 2002).

In the Hazard Identification step, agents known to infect ducks are identified, and their potential as hazards to New Zealand's biosecurity is considered. It is concluded that the only potential hazard is infectious bursal disease virus (IBDV), which is present in Australia, can infect ducks and is unlikely to be inactivated by the cooking processes used in the preparation of the commodities under consideration. Exposure of this organism to susceptible species in New Zealand would require the feeding of scraps to poultry, which are the only species affected by this disease. However, there are no potential effects on humans or the environment of New Zealand.

Since the consequences of introduction of IBDV into New Zealand poultry would be significant, it is concluded that the risk of IBDV in the commodities is non-negligible and risk management measures are justified.

Risk management measures are recommended to establish and maintain compartmental freedom from IBDV for the farm of origin. The measures cover farm management, testing of the birds for IBD prior to slaughter, the slaughtering system, and processing of the final product.

Farm management system

The ducks from which product for export to New Zealand is derived should be sourced from a single farm (specified in appendix 1) that operates a management system based on:

- physical isolation from other poultry farms
- an 'all-in all-out' production system
- biosecurity measures covering visitors, staff, bird-proofing, feed delivery, recording, and cleaning of facilities and equipment
- auditing of the farm management system by the appropriate Australian authority.

Slaughtering

The birds should be slaughtered as the first batch following cleaning disinfection and drying of a plant that is operated by the supplier of the birds, and is operating to quality standards approved and audited by the Victorian Meat Authority

Manufacturing

The Victorian Meat Authority should be responsible for auditing the manufacturer's cooking and production procedures and in particular for ensuring that all product is heated to achieve a minimum core temperature of 60⁰C for 30 minutes and 80⁰C for 10 minutes.

Testing.

At slaughter, each batch of birds should be sampled and tested, with negative results, by a serological test for IBDV antibodies and by a polymerase chain reaction (PCR) test for IBDV in bursal tissues.

Any positive test results would be interpreted as indicating that the compartment was not free from IBD, and this would make the product non-compliant with New Zealand's certification requirements.

2 INTRODUCTION

This risk analysis is being conducted to assess the risk of a range of cooked duck products, produced by the Australian company “Luv-A-Duck Pty Ltd”.

2.1 COMMODITY DEFINITION

This risk analysis covers the following six duck meat products produced by the company Luv-A-Duck:

- Luv-A-Duck Confit – Plain.
- Luv-A-Duck Confit – Flavoured.
- Luv-A-Duck Magret (Roast duck breast) – Plain.
- Luv-A-Duck Magret (Roast duck breast) – Flavoured.
- Luv-A-Duck Roast half-duck – Plain.
- Luv-A-Duck Roast half-duck – Flavoured.

2.2 SOURCE OF PRODUCTS

The source farm of the ducks used to produce the products is described in detail in Appendix 1. It is important to note that all products for export to New Zealand will be derived from ducks from a single farm. The management and testing methods will be fully audited. All batches of ducks for export to New Zealand will comprise single batches from an all-in, all-out system and tested as described in Appendix 1.

On the source property the following organisms have occasionally been isolated from grower ducks, or the diseases they cause diagnosed in grower ducks on the original multi-age property but not on single age, all-in, all-out properties:

- Lentogenic strains of Newcastle disease virus.
- Non-pathogenic avian influenza
- Non-pathogenic *Riemerella anatipestifer*
- Pasteurella multocida*
- Mannheimia (Pasteurella) haemolytica*
- Salmonella* Typhimurium
- Mycoplasma anatis*
- Chlamydophila psittaci*

2.3 PRODUCTS AND MANUFACTURING PROCESSES

A detailed description of the products to be imported is given in Appendix 2.

As can be seen from the process flow diagrams in Appendix 2, during manufacture all products are heated to a core temperature of 60°C for at least 30 minutes and reach a core temperature of 80°C for at least 10 minutes.

2.4 RISK ANALYSIS METHODOLOGY

The framework of this qualitative risk analysis follows OIE recommendations in the *Terrestrial Animal Health Code*, and the MAF publication, *Import Risk Analysis – Animals and Animal Products* (Murray 2002).

This risk analysis includes the following steps:

- **Hazard identification** consists of two segments:
 - *Identification of agents of potential concern*: Agents of possible concern are identified and analysed.
 - *Conclusions*: Conclusions are made about which agents are potential hazards.

- **Risk assessment** comprising four segments to be addressed for each disease/agent that is identified as being a potential:
 - *Release assessment*: Describes the biological pathways necessary for the product to become infected or contaminated and estimates the likelihood of the commodity being infected or contaminated on arrival in New Zealand.
 - *Exposure assessment*: Describes the biological pathways necessary for exposure of animals and humans in New Zealand to the potential hazard and estimates the likelihood of exposures.
 - *Consequence assessment*: Identifies the potential biological, environmental and economic consequences associated with the entry, establishment or spread of the potential hazard.
 - *Risk estimation*: Expresses the likelihood of the agent entering, becoming established and causing adverse consequences.

- **Risk management** suggests sanitary measures that could be implemented to reduce the risk to an acceptable level. It includes three segments:
 - *Risk management objectives*: Risk management objectives are defined.
 - *Options evaluation*: Options for risk management is considered.
 - *Recommendations*: Risk management recommendations are made

3 HAZARD IDENTIFICATION

3.1 *Organisms of potential concern*

The hazard identification process begins by identifying the organisms that are known to be associated with ducks. This was done using the OIE listed diseases as a starting point, and including other organisms that are discussed in the scientific literature. Many of the organisms considered as a result are not be pathogenic to ducks but are included because they have been isolated from ducks and may be harmful to other species.

The significance of each organism on this initial hazard list is further considered according to the following criteria:

- 1) if it is present in New Zealand,
 - a) is it is "under official control" in New Zealand, which could be by government departments, by national or regional pest management strategies or by a small-scale programme?, or
 - b) are more virulent strains known to exist in the exporting country?
- 2) if it is exotic to New Zealand, then is it present in the exporting country?
- 3) can the commodities act as a vehicle for the introduction of the organism – in particular, is the organism likely to survive the temperatures involved in the manufacturing process for the commodities?

At each step of the above process, organisms may be removed from the list of potential hazards.

Given the nature of the commodities, there are no hitchhiker pests considered to be potential hazards.

Since the commodities are produced according to joint Australian and New Zealand food safety standards (FSANZ), there are no human food safety concerns.

The organisms in Table 1 are excluded from further consideration on the grounds that they are present in New Zealand, they are not under official control, and there is no evidence that more pathogenic strains exist in Australia.

The organisms in Table 2 are excluded from further consideration on the grounds that they are not present in Australia.

Organisms that are inactivated by cooking at the time/temperature combinations to which the commodities are subjected during processing can also be excluded from further consideration. These are listed in Table 3.

Table 1. Organisms present in New Zealand, not under official control, and for which there is no evidence of more pathogenic strains existing in Australia

Common Disease Name	Organism	Reference
Aspergillosis	<i>Aspergillus fumigatus</i>	Kunkle 2003
Avian chlamydiosis	<i>Chlamydophila psittaci</i>	OIE 2003
Avian influenza – MPAI	Avian influenza virus A	Stanislawek 1990 and 1992
Avian mycoplasmosis	<i>Mycoplasma gallisepticum</i>	OIE 2003
Avian tuberculosis	<i>Mycobacterium avium</i>	OIE 2003
Campylobacteriosis	<i>Campylobacter jejuni</i>	Anon 2000
Coliform septicaemia of ducks	<i>Escherichia coli</i>	Barnes et al 2003
Duck hepatitis B virus	Hepadnavirus	Woolcock 2003
Egg drop syndrome	Adenovirus subgroup III	Howell 1992
Erysipelas	<i>Erysipelothrix rhusiopathiae</i>	Alley 2002
Fowl cholera	<i>Pasteurella multocida</i>	OIE 2003
Intracellular yeast like infection	Intracellular yeast like organisms	Wobeser 1997
<i>M. haemolytica</i> infection	<i>Mannheimia haemolytica</i>	Howell 1992
Newcastle disease – avirulent	Avian paramyxovirus 1	Howell 1992
Pseudotuberculosis	<i>Yersinia pseudotuberculosis</i>	Cork et al 1999
Reovirus infection	Reoviruses	Howell 1992
Reticuloendotheliosis	Reticuloendotheliosis virus	Howell 1992
Salmonellosis (paratyphoid)	<i>S. Typhimurium</i> and others	ESR 2003, 2004a and 2004b
<i>S. Enteritidis</i> infection	<i>Salmonella</i> Enteritidis	ESR 2003, 2004a and 2004b

Table 2. Organisms known to be exotic to Australia.

Common Disease Name	Organism	Reference
Arizonosis	<i>Salmonella</i> Arizonae	Anon 2000
Avian influenza – HPAI	Avian influenza virus A	OIE 2003
Avian pneumovirus infection	Pneumovirus	Bell and Alexander 1990
Derszy's disease	Goose parvovirus	Anon 2000
Duck hepatitis type 2	Astrovirus	Anon 2000
Duck hepatitis type 3	Picornavirus	Anon 2000
Duck virus enteritis	Anatid herpesvirus 1	OIE 2003
Duck virus hepatitis type 1	Picornavirus	OIE 2003
Eastern equine encephalitis	Eastern equine encephalitis virus	Anon 2000
Fowl typhoid	<i>Salmonella</i> Gallinarum	OIE 2003
Newcastle disease – virulent	Avian paramyxovirus 1	OIE 2003
Ornithobacteriosis	<i>Ornithobacterium rhinotracheale</i>	Anon 2000
Rhinosporidiosis	<i>Rhinosporidium</i> spp	Mendoza et al 2001

Table 3. Organisms inactivated at the time/temperature combinations used in the manufacture of the commodities.

Common Name of disease	Organism	Inactivation by cooking?
Cryptosporidiosis	<i>Cryptosporidium bayleyi</i>	Yes (McDougald 2003)
Fowl tick fever	<i>Borrelia anserina</i>	Yes (Chia and Taylor 1978)
Intestinal spirochaetosis	<i>Angullina coli</i>	Yes (Chia and Taylor 1978)
Mycoplasmosis	Exotic <i>Mycoplasma</i> spp. (e.g. <i>Mycoplasma anatis</i>)	Yes (Bradbury 2002)
Paramyxoviruses	Avian paramyxoviruses 2-9	Yes (MAF 1999)
Pullorum disease	<i>Salmonella</i> Pullorum	Yes (Shivaprasad 2003)

The final list of organisms of potential concern in the commodities is shown in Table 4.

Table 4. Organisms of potential concern.

Common Name of disease	Organism	Potential hazard?
Bursal disease of ducks	Undefined (<i>Herpes virus?</i>)	No
Duck septicaemia	<i>Riemerella anatipestifer</i>	No
Infectious bursal disease	<i>Birnavirus</i>	Yes
Necrotic enteritis	<i>Clostridium</i> spp	No

Three of the four organisms in the above list can be excluded from further consideration for different reasons, as follows:

Bursal disease of ducks

Bursal disease of ducks has only been described once (Smyth and McNulty 1994). Although it was suspected to have been caused by a Herpesvirus, the etiological agent was not isolated. However, as this condition has never been described in Australia, it is not considered to be a potential hazard in this risk analysis.

Duck septicaemia

Although duck septicaemia due to *Riemerella anatipestifer* was recorded in New Zealand in 1974, there have been no convincing reports of this disease since then. Although meningoencephalitis in six-week-old ducks, typical of this organism, was reported in 1990, it is unclear whether *R. anatipestifer* was recovered from this case (Anon 1990). Correspondence with the Investigation and Diagnostic Centre (Wallaceville), and with specialist poultry veterinarians in New Zealand suggests that this disease is not now present in commercial ducks in New Zealand. Duck septicemia is present in Australia (Anon 2000).

The thermal stability of *R. anatipestifer* has not been extensively researched. Bangun et al (1980) demonstrated that cultures of this organism growing on agar plates were rendered non-viable after exposure to 55°C for a period of between 12 and 16 hours. Another study (Harry and Deb 1979) indicated that a suspension of *R. anatipestifer* was inactivated following exposure to 60°C for 1 hour. As the processing conditions for the commodities includes cooking to reach a core temperature exceeding 60°C for 30 minutes and reaching 80°C for at least 10 minutes, it is considered unlikely that *R. anatipestifer* would remain viable after processing (Rob Davies, VLA Weybridge, personal communication). Therefore this organism is not considered to be a potential hazard in the commodities.

Necrotic enteritis

Necrotic enteritis has only been described once in ducks (Leibovitz 1973) and its cause is uncertain. However, the same condition in chickens and turkey is caused by *Clostridium perfringens* types A or C (Wages and Opengart 2003), and it is assumed that the disease in ducks is also caused by *Clostridium spp.* *Clostridium perfringens* is a ubiquitous environmental contaminant that is frequently found in faeces, dust, soil, poultry litter and the intestinal contents of clinically normal birds (Wages and Opengart 2003). Both type A and type C are present in New Zealand (McDonald, IDC, personal communication). Therefore this organism is not considered a potential hazard in this risk analysis.

3.2 Hazard identification conclusion

The only organisms that is considered to be a potential hazard in this risk analysis is infectious bursal disease virus (IBDV).

4 INFECTIOUS BURSAL DISEASE

4.1 Hazard identification

4.1.1 Aetiological agent

Family Birnaviridae; Genus Avibirnavirus. Serotypes 1 and 2 are recognised.

4.1.2 OIE List

Listed

4.1.3 New Zealand Status

Exotic, notifiable disease (MAF 2004).

4.1.4 Australian status

Both classic and variant strains of IBDV are present in Australia (Ignjatovic and Sapats, 2002) and vaccines are regularly used (Reece et al, 1982). The virus is listed as a notifiable disease with control of wildlife reservoirs and precautions at the border. Controls applied in Australia are aimed at the highly virulent strains that do not occur in that country.

4.1.5 Source farm status

The source population has been tested for the presence of antibodies and found negative. A small sample (30 birds) has been tested on two occasions by the commercially available ELISA test for IBDV antibody (IDEXX test kit) with negative results (Williamson, 2002a).

4.1.6 Epidemiology

Chickens and turkeys are the natural hosts of IBDV (Luckert and Saif 2003). Serotype 1 virus is common in chickens throughout the world (Luckert and Saif, 2003), with the exception of New Zealand (Chai et al 2001).

Serotype 1 occurs in numerous pathotypes ranging from avirulent, through "classic" and "variant", to "very virulent" (Luckert and Saif, 2003). Serotype 1 primarily affects chickens, but natural infections in turkeys and ducks have been reported (McFerran et al, 1980).

Serotype 2 infections occur in chickens, turkeys and ducks (McFerran et al, 1980; Smyth and McNulty, 1994). IBDV type 2 is believed to be non-pathogenic.

Infection with IBDV is rare in free flying birds and in the cases that are reported it is not clear (especially in earlier reports) which serotype is involved (Smyth and McNulty, 1994; McFerran et al, 1980; Ogawa, 1998; Wang et al, 1997; Wilcox, 1983; Yamada et al, 1982; Zhou et al, 1998).

A limited study of wild Antarctic penguins reported low levels of seropositivity (about 2%) in adult Adelie penguins, and 65% seropositivity in fledgling Emperor penguins (Garner et al, 1997). A serotype 2 virus was isolated from two species of penguin in a zoo in the UK (Gough et al, 2002). Disease was not seen in any of these birds. IBDV has been isolated once from ostriches (Luckert and Saif, 2003).

4.1.7 Conclusion

IBDV is present in Australia but is not present in New Zealand. It is an exotic notifiable organism and is therefore considered to be a potential hazard in this risk analysis.

4.2 Risk assessment

4.2.1 Release assessment

IBDV is a very resistant virus that is transmitted mainly by the faecal-oral route. The virus infects lymphoid cells in the intestine and spreads to the liver, resulting in viraemia. It is distributed to other organs including the bursa. The bursal infection is followed by a second massive viraemia (Luckert and Saif, 2003). The virus may be present in the muscles and viscera, especially the bursa, for some weeks after infection (Elankumaran et al, 2002). Depending on age of infection and presence of maternal antibodies virus may persist in the tissues beyond 40 days of age (Wyeth and Cullen, 1979). The faeces may carry a high virus load.

Serotype 1 IBDV is widespread in chickens in Australia and vaccination of breeders is regularly practised (Ignjatovic and Sapats, 2002). The organism contaminates chicken farms and slaughter age chickens may be infected.

Infection of ducks with IBDV is not well studied, but disease by this organism has not been reported in ducks (Gilchrist, 2005). There are a few reports of Serotype 1 infection in ducks (McFerran et al, 1980; Wang et al, 1997; Yamada et al, 1982; Zhou et al, 1998), but in all cases the ducks were clinically healthy.

In one case IBDV was isolated from the faeces of clinically healthy ducks. Test results suggested that it was virtually identical to a vaccine strain studied in the same work (McFerran et al, 1980).

In the second case, chicken isolates of two strains of Serotype 1 IBDV were administered by nasal and oral route to ducks of varying ages from 1 to 180 days of age. No clinical signs or gross or microscopic pathology suggestive of IBD was observed in any ducks. Bursa/body weight ratios were not different from controls. All attempts to isolate IBDV from infected ducks failed. Seroconversion indicated that virus replication had occurred, but no attempt was made to detect virus shedding (Yamada et al, 1982).

The third and fourth reports are English language summaries of Chinese work, both of which appear to deal with the same investigation. Serum samples from 380 ducks produced 363 (95.5%) seropositive results (Wang et al, 1997). The other paper reported on the isolation of a Serotype 1 IBDV from these ducks (Zhou et al, 1998).

IBDV is highly heat-resistant. Heat inactivation of a field strain of IBDV (52/70) in peptone broth was assessed (Alexander and Chettle, 1998). Approximate times to reduce the infectivity by 1 log₁₀ were 18.8 min at 70°C, 11.4 min at 75°C and 3.0 min at 80°C. This work, with relatively unprotected virus, was further investigated with virus incorporated in tissues. Protein and skin in poultry meat are normally expected coagulate with rising temperature, resulting in uneven exposure of viral particles to heat through insulation and possible protection of the virus. Cooking at 80°C for 120 minutes was required to eliminate all virus (Anonymous, 1997). It is probable that IBDV would survive the cooking processes used to prepare the products.

Since IBDV is a highly heat resistant virus that has been found in tissues of chickens and has also been isolated from ducks, the likelihood that virus could be present in cooked duck meat from Australia is considered to be non-negligible.

4.2.2 *Exposure assessment*

For IBDV to infect susceptible birds in New Zealand via the commodities, they would have to consume infected or contaminated cooked duck meat. This would require the discarding of scraps cut from a package of meat that had been further trimmed before being prepared for human consumption. However, the "ready to cook" nature of the commodities considered in this risk analysis make it highly unlikely that any trimming would be carried out.

If trimming were carried out, the scraps would have to be disposed of in a way that would enable them to be consumed by susceptible avian species in New Zealand.

The likely sites for disposal of such wastes where access by susceptible avian species may occur are:

- Commercial waste tips allowing access of free-flying wild birds.
- Home disposal, allowing access of backyard poultry.

Therefore the likelihood of exposure to susceptible species in New Zealand is considered to be non-negligible.

4.2.3 Consequence assessment

IBD is considered to be one of the most significant diseases of commercial poultry worldwide (Luckert and Saif, 2003). Although the most pathogenic strains of the IBDV are not present in Australia, the strains that are present do cause significant economic losses due to reduced immunocompetence, disease and vaccination costs. In the case of entry of these strains of IBDV into New Zealand poultry flocks, the cost of eradication could be considerable.

Since disease caused by IBDV occurs only in poultry, there would be no consequences to either humans or native animal species in New Zealand.

Therefore the consequences of entry of Australian strains of IBDV into New Zealand are considered to be non-negligible.

4.2.4 Risk estimation

The likelihood of release and exposure are non-negligible and the consequences of introduction are also considered to be non-negligible. Therefore the risk of IBDV introduction is considered to be non-negligible and the organism is considered to be a hazard in the commodities.

4.3 Risk management

4.3.1 Risk evaluation

Since the risk estimate of IBDV introduction is considered to be non-negligible, and since IBDV is subject to official control in New Zealand, risk management measures are justified.

4.3.2 Option evaluation

4.3.2.1 Risk management objective

To effectively manage the risk of IBD virus, sanitary measures need to ensure that the likelihood of this virus being introduced in the commodity is negligible.

4.3.2.2 Options

The available options for risk management are:

a) Cooking at higher temperatures

IBDV is highly heat resistant, and cooking to temperatures that would inactivate the virus is not practical as product quality would be reduced to an unacceptable level.

b) IBDV free country

Australia is not IBDV free.

c) IBDV free zone

The high incidence of IBDV in chickens makes zoning impossible.

d) Farm freedom accreditation

No scheme is available for IBDV.

e) Specific offshore or on-shore processing

Processing (such as deboning, irradiation or hydrostatic pressure) cannot be relied on to ensure destruction of the virus, as measures are ineffective or unproven.

f) Compartmental freedom

In the OIE *Terrestrial Animal Health Code* zoning and compartmentalisation are procedures implemented by a country with a view to defining *subpopulations* of different *animal health status* within its territory for the purpose of disease control and/or *international trade*. Compartmentalisation applies to a *subpopulation* when management systems related to biosecurity are applied, while zoning applies when a *subpopulation* is defined on a geographical basis.

A compartment means one or more establishments under a common biosecurity management system containing an animal sub-population with a distinct health status with respect to a specific disease for which required surveillance, control, and biosecurity measures have been applied for the purpose of international trade.

By defining a single farm as a compartment, and designing the compartmentalisation case accordingly, it is possible to achieve a negligible likelihood of entry of IBDV.

Hygiene discipline is thus more likely to be observed and is more easily monitored. It is also only necessary to apply surveillance and monitoring to the designated farm.

The quantity of product to be exported to New Zealand is likely to be small and could easily be supplied by a single farm. A flock could be designated for this purpose and, after slaughter, the cooked product could be held frozen pending test results.

This option could rely on the following components being applied for the cycle preceding the batch intended for export and each export batch of birds.

The components of the recommended sanitary measures could be:

- Biosecurity management system.
- Independent auditing of husbandry, slaughter and further processing systems.
- Testing samples of birds to confirm compartmental freedom from IBDV.

An IBDV management system could recognise the following epidemiological elements:

- Egg transmission does not occur.
- Transmission is primarily by the faecal/oral route.
- IBDV infection is rare in ducks.
- Infected ducks soon develop a high prevalence of infection.
- The bursa is the principal site of virus multiplication.
- The serum titres detected by the Australian Animal Health Laboratory (AAHL) in developing the duck serological tests indicated that that the prevalence of seroconversion was high and that titres remained high for the 21 days duration of the study. It is expected that serological titres would remain high for the 42 days of life of commercial ducks.

In this case, a compartment could be a single production unit designated as the export farm. The management procedures applied to this compartment could include conditions of operation of the farm, slaughtering and slaughter premises, further processing operations, and specific diagnostic tests applied to samples of birds.

4.3.3 *Recommended sanitary measures*

The biosecurity measures recommended to demonstrate and maintain compartmental freedom from IBDV are:

i) Farm management system

All ducks from which product for export to New Zealand is derived should be sourced from a single farm (specified in appendix 1), which should operate a management system that includes the following elements:

Farm

- Farm located at least 5 km from other poultry farms.
- Single age, all-in, all-out operation (including single pick-up).
- Locked farm - residences located outside dedicated area.
- Locked and effectively bird-proofed sheds.
- Extensive hygiene clean-out between batches.
- Equipment dedicated to the export farm.
- Log book for recording of people and equipment movements.
- Records kept of production data, mortalities and relevant events.
- Shower-in and change of clothes.

- Separate feed delivery (first of the week) by washed truck or delivery tube sited outside perimeter fence.

Staff

- Staff dedicated to the export farm.
- A condition of employment is for staff to have no access to other poultry farms, backyard poultry or to pet birds.
- Educational program for staff.

The farm management system should be audited at least once each export cycle by AQIS or an independent authority approved by AQIS.

ii) Slaughtering and processing

Slaughter of birds should be done at the plant that is operated by the supplier of the birds.

Birds for export to New Zealand should be the first batch of birds slaughtered at the plant after approved end of the week cleaning, disinfection and drying.

The plant and processing should operate quality assurance systems based on:

- ISO 9000,
- The Australian Standard for construction of premises and hygienic production of poultry for human consumption (Second Edition) AS 4465: 2001
- The Australian Standard: Guide to cleaning and sanitising of plant and equipment in the food industry. AS 4709: 2001

The Victorian Meat Authority should be responsible for supervision of the plant and for auditing these procedures.

The Victorian Meat Authority should also be responsible for auditing the manufacturers cooking and production procedures as specified in Appendix 3 including certification that all products are heated to a minimum core temperature of 600C for 30 minutes and 800C for 10 minutes.

iii) Testing for IBDV

At slaughter, each batch of birds should be tested as follows:

- A randomly collected sample of birds should be tested, with negative results, by the AAHL ELISA (Appendix 3). The sample should be of sufficient size to detect a prevalence of 10% of birds with IBD titres, with a confidence of 99%.
- A randomly sample of bursa from the birds should be collected and tested for the presence of IBD RNA by a PCR test that has been approved by AAHL, with negative results. The sample should be of sufficient size to detect a prevalence of 20% with 99% confidence.

Any positive test results would be interpreted as indicating that the compartment was not free from IBD, and this would make the product non-compliant with New Zealand's certification requirements.

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6 APPENDICES

6.1 Appendix 1: Source of supply

The duck meat is produced and processed on the property of:

Luv-A-Duck Pty Ltd,
Rupps Road (PO Box 205)
NHILL VIC 3418
Australia

The property is situated on the outskirts of the small town of Nhill in western Victoria. The processing plant is licensed by the Victorian Meat Authority. Despite the plant being licensed to process poultry and rabbits, ducks are and will remain the only animals processed at this plant. This is further considered in the risk management section.

The company owns all ducks processed in the plant. They may be broiler ducks or culled-for-age breeder ducks. The processed duck output is 45,000 weekly.

Nucleus and grandparent breeder ducks are housed on a breeding property located 45 km from the processing plant.

Young breeder ducks are reared in single-age sheds on a multi-age site located 30 km from the processing plant.

Adult parent breeder ducks are housed at one location. It is a multi-age site 30 km from the processing plant.

The broiler ducks are grown under two sets of conditions. The first set comprises a number of single age, all-in, all-out sheds on various contract or company owned farms in the local area, but all located over 15 km from the processing plant.

The second is a multi-age site on the property adjacent to the processing plant. This section is progressively being converted to three isolated units operating on an all-in, all-out, single age basis. Housing on this property is in open sided sheds without bird proofing and with partial curtaining for protection against the prevailing wind. Sheds are separated from one another by a laneway about 10 m wide.

TABLE 1 summarises the operation.

Table 1. Luv-A-Duck breeding and growing operation

No	Unit	Distance Isolation	Source of replacements	Biosecurity
1	Nucleus breeders	45 km from base	Self replacing. New imports of fertile eggs from UK via Quarantine.	Shower in. Separate staff. Truck wash
2	Nucleus hatchery	On Nucleus farm	N/A	Eggs washed in chlorine disinfectant
3	Parent breeder juveniles	30 km from base	Day-olds from hatchery located on nucleus farm	Shower in. Separate staff
4	Parent breeder adults	30 km from base	18 week olds from juvenile farm	Shower in. Separate staff. Truck wash.
5	Commercial hatchery	In local township (Nhill)	N/A	Eggs washed in chlorine disinfectant. Separate staff
6	Contract Commercial meat ducks	Various sites 15 km to 200 km	Day-olds from commercial hatchery	Restricted access Separate staff
7	Company commercial meat ducks	One site 15 km from base	Day-olds from commercial hatchery	Restricted access Separate staff
8	Fattening meat ducks	500m from base	5 week old ducks moved from sites 6 & 7.	Restricted access
9	Processing plant and Office	Base site. 5 km from town centre.		AQIS supervised MSQA quality assurance program in place
10	Feed mill	In local township (Nhill)		Separate commercial supplier

- All birds are in bird-proofed sheds.
- Log book kept on each unit.
- No. 6 - each farm is single aged.
- No. 7 has four environmentally controlled sheds. Each shed is single aged.

6.2 Appendix 2: Products to be imported

Product identification

There are 6 products involved, listed below.

- 1) Luv-A-Duck Confit – Plain
- 2) Luv-A-Duck Confit – Flavoured
- 3) Luv-A-Duck Magret (Roast duck breast) - Plain
- 4) Luv-A-Duck Magret (Roast duck breast) - Flavoured
- 5) Luv-A-Duck Roast half-duck - Plain
- 6) Luv-A-Duck Roast half-duck – Flavoured

These pre-cooked, value-added products are designed for the restaurant and elite hotel trade and will be shipped in one or two 1.2 tonne AV air containers.

Each container will hold

- 400 kg of Confit (Product 1 & 2)
- 400 kg of duck breasts (Products 3 & 4)
- 400 kg of half duck (Products 5 & 6)

The whole 1200 kg is derived from 2280 ducks.

Product details

Luv-A-Duck Confit – Plain

Also called “Traditional Confit”

Comprised of Duck meat and Marinade.

Duck meat is breast and half the wing, involving the humerus bone

Marinade contains no meat and is composed of water, salt, mineral salts (451, 452, 450), dextrose, vegetable powders, canola oil.

Luv-A-Duck Confit – Flavoured

Also called “Peking Flavoured Confit”

Comprised of Duck meat, Marinade and Peking Flavour.

Duck meat is breast and half the wing, involving the humerus bone.

Marinade contains no meat and is composed of water, salt, mineral salts (451, 452, 450), dextrose, vegetable powders, canola oil.

Peking Flavour contains no meat and is comprised of Sugar, Salt, Tomato Powder {anti-caking agent (551), Soy Sauce Powder {Fermented Soy Sauce (Defatted soy beans, Wheat, Salt), Maltodextrin, Vegetable oil (Palm)}, Malt extract, Vegetable powders, Breadcrumbs, {Emulsifiers (481, 471), Preservative (282), Fermented rice, Colours (102, 110, 150, 120) Spice extract, Food Acid (330) Flavour enhancer, (635), Spice, Vegetable gum (415).

Luv-A-Duck Magret (Roast duck breast) – Plain

Also called “Roasted Magret of Duckling”

Comprised of Duck meat and Marinade.

Duck meat is breast meat with no bone or cartilage

Marinade contains no meat and is composed of water, salt, mineral salts (451, 452, 450), dextrose, vegetable powders, canola oil.

Luv-A-Duck Magret (Roast duck breast) – Flavoured

Also called “Peking Flavoured Roasted Magret of Duckling”.

Comprised of Duck meat, Marinade and Peking Flavour.

Duck meat is breast meat with no bone or cartilage

Marinade contains no meat and is composed of water, salt, mineral salts (451, 452, 450), dextrose, vegetable powders, canola oil.

Peking Flavour contains no meat and is comprised of Sugar, Salt, Tomato Powder {anti-caking agent (551), Soy Sauce Powder {Fermented Soy Sauce (Defatted soy beans, Wheat, Salt), Maltodextrin, Vegetable oil (Palm)}, Malt extract, Vegetable powders, Breadcrumbs, {Emulsifiers (481, 471), Preservative (282), Fermented rice, Colours (102, 110, 150, 120) Spice extract, Food Acid (330) Flavour enhancer (635), Spice, Vegetable gum (415).

Luv-A-Duck Roast half-duck – Plain

Also called “Roasted duck half”

Comprised of Duck meat and Marinade.

Duck meat is breast and half the wing, involving the humerus bone, plus the leg including the femur, tibia and fibula.

Marinade contains no meat and is composed of water, salt, mineral salts (451, 452, 450), dextrose, vegetable powders, canola oil.

Luv-A-Duck Roast half-duck – Flavoured

Also called “Chinese Style Roasted duck half”

Comprised of Duck meat and Marinade.

Duck meat is breast and half the wing, involving the humerus bone, plus the leg including the femur, tibia and fibula.

Marinade contains no meat and is composed of water, salt, mineral salts (451, 452, 450), dextrose, vegetable powders, canola oil.

Peking Flavour contains no meat and is comprised of Sugar, Salt, Tomato Powder {anti-caking agent (551), Soy Sauce Powder {Fermented Soy Sauce (Defatted soy beans, Wheat, Salt), Maltodextrin, Vegetable oil (Palm)}, Malt extract, Vegetable powders, Breadcrumbs, {Emulsifiers (481, 471), Preservative (282), Fermented rice, Colours (102, 110, 150, 120) Spice extract, Food Acid (330) Flavour enhancer (635), Spice, Vegetable gum (415).

Processing of products

Duck Confit products (Products 1 and 2) are:

- Vacuum sealed and cooked in an 85°C water bath to reach a core temperature exceeding 60°C for 30 minutes and reaching 80°C for at least 10 minutes.
- Chilled and packed in corrugated cardboard cartons, with a plastic liner
- Frozen.

Roasted products (Products 3, 4, 5 and 6) are:

- Cooked (browned) in a chain oven at 300°C for 15 minutes reaching a core temperature of 60°C.
- Chilled.
- Vacuum sealed and cooked in an 85°C water bath to reach a core temperature exceeding 60°C for 30 minutes and reaching 80°C for at least 10 minutes.
- Frozen

Core temperatures during cooking are probe tested.

The process flow diagrams for Confit and Roasted Product are provided in Diagrams 1 and 2 below.

Quality Assurance is based on ISO 9000 as required by Meat Safety Quality Assurance prescribed by the licensing authority, the Victorian Meat Authority.

The Australian Quarantine and Inspection Service and the Victorian Meat Authority are responsible for supervision of processing and further processing. The objectives of these authorities are to ensure adherence to the relevant Australian Standards which are:

The Australian Standard for construction of premises and hygienic production of poultry for human consumption (Second Edition) AS 4465: 2001

Australian Standard: Guide to cleaning and sanitising of plant and equipment in the food industry. AS 4709: 2001

The description of the cooking process described in the process flow diagrams below is copied from the company's internal documents and is based on the conditions of the above standards.

DIAGRAM 1
PROCESS FLOW DIAGRAM – CONFIT PRODUCTION

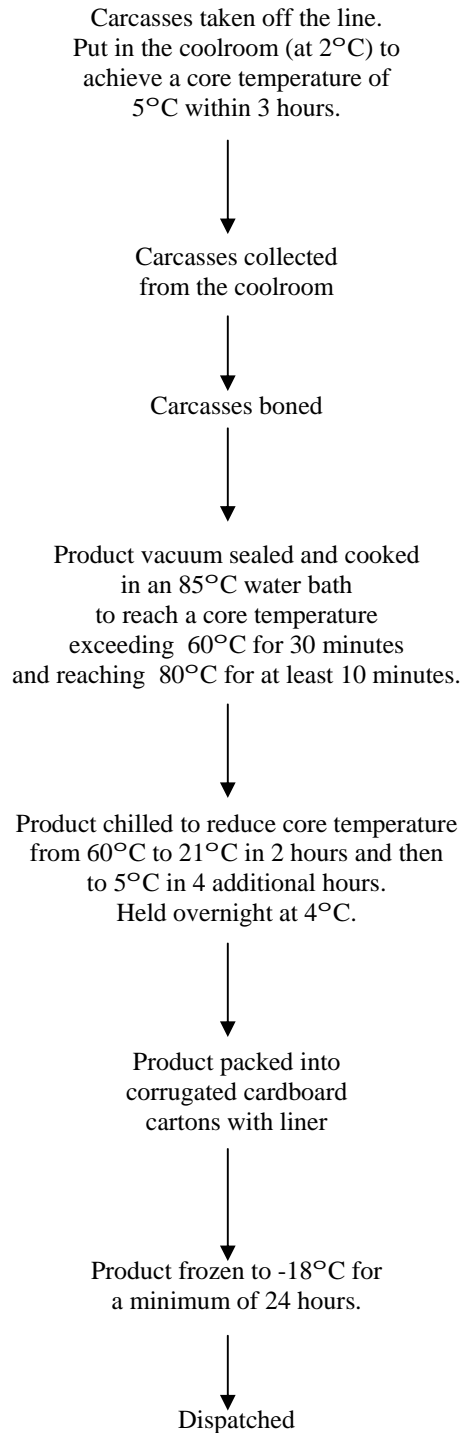
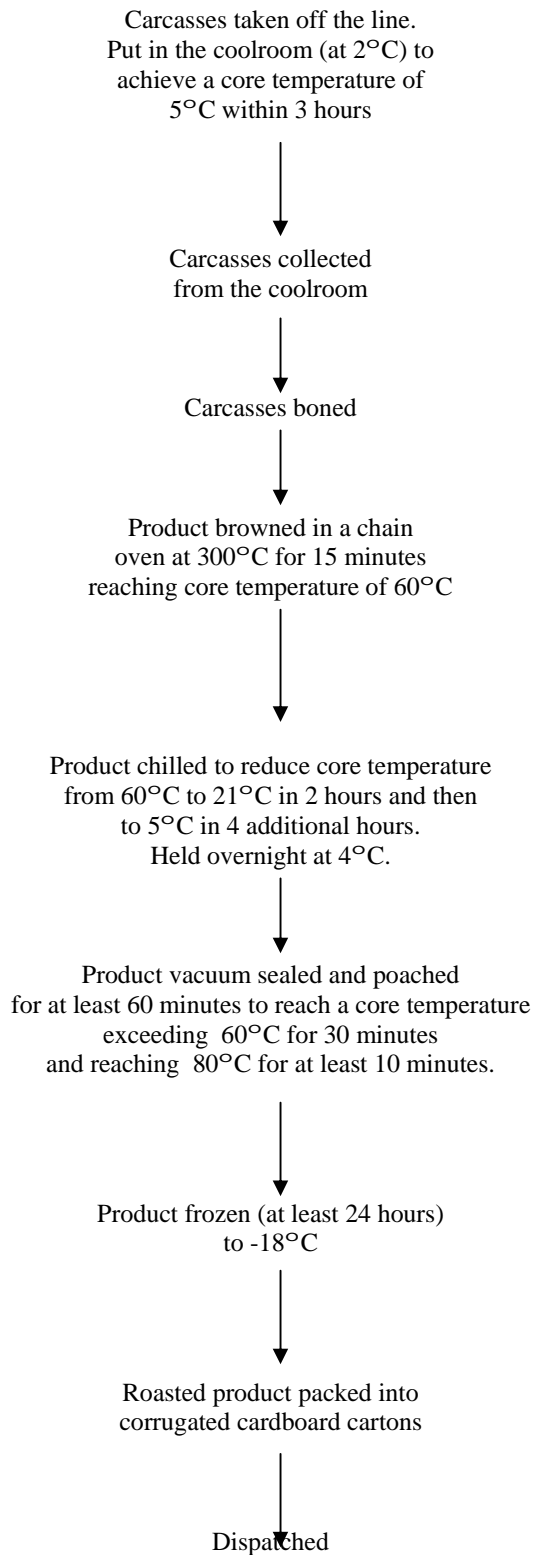


DIAGRAM 2
PROCESS FLOW DIAGRAM –ROASTED PRODUCT PRODUCTION



Cooking instructions

The cooking instructions issued with the products are:

Confit portions (Products 1 and 2):

1. To warm meat. After removing from bag, place skin side down under a hot grill for 5 minutes.
2. To crisp skin. Place skin side up until golden brown and crispy, approx 5 mins.

Magret Roast Duck Breast and half duck (Products 3, 4, 5 and 6):

To Heat in Oven. Place skin side up in an oven dish and into a hot oven until hot, approx 5-8 mins.

6.3 Appendix 3: Validation of ELISA test

Preliminary validation of a competitive ELISA, and two Serum Neutralisation Tests (1 and 2) for serological diagnosis of IBDV in ducks

Aim

The Australian Duck Meat Industry is attempting to establish an export market in New Zealand, a country whose commercial poultry industry is free of classical, very virulent, and variant strains of Infectious Bursal Disease Virus (IBDV). There is no well-validated, widely accepted or established serological test for assessment of exposure of duck flocks to IBDV. Such a test is a prerequisite for ongoing surveillance of Australian commercial duck flocks for confirmation of their freedom or otherwise from IBDV infection and to allow importing countries to manage the risk of Australian duck meat exports.

Objective

The objective of this study was to assess the serum of ducks by neutralization test (SNT) and by competitive ELISA (cELISA) prior to, and following, natural exposure to an Australian classical strain of IBDV. Assessment of the test data in “non-infected” and “infected” animals would provide preliminary validation data for these tests in detection of IBDV antibodies in duck sera.

Materials and Methods

Birds

Three week old commercial Pekin ducks were used in the study. These were derived from an elite great-grandparent and grandparent flock established 9 years ago from imported birds and located in isolation from commercial breeders, meat birds, and poultry.

A panel of duck sera (collected at slaughter of meat birds reared in isolation from poultry) were obtained from Luv-a-duck, Nhill. Specific Pathogen Free chickens were supplied by SPAFAS

Virus

An Australian classical strain of IBDV - IBDV 002/73 (AAHL reference 0406-15-0273) was used to inoculate SPF chickens by the oculo-oral route. On day 3 following challenge, bursae were collected from infected chickens, pooled and a 20% homogenate was prepared to provide the challenge inoculum. This was stored as 1 ml aliquots at -80°C until use.

Cells

CEF (chicken embryo fibroblast) cells were prepared from 11-day-old SPF chicken embryos by trypsinization. Cells were cultured in 150 cm² plastic tissue culture flasks

and used as tertiary cells in the VNT. BGM (African Green monkey) cells were obtained from ECACC.

Serology

cELISA - see Appendix 1
SNT - see Appendix 2

Virus Isolation

Virus isolations were done in 9-11 day-old, embryonated, SPF chicken eggs. The chorioallantoic membrane (CAM) was prepared and 0.2 ml of swab transport medium inoculated onto each egg. Inoculated eggs were incubated at 37°C for 7 days, after which time the CAMs were harvested and pooled. Each CAM pool was tested by antigen capture ELISA. Negative CAMS were passed through eggs a second time. Samples that tested negative by ELISA after two passes were considered negative.

Experimental Infection

Five, 3-week-old ducks and five, 3-week-old SPF chickens were infected by ocular, nasal and oral instillation of 0.5 ml of a 1:10 dilution of stock IBDV 002/73 virus. After 48 hours thirty, 3-week-old ducks and five, 3-week-old SPF chickens were placed in contact with the infected birds.

Blood samples were collected from each bird prior to exposure, and from in-contact chickens and ducks at day 10, day 14 and day 21 post exposure. Chickens were euthanased at day 6 post-challenge and blood and bursal tissue collected for serology and histological examination. Serum was harvested and stored at -20°C until tested.

Cloacal swabs were collected from directly infected birds on days 3 and 4 post infection, and from all birds on days 5, 6 and 7 post-infection. These were stored at -80°C (in swab transport medium) until the conclusion of the experiment and processed in pools from 3 animals.

Results and Data analysis

Virus Titrations

IBDV (0004-13-0050) had an infectivity titre of 2×10^4 TCID₅₀/50 µl in CEF cells and (0309-11-1501) 4×10^5 TCID₅₀/50 µl in BGM cells.

Positive Reference Population

Positive reference sera were available from between 26 and 29 ducks infected naturally with IBDV by contact both prior to infection and 10, 14 and 21 days after exposure (PE). These sera were tested by c-ELISA and two different SNTs. Pooled cloacal swabs from in-contact ducks were each positive for the presence of IBDV virus, confirming viral replication occurred in the in-contact ducks and accounting for the seroconversion.

In view of the suitability of application of the cELISA to perform the function of a screening test, emphasis for this preliminary evaluation has been given to the ELISA.

Fig 1a c-ELISA Serology of infected ducks on day 0, 10, 14 and 21 PE

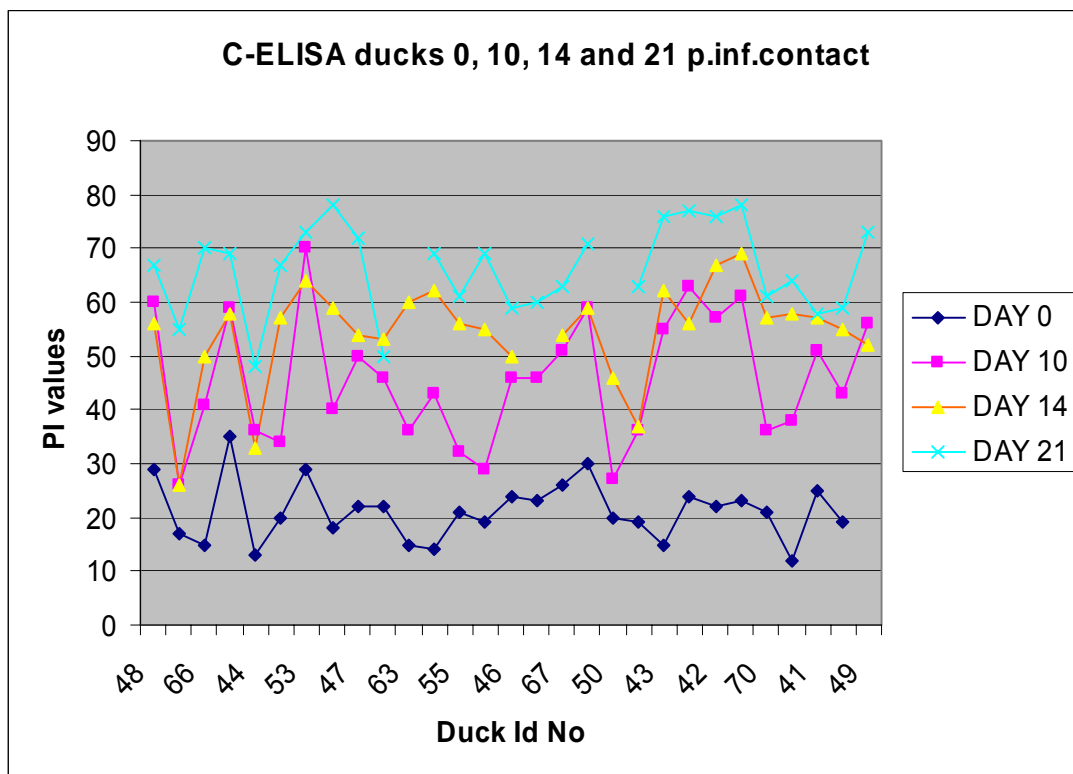


Fig 1b SNT 1 Serology of infected ducks on day 0, 10, 14 and 21 PE

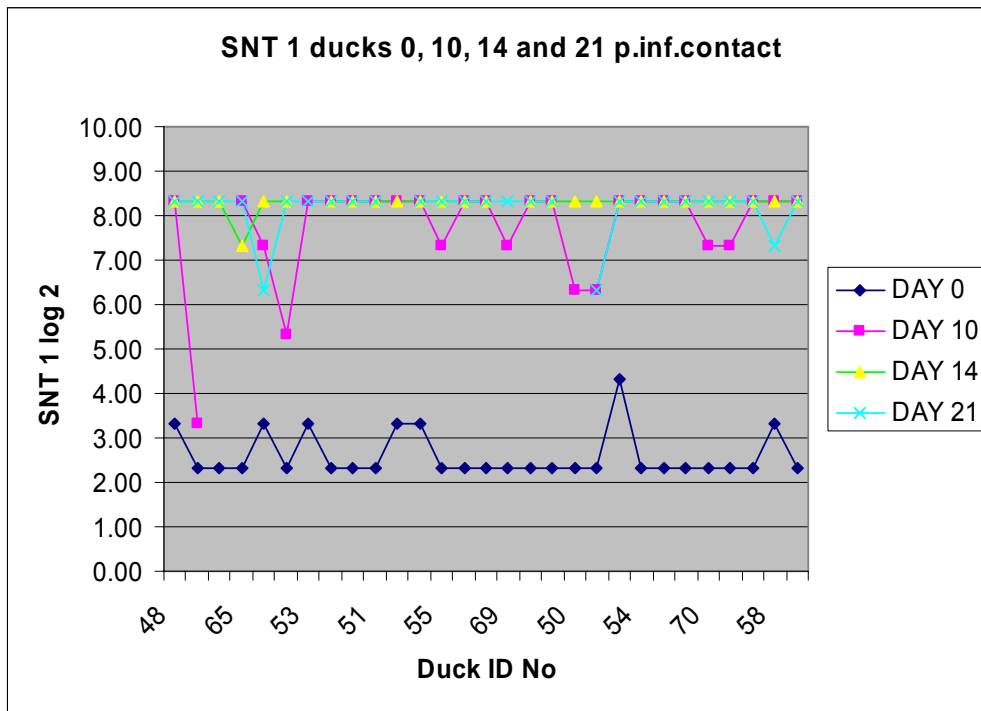


Fig 1c SNT 2 Serology of infected ducks on day 0, 10, 14 and 21 PE

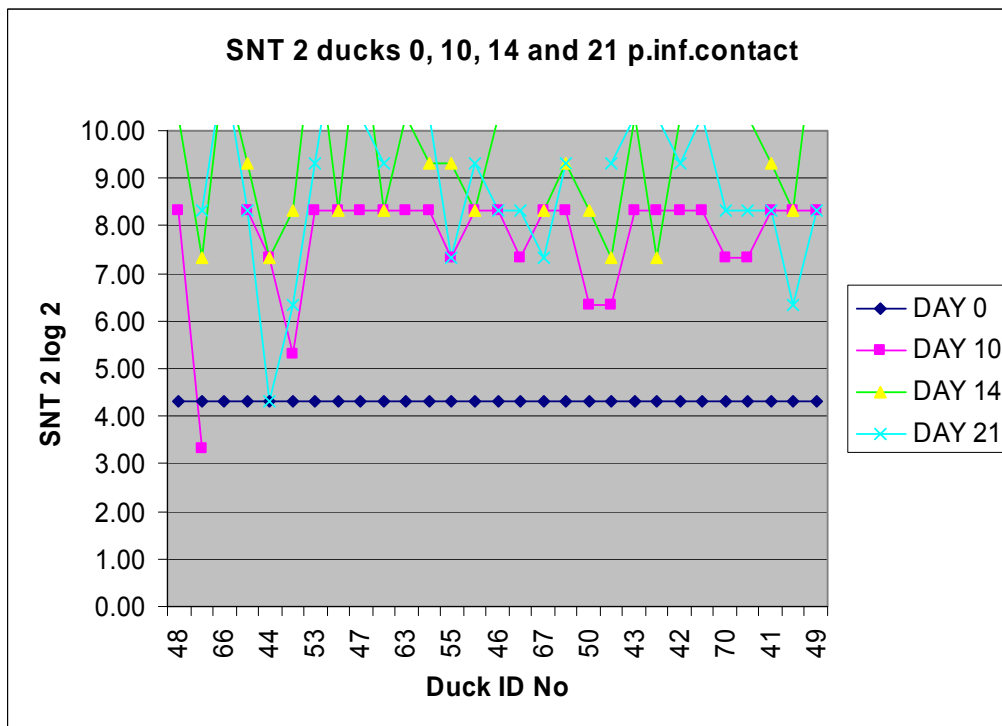


Table 1. In-contact ducks antibody response in SNT 1, 2 (*) and c-ELISA PE

	Day 0			Day 10			Day 14			Day 21		
	SNT/1*	SNT/2*	ELISA	SNT/1*	SNT/2*	ELISA	SNT/1*	SNT/2*	ELISA	SNT/1*	SNT/2*	ELISA
Mean	2.60	4.32	21.14	7.71	8.22	45.76	8.28	9.46	54.36	8.14	8.82	66.15
Standard Error	0.10	0.00	1.04	0.22	0.38	2.20	0.04	0.29	1.78	0.11	0.33	1.59
Median	2.32	4.32	21.00	8.32	8.32	46.00	8.32	9.32	56.00	8.32	8.82	67.00
Mode	2.32	4.32	15.00	8.32	8.32	36.00	8.32	10.32	56.00	8.32	8.32	69.00
Standard Deviation	0.53	0.00	5.50	1.17	2.06	11.84	0.19	1.51	9.41	0.56	1.68	8.28
Sample Variance	0.28	0.00	30.20	1.36	4.24	140.12	0.04	2.28	88.61	0.31	2.82	68.59
Kurtosis	2.71	-2.15	0.20	7.05	-0.22	-0.92	27.00	-0.57	2.77	7.76	1.17	-0.46
Skewness	1.81	-1.06	0.50	-2.52	-0.30	0.15	-5.20	0.37	-1.48	-2.96	-0.49	-0.43
Range	2.00	0.00	23.00	5.00	8.00	44.00	1.00	5.00	43.00	2.00	8.00	30.00
Minimum	2.32	4.32	12.00	3.32	4.32	26.00	7.32	7.32	26.00	6.32	4.32	48.00
Maximum	4.32	4.32	35.00	8.32	12.32	70.00	8.32	12.32	69.00	8.32	12.32	78.00
Sum	75.34	125.34	592.00	216.01	238.34	1327.00	223.69	265.01	1522.00	219.69	229.37	1786.00
Count	29.00	29.00	28.00	28.00	29.00	29.00	27.00	28.00	28.00	27.00	26.00	27.00
Confidence Level(95.0%)	0.20	0.00	2.13	0.45	0.78	4.50	0.08	0.58	3.65	0.22	0.68	3.28
Coefficient of Variation (CV)	20.31	0.00	25.99	15.11	25.05	25.87	2.32	15.94	17.32	6.85	19.04	12.52

*SNT titers are expressed as log 2

Negative reference population

Sera from 515 putatively non-infected ducks (see Materials and Methods) were examined in the c-ELISA. Basic statistical parameters for the distribution of results in the negative indicate are given in table 2a.

Table 2a. Basic Statistics Normal Duck Sera

<i>c-ELISA PI values normal duck sera</i>	
Mean	10.93
Standard Error	0.61
Median	12.00
Mode	14.00
Standard Deviation	13.89
Sample Variance	192.98
Kurtosis	0.73
Skewness	-0.53
Range	94.00
Minimum	-39.00
Maximum	55.00
Sum	5627.00
Count	515.00
Confidence Level(95.0%)	1.20
CV	127.14

Fig 2a. Frequency distribution of IBDV negative ducks in cluster

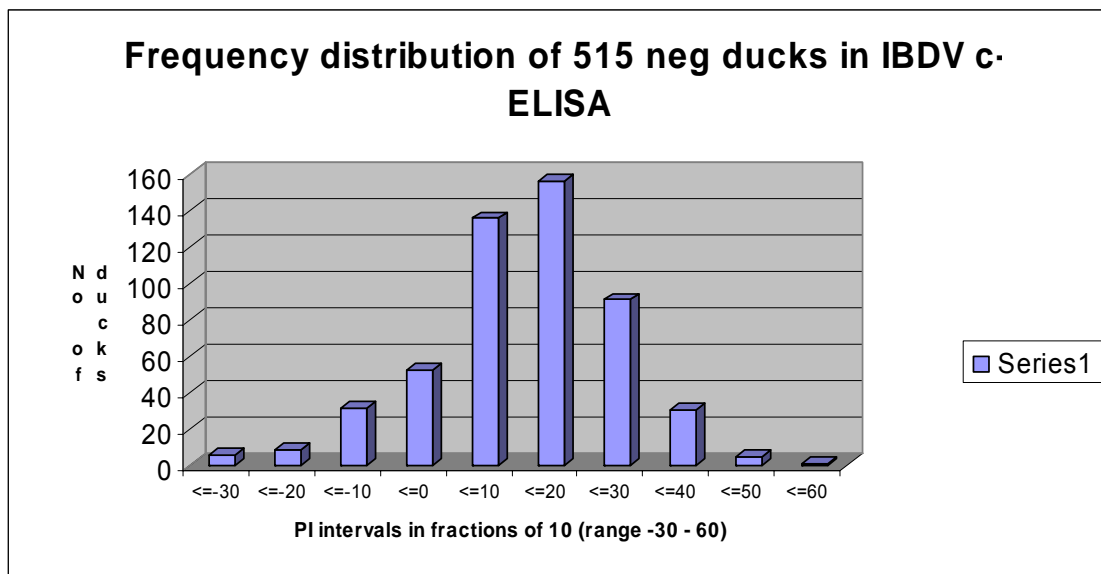


Fig 2b. Frequency distribution of IBDV negative ducks individually.

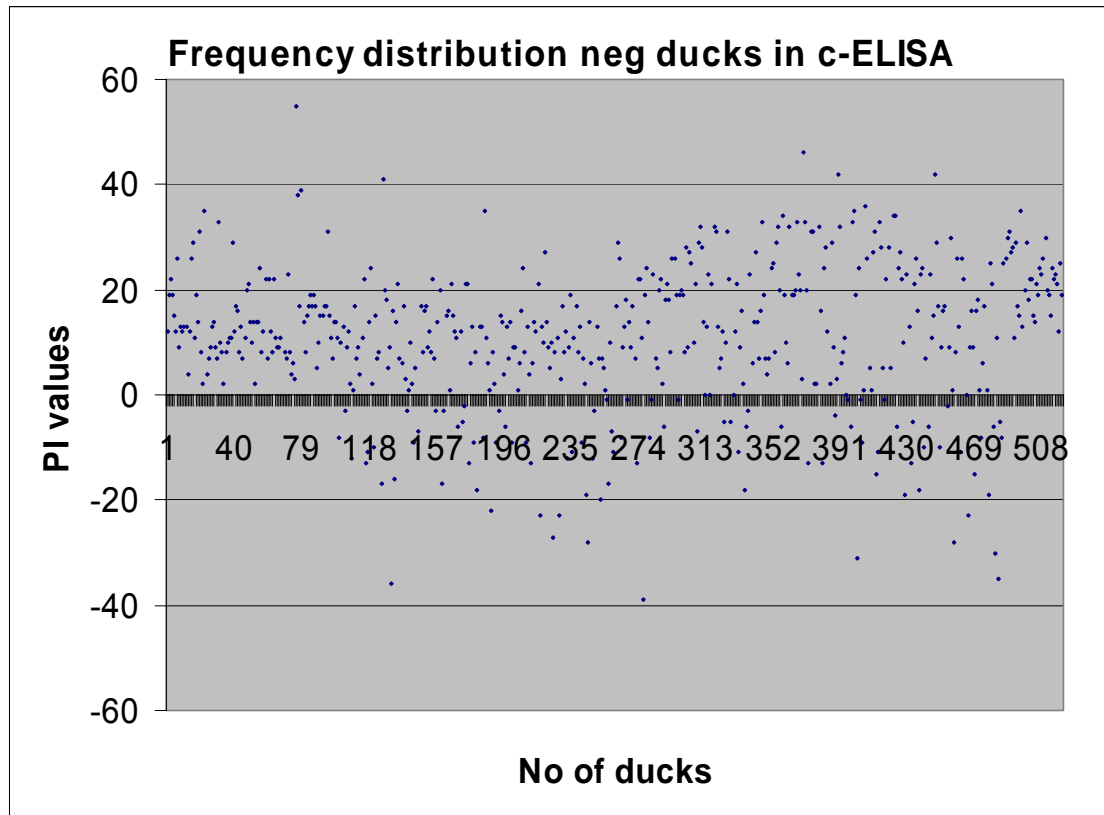


Table 2b. Frequency distribution of IBDV negative ducks

Interval	No of reactors
<=-30	5
<=-20	8
<=-10	31
<=0	52
<=10	136
<=20	157
<=30	91
<=40	30
<=50	4
<=60	1
Total	515

Precision

a) QC/QA Repeatability

Preliminary repeatability results based on internal quality controls of 5 ELISA plates are given in table 3a.

Table 3a. Repeatability c-ELISA

	<i>mab PI</i>	<i>mab OD</i>	<i>pos 1:10</i>	<i>pos 1:100</i>	<i>pos 1:1000</i>	<i>neg</i>
Mean	-0.13	0.72	78.70	74.00	69.10	1.10
Standard Error	2.50	0.03	1.24	2.33	2.58	4.50
Median	1.50	0.66	79.00	74.00	68.00	1.00
Mode	10.00	0.65	79.00	74.00	68.00	#N/A
Standard Deviation	15.82	0.16	3.92	7.38	8.16	14.22
Sample Variance	250.27	0.03	15.34	54.44	66.54	202.32
Kurtosis	5.10	0.16	0.65	-0.89	-0.56	-0.25
Skewness	-1.46	0.77	-0.44	-0.07	-0.17	-0.67
Range	90.00	0.69	14.00	22.00	25.00	43.00
Minimum	-63.00	0.48	71.00	62.00	55.00	-25.00
Maximum	27.00	1.17	85.00	84.00	80.00	18.00
Sum	-5.00	28.65	787.00	740.00	691.00	11.00
Count	40.00	40.00	10.00	10.00	10.00	10.00
Confidence Level(95.0%)	5.06	0.05	2.80	5.28	5.84	10.18
CV	12655.84	22.98	4.98	9.97	11.81	1293.09

Preliminary upper and lower control limits for internal controls e.g. mab (PI and OD values), positive (C+ in three different dilutions 1:10, 1:100 and 1:1000) and negative (C-) controls are based on mean values +/- 3 STD of 5 plates are given in Table 3b.

Table 3b. Preliminary upper and lower control limits for c-ELISA

	<i>mab PI</i>	<i>mab OD</i>	<i>pos 1:10</i>	<i>pos 1:100</i>	<i>pos 1:1000</i>	<i>neg</i>
LCL	-47.58	0.22	66.95	51.86	44.63	-41.57
UCL	47.33	1.21	90.45	96.14	93.57	43.77

b) Reproducibility

Reproducibility studies have not been undertaken as part of this validation exercise.

Cut-off

General considerations:

The cut-off partitions the results of non-infected individuals as true negative (TN) and false positive (FP), unless the test is perfectly specific. The cut-off also partitions the results of infected individuals as false negatives (FN) and true positive (TP) results, unless the test is perfectly sensitive.

Preliminary cut-offs for the IBDV cELISA are given in Tables 4a and 4b. New Zealand is free of IBDV in ducks and chickens, and introduction of the virus by infected or contaminated poultry meat could have serious consequences if the agent were able to infect local susceptible hosts and subsequently spread to commercial poultry or native birds". Accordingly, the emphasis on the test system should be to avoid false negative results. This can be also regarded as the "main purpose of the test". A desired high sensitivity can be achieved by selecting a low cut-off. In turn, the probability of getting an increased number of false positive results can be counterbalanced by a second more specific test or through sequential testing. Ultimately, the selection of cut-off points lies in the hands of the end-user and should be matched to the purpose of the test.

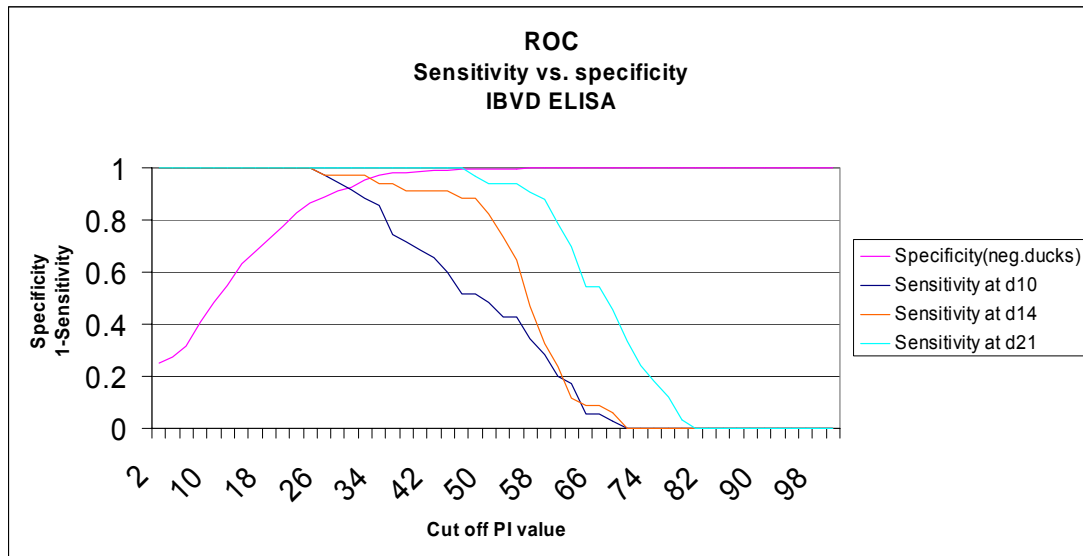
A range of different cut-offs in relation to Sensitivity (Sn) and Specificity (Sp) is displayed in table 4a. Specificity and sensitivity relate to diagnostic specificity (DSp) and diagnostic sensitivity (DSn).

Different cut-off scenarios can be helpful to maximize diagnostic performance:
ELISA screening cut-off value: for routine screening of samples a low cut-off is used favouring high sensitivity and high negative predictive value. This approach favors fractions of maximum true positive samples, and false positive samples. The cut-off value is preferably based on negative duck serum samples. For example a cut-off of 24 PI gives a DSn of 100% and a specificity of 86% (including infected and contact animals).

This serum panel comprising both true and false positive samples should then be examined using the **confirmatory cut-off value**. The higher cut-off value is set to increase the specificity and the positive predictive value of the ELISA system. To determine the confirmatory cut-off value, it must be demonstrated that samples above higher cut-off represent true positive samples. Thus, all samples originating from truly infected ducks must test positive at this cut-off setting. For example, all animals test positive at day 10 and 14 PE with a cut-off of greater than 26 PI, and at day 21 p.inf. all birds test positive with a cut-off greater than 48 PI. It is important to note that due to disease dynamics virus isolation (and PCR) may not give a positive result in the presence of circulating antibodies. Therefore, virus isolation and PCR results won't always confirm a positive infection status in an animal positive by ELISA.

The use of two cut-off values is helpful in situations where direct techniques for confirmation can't be used. It allows the reporting of results of samples as reflecting a true positive result and consequently justification for ensuing regulatory decisions. In addition, a proportion of positive samples could be identified which are regarded as suspicious. In order to minimise the risk of importing the disease this population of birds should also be identified.

Fig 4a. ROC curve for c-ELISA 0, 10, 14 and 21 PE.



Using the ROC curve a cut-off of 24PI gives a sensitivity of 100% and a specificity of 86% (including infected and contact animals).

Other considerations:

Vaccination of animals will impact on the cut-off. Should this occur the cut-off should be re-assessed.

If cost values for miss-classification of animals can be obtained a miss-classification cost term (MCT) scenario based on different cut-offs and tests (SNT1, SNT 2 and c-ELISA) can be produced following further consultation.

Specificity (Table 4a)

Non-infected ducks:

D_{Sp} was calculated using 515 normal ducks. Test validation is a continuous process and longitudinal data collection, as will occur with routine usage of the test, will allow ongoing refinement of D_{Sp}.

Sensitivity (Table 4a)

Sensitivity estimates were calculated using 26 to 29 ducks on each of several days following natural infection by in-contact challenge. Sensitivity estimates were calculated taking into consideration the advantage of having information about the “diagnostic window”; there is potential for overestimation of this parameter when transferring experimental infection data to the field situation.

Identification of commercial duck populations of naturally-occurring known positive infection status to add to this data will be difficult as clinical infection is unlikely to be recognised in the field.

Table 4a. Various c-ELISA cut-off scenarios depending on DS_n and DS_p and diagnostic window (PE) including predictive value estimates at prevalences of .01 and .1

cut-off	10 PE		14 PE		21 PE.		.1 % prev		1% prev	
	DS _n	DS _p	DS _n	DS _p	DS _n	DS _p	PPV*	NPV*	PPV**	NPV**
20	1.0000	0.7553	1.0000	0.7553	1.0000	0.7553	0.004	1.000	0.040	1.000
25	1.0000	0.8816	1.0000	0.8816	1.0000	0.8816	0.008	1.000	0.079	1.000
30	0.8966	0.9320	0.9643	0.9320	1.0000	0.9320	0.015	1.000	0.129	1.000
35	0.8276	0.9845	0.9286	0.9845	1.0000	0.9845	0.061	1.000	0.394	1.000
40	0.6207	0.9903	0.8929	0.9903	1.0000	0.9903	0.093	1.000	0.510	1.000
45	0.5172	0.9961	0.8929	0.9961	1.0000	0.9961	0.205	1.000	0.722	1.000

Prevalence *.01, **.1

PPV Positive predictive value

NPV Negative predictive value

Table 4b. Preliminary cut-offs using the mean plus 2 or 3 STD of 29 negative duck sera for SNT 1 and 2, and 515 negative duck sera for the c-ELISA.

	x	2STD	3STD
SNT 1*	2.60	3.65	4.18
SNT 2*	4.32	4.32	4.32
c-ELISA	10.93	38.71	52.60

*Values for SNT are expressed as log 2

Positive predictive value (PPV) and negative predictive value (NPV)

General considerations

Correct interpretation of test results is the ultimate goal. The predictive value reflects the proportion of animals that test positive/negative in an assay and are truly infected/uninfected. The PPV rather than the NPV of a test is a function of the prevalence of the disease. If the prevalence of IBDV in the target population is very low e.g. 1% or .1% this will have a substantial impact on the positive predictive value of the test even if it has good DS_n and DS_p parameters.

For example, if we assume that the test has 99% DS_n and 99%DS_p its predictive value at a given prevalence of 1% (or 0.1%) will be 50.3 (or 9.1 %). This means that at a prevalence of 1% a positive test result will be a false positive in every second case and at a 0.1 prevalence 11 out of 10 positive test results will be false positives.

Diagnostic problems or large sampling requirements related to low prevalence can be compensated by sampling design or by combining multiple diagnostic assays into parallel or serial testing regimes. The selection of the assays, the sampling process, the combination of multiple assays into a testing regimen and the interpretation rules for the results define the diagnostic process.

Specific considerations

On the basis of the history of the source of the ducks of interest, location of the property remote from chicken meat and egg industries, and other epidemiological considerations then the prevalence of IBDV infected birds likely to be very low (<1% or even less, 0.1 %). When considering the test result from a single sample, a low prevalence will dramatically lower the PPV and increase the probability of getting false positive results.

In table 4a, PPV and NPV estimates are given for two different prevalence estimates e.g. 0.1 and 1% in relation to the performance characteristics of the test (DSn and DSp). The influence of prevalence is evident, e.g. at a cut-off of 20% and with an estimated prevalence of .1% the test has a PPV of .4%. That means that in over 99.96% of the times a positive test will reflect a false positive result. At the same time the NPV is 100%. When the prevalence estimate rises to 1% the same test at a setting of 20% cut-off will have a PPV of 4%, which means that still in more than 96% a positive test result will be a false positive result.

However, estimates of PPV and NPV should reflect the likely realities of a disease profile in a flock e.g. either the prevalence will be close to 0% (which is the case in a disease free flock) or the prevalence will be close to 30%-50% (which will be the case in an infected duck/chicken flock); probabilities will vary between single testing and multiple testing. The PPV and NPV calculated above relate to testing of one animal. In the context of this particular exercise several birds e.g. 5-20 or more would be tested. This approach will change the equation considerably.

Probability of detection of a condition

Given a population size of 3000 animals, table 6a indicates the number of samples needed at different levels of prevalence and probabilities of detection. Bearing in mind the likely biological behaviour of IBDV should it enter the duck flock in question, an appropriate disease prevalence and % probability of detection can be selected from this table to provide an estimate of suitable sample size.

Table 6a. Probability of detection of a condition

	Pop. 3,000												
Prevalence	50%	25%	20%	15%	10%	7.5%	5%	2.5%	1%	0.5%	0.1%	0.05%	0.01%
99% prob det	7	17	21	29	44	59	89	177	425	792	2,353	2,970	2,970
95% prob det	5	11	14	19	29	39	58	117	284	542	1,895	2,850	2,850
90% prob det	4	9	11	15	22	30	45	90	221	426	1,607	2,701	2,701
80% prob det	3	6	8	10	16	21	32	63	156	305	1,246	2,401	2,401
70% prob det	2	5	6	8	12	16	24	48	118	231	992	2,100	2,100
60% prob det	2	4	5	6	9	12	18	36	90	178	790	1,800	1,800
60% prob det	2	3	4	5	7	9	14	28	69	136	619	1,501	1,501

Addendum 1. Competitive ELISA Method

Coat NUNC Maxisorp plates with 50 µl of IBDV antigen (0108-24-0273) diluted 1:1000 in ELISA coating buffer

Incubate at 37°C for 1 hour on a plate shaker at 500 RPM.

Wash plates 4 times with PBST.

Dilute each test serum, and the negative control serum (9903-24-0168) 1:10 in PBST containing 1% skim milk powder.

Dilute the positive control serum (0201-03-1630) 1:10, 1:100 and 1:1000 in PBST containing 1% skim milk powder.

Add 50 µl of each serum dilution to 1 or 2 wells of a coated microtitre plate.

Add 50 µl of each control serum dilution to 2 wells of a coated microtitre plate.

Add PBST containing 1% skim milk powder to 8 wells as a Mab control.

Dilute the anti-IBDV mab (0009-22-0968) 1:1000 in PBST containing 1% skim milk powder and add 50 µl to each well.

Incubate at 37°C for 1 hour on a plate shaker at 500 RPM.

Wash plates 4 times with PBST.

Dilute the anti-mouse HRPO conjugate (8908-11-1700) 1:1000 in PBST and add 50 µl to each well.

Incubate at 37°C for 1 hour on a plate shaker at 500 RPM.

Wash plates 4 times with PBST.

Add 50 µl of TMB substrate to each well and incubate for 5 minutes at room temperature on a plate shaker at 500 RPM.

Stop the reaction by the addition of 50 µl of 1M H₂SO₄ to each well.

Read the optical density at 450 nm on a plate reader. Calculate the percentage inhibition or each serum

Percentage inhibitions of greater than 40% are considered positive.

Addendum 2. Virus Neutralization Method

Add 50 µl of EMEM to every well of a flat-bottom, tissue culture grade, microtitre plate. Serial, 2-fold dilutions of test sera are made starting at 1:5 and continuing to the end of the row or column.

A known positive serum and negative serum titration are included in each test. IBDV (XXX) is diluted to contain 100 TCID₅₀/50 µl and 50 µl added to each well. A back titration is included in each test to ensure the correct amount of virus is used.

Plates are incubated at 37°C in a humid atmosphere containing 3 – 5% CO₂ for 1 hour.

CEF or BGM cell suspensions are prepared and adjusted to contain 10⁵ cells/ml. 100 µl of cell suspension is added to each well.

Plates are incubated at 37°C in a humid atmosphere containing 3 – 5% CO₂ for 7 days after which they are examined for the presence of specific viral CPE.

The serum antibody titre is the reciprocal of the highest dilution to completely neutralize the growth of the virus.

As a confirmation of the endpoints a peroxidase linked assay can be carried out.