Australian Government

Biosecurity Australia

23 November 2005

CONDITIONS FOR THE IMPORTATION OF FERTILE EGGS (DOMESTIC TURKEY)
(Adopted Dec 1998, amended July 2004, amended November 2005)

1. DOCUMENTATION

a) Prior permission in writing to import fertile turkey eggs must be obtained from the Australian Quarantine and Inspection Service (AQIS). The completed application should be posted or faxed to AQIS - Live Animal Imports in Canberra.

AQIS - Live Animal Imports GPO Box 858 Canberra ACT 2601 Australia	Fax 61 2 6272 3110 Phone 61 2 6272 4454

b) All consignments must be accompanied by a "Permit to Import" and the appropriate Certificates which must not be modified without the written permission of AQIS. These documents must be provided to the Australian Quarantine Officer at the port of entry.

c) Certification and post arrival quarantine requirements vary, depending on the disease status of the country of origin, and vaccination status of the source flock. A key which will assist in determining the appropriate requirements for a particular set of circumstances is included at Appendix 1.

2. ELIGIBILITY

a) Approved countries

Importation is only permitted from countries approved by AQIS. To be considered for approval, countries need to demonstrate an effective veterinary service, have in place appropriate surveillance programs for avian diseases and practice a policy of active eradication by stamping out of all outbreaks of virulent Newcastle disease and avian influenza. AQIS may also consider the pathogenicity of strains of Newcastle disease and avian influenza known to be present in a prospective exporting country, in determining the quarantine conditions to be applied.

b) Highly pathogenic avian influenza (HPAI)

Importation is only permitted from countries officially free of highly pathogenic notifiable avian influenza in poultry, as defined in the OIE Terrestrial Animal Health Code.

c) Outbreaks of Newcastle disease (ND) and avian influenza (AI)

Definition of Occurrence of Newcastle disease or avian influenza:

For the purposes of this protocol, reference to a time interval after the occurrence of ND or AI is to be interpreted as the stipulated time interval after the last case of the disease has been reported and following the completion of a stamping-out policy and disinfection procedures. Thus, the occurrence of a disease includes the stamping out and disinfection procedures.

3. REQUIREMENTS RELATING TO THE SOURCE FLOCK

a) The source flock must not have been vaccinated against avian influenza. Vaccination of the source flock against Newcastle disease is permitted, but must not have been conducted within 10 weeks of the date of commencement of pre-collection testing. The source flock may be vaccinated against avian pneumovirus (APV), also known as turkey viral rhinotracheitis, with a single dose of vaccine at 16 to 18 weeks of age. The source flock, if vaccinated against Newcastle disease or APV, will require additional testing, to demonstrate no significant rise in antibody titres. Where any bird in the source flock has been vaccinated against Newcastle disease or APV, the entire flock shall be considered to have been vaccinated and requirements for vaccinated source flocks shall apply. There are no restrictions concerning the vaccination of the source flock against infectious bursal disease and/or Marek's disease.

b) The eggs shall be laid by a source flock which has a maximum age range of six weeks, the youngest birds being not less than 35 weeks old when eggs are collected, and which has been a closed flock from the onset of sexual maturity.

c) The source flock shall be housed in secure rodent-proof and bird-proof buildings and shall be isolated by 400 metres from all poultry unless these are shown by testing to be of a health status equal to the source flock.

d) The source flock may be exempted from testing for specified diseases where AQIS is satisfied that an official flock health monitoring program provides sufficient assurance of freedom from disease.

4. EGG COLLECTION AND TRANSPORT

a) The eggs shall be collected, indelibly marked and dispatched under the supervision of a Government Veterinary Officer of the country of export. The eggs shall undergo fumigation or disinfection and then shall be packed and sealed in air-tight, leak-proof containers for transport to Australia.

b) The eggs shall be packed in such a way that there will be no leakage in the event of the eggs breaking during transport.

c) The eggs must be consigned to Australia by air, by a route approved by AQIS. They may be accompanied in transit by other eggs or birds only with the approval of AQIS. Any transhipment requires the prior approval of AQIS.

d) In the event of a consignment arriving in Australia in an unsealed container, or in a container the seal of which has been broken, or with inadequate certification, the consignment may not be permitted entry into Australia.

5. QUARANTINE

a) The imported eggs shall be hatched in quarantine at either the Torrens Island Animal Quarantine Station or Quarantine Approved Premises (QAP). The quarantine flock of turkeys hatched from these eggs will remain in quarantine for a period of 9 weeks, and will only be released subject to satisfactory results of a program of testing during quarantine as prescribed by AQIS.

b) AQIS may approve a private quarantine facility based on criteria set out in AQIS' guidelines on the location and construction of such a facility. The use of the facility for the quarantine of hatching eggs shall be subject to Quality Assurance based-systems approved by AQIS; approval will be dependent on the importer agreeing to comply with policies, procedures and specifications set out in an Approved Quarantine Directive Manual.

c) In circumstances where a consignment of eggs is permitted into a partially HEPA filtered post-arrival quarantine facility, the facility must be so constructed as to ensure that the eggs during incubation and hatching, and the turkeys after hatching, are contained within a ventilation system that is HEPA filtered until the successful completion and reporting of the results of all post-egg collection testing of the source flock.

d) In the circumstances where a consignment of eggs is permitted into a fully HEPA filtered post-arrival quarantine facility, the facility must be so constructed as to ensure that the eggs during incubation and hatching, and the turkeys during the entire nine weeks of brooding, are contained within a ventilation system that is HEPA filtered.

e) A sentinel flock of chickens shall be hatched and reared concurrently with the imported flock (in PAQ) in a ratio of 1 sentinel to 50 quarantine birds. In the case where the 1:50 ratio results in the number of sentinel chickens being less than 100, a minimum of 100 sentinel chickens shall be reared concurrently with the quarantine flock.

f) In these requirements, the word 'disease' means a disease as listed below:

Arizona disease (*Salmonella* Arizona)
Avian influenza
Avian paramyxovirus type 2 infection
Avian paramyxovirus type 3 infection
Fowl typhoid (*Salmonella* Gallinarum)
Infectious bursal disease
Mycoplasma iowae infection
Newcastle disease
Ornithobacterium rhinotracheale infection
Pullorum disease (*Salmonella* Pullorum)
Runting/stunting syndrome
Salmonella Enteritidis infection
Turkey Lymphoproliferative disease
Turkey meningoencephalitis
Turkey viral hepatitis
Avian pneumovirus infection

g) Specifications for the tests described in Appendices 3, 4, 5 and 6 are detailed in Appendix 8. The sample size required to provide a 99% confidence of detecting a disease if there is a 5% or 0.5% disease prevalence in a source flock is indicated in Appendix 9.

6. IMPORTER'S / AGENT'S RESPONSIBILITIES

a) The importer or the agent coordinating the importation must be Australian based and must nominate a person who will be accessible to Departmental officers if any problems or emergencies arise.

b) The importer should be aware that if, during the process of quarantine, it is found that the pre-export testing or certification requirements have not been fully met, the consignment may be re-exported or destroyed.

c) The agent and the aircraft operator are responsible for the safe transportation of the eggs.

d) All costs associated with the testing, transport, quarantine and veterinary supervision during the importation program must be met by the importer/agent.

e) If any eggs or birds are destroyed during any period of control, compensation will not be paid by the Government.

f) The diseases included in the attached certificates are of quarantine concern. It is the prerogative of the importer to arrange for any other health certification or testing of the fertile turkey eggs for export (or the birds hatched from the imported eggs) eg avian encephalomyelitis, fowl cholera, infectious bronchitis, Marek's disease, haemagglutinating avian adenovirus disease, haemorrhagic enteritis virus disease of turkeys, reticuloendotheliosis virus disease, *Salmonella* Hadar infection, *Mycoplasma gallisepticum* infection, *Mycoplasma synoviae* infection.

7. ACTION TO BE TAKEN FOLLOWING THE DETECTION OF A PATHOGEN IN BIRDS IN QUARANTINE IN AUSTRALIA

If any investigation or specified test indicates the presence of a pathogen/disease (as defined in Section 5(f)) in the quarantine flock (including sentinel birds), AQIS shall be notified and the flock shall remain in quarantine. At the discretion of AQIS and in consultation with the laboratory carrying out the investigations or test, and where necessary, other relevant authorities, further investigations and additional testing may be carried out to ascertain the cause of the positive result. The quarantine flock may be destroyed if it is confirmed that it is infected with any of the diseases listed in Section 5(f) of these conditions or, at the discretion of AQIS, with any other pathogen. Any decision by AQIS shall be made in consultation with the Australian States, industry and scientific organisations.

8. REVIEW

The conditions of importation may be reviewed if there are any changes in the import policy of the exporting country or at any time at the discretion of the Director of Animal and Plant Quarantine.

ROBYN MARTIN
General Manager
Animal Biosecurity

APPENDICES

Appendix 1. Key for determining post-arrival quarantine requirements.

Appendix 2. Declaration by Owner or Manager of the Source Flock.

Appendix 3. **First** Veterinary Certificate Relating to Export of Hatching Eggs of Domestic Turkeys to Australia (**Source flock not vaccinated** against ND).

Appendix 4. **First** Veterinary Certificate Relating to Export of Hatching Eggs of Domestic Turkeys to Australia. (**Source flock vaccinated** against ND).

Appendix 5. **Second** Veterinary Certificate Relating to Export of Hatching Eggs of Domestic Turkeys to Australia (**Source flock not vaccinated** against ND).

Appendix 6. **Second** Veterinary Certificate Relating to Export of Hatching Eggs of Domestic Turkeys to Australia (**Source flock vaccinated** against ND).

Appendix 7. Certificate from the Supervising Veterinary Officer, Torrens Island Quarantine Station or the Australian Government Veterinary Officer Supervising the Quarantine Approved Premises.

Appendix 8. Approved Tests For The Importation Of Fertile Eggs.

Appendix 9. Sample size required to provide a 99% confidence of detecting a disease if there is a 0.5% or 5% disease prevalence in a source flock.

APPENDIX 1.

Key for determining post-arrival quarantine requirements

COUNTRY APPROVAL

1	Does the country have an effective veterinary service?	YES	GO TO 2
		NO	IMPORT PROHIBITED
2	Does the country have effective surveillance programs for Newcastle disease (ND) and avian influenza (AI)?	YES	GO TO 3
		NO	IMPORT PROHIBITED
3	Does the country practice stamping out of all outbreaks of Newcastle disease and avian influenza in commercial poultry or pet birds?	YES	GO TO 4
		NO	IMPORT PROHIBITED
4	Does the country have strains of ND virus or AI virus which are more pathogenic than those found in Australia, which are not routinely stamped out?	YES	GO TO 8
		NO	GO TO 5

DISEASE STATUS OF COUNTRY

5	Is the country officially free of HPAI?	NO	IMPORT PROHIBITED
		YES	GO TO6
6	Is the country officially free of ND and notifiable AI (NAI)? (6 months since the last case)	YES	GO TO 7
		NO	GO TO 8
7	Are the birds vaccinated against ND?	YES	Partial HEPA[A]
		NO	Routine[B]
8	Is there active infection with ND or NAI in the country? (outbreak within 21 days)	YES	GO TO 9
		NO	GO TO 11

ACTIVE INFECTION IN THE COUNTRY

9	Is the source flock in a declared ND- or NAI- infected zone (less than 10 kms from infected premises)	YES	IMPORT PROHIBITED
		NO	GO TO 10
10	Are the birds vaccinated against ND?	YES	Full HEPA[A]
		NO	Partial HEPA[B]

NO CURRENT ACTIVE INFECTION

11	Are the birds vaccinated against ND?	YES	Partial HEPA[A]
		NO	GO TO 12
12	Are the birds from an area previously at risk of windborne infection with ND or NAI? (less than 40 kms from previously infected premises, but more than 21 days after stamping out and disinfection)	YES	Partial HEPA[B]
		NO	Routine[B]

[A] Use certificates for source flocks, which are vaccinated against Newcastle disease (Appendices 2, 4, 6 and 7).

[B] Use certificates for source flocks, which are not vaccinated against Newcastle disease (Appendices 2, 3, 5 and 7).

NOTES:
Where birds are vaccinated against ND, paired serological tests for ND are required
Where paired serology is required (ie for vaccinated birds) sentinel birds may be used in the source flock as an alternative

DECLARATION BY THE OWNER OR MANAGER OF THE SOURCE FLOCK
(This certificate to accompany the consignment of eggs)

I, ... (please print name), the owner/manager (delete one) of the source flock from which the eggs to be exported to Australia were derived, hereby declare that:

1. The source flock has not been vaccinated against avian influenza.

2. EITHER:

   * The source flock has not been vaccinated against Newcastle disease.

   OR

   * The source flock has not been vaccinated against Newcastle disease later than 10 weeks prior to the date of commencement of pre-collection testing of the flock.

*** Delete whichever is not applicable**

3. The vaccination history of the source flock is as follows;

Disease	Date(s) of Vaccination	Type of Vaccine

4. The eggs have been laid by a source flock which has a maximum age range of six weeks, the youngest birds being not less than 35 weeks old when eggs were collected, and which has been a closed flock since the onset of sexual maturity.

5. The source flock is housed in secure rodent-proof and bird-proof buildings and is isolated by 400 metres from all poultry unless these are shown by testing to be of a health status equal to the source flock. All buildings containing feed and feeding equipment are also bird-proofed.

6. All water supplies are secure against contamination by wild birds.

7. A comprehensive biosecurity program has been in place prior to and during egg collection to minimise the introduction of disease. This included the use of dedicated staff for the source flock during this period. There has been no epidemiological contact between the source flock and any premises on which clinical Newcastle disease or avian influenza has occurred during the past 3 months.

8. The source flock has been free from clinical signs of the diseases listed below for the 90 day period prior to collection of the eggs for export to Australia and has not come into contact with any birds showing evidence of these diseases:

   Arizona disease (*Salmonella* Arizona)
   Avian influenza
   Avian paramyxovirus type 2 infection
   Avian paramyxovirus type 3 infection
   Fowl typhoid (*Salmonella* Gallinarum)
   Infectious bursal disease
   Mycoplasma iowae infection
   Newcastle disease
   Ornithobacterium rhinotracheale infection
   Pullorum disease (*Salmonella* Pullorum)
   Runting/stunting syndrome
   S*almonella* Enteritidis infection
   Turkey Lymphoproliferative disease
   Turkey meningoencephalitis
   Turkey viral hepatitis
   Avian pneumovirus infection

9. The eggs for export to Australia were collected over a period of fourteen (14) days or less. The eggs for export to Australia were collected separately to floor and dirty eggs. No floor or dirty eggs are included in this consignment of eggs for export to Australia.

10. The eggs for export were clean and were not washed or cleaned after collection.

11. After collection, the eggs for export were stacked on new egg flats so as to permit air circulation and, within 8 hours of lay, were fumigated with formaldehyde gas or disinfected with an egg sanitizer/disinfectant (provide details of procedure used).

12. The eggs for export were packed in the room in which they were fumigated/disinfected. After fumigation/disinfection and cooling to storage temperature, the eggs were packed into new crates with new, unused separators and sealed in air-tight egg boxes for transport to Australia. The eggs were handled and packed in a manner to avoid possible recontamination. The eggs were placed in plastic bags or the approved containers were lined with plastic to prevent any leakage if damage to the eggs occurs during transport. The sealed boxes were held in a cool room (less than 18 ^{o}C [65 ^{o}F] and 65% relative humidity) in isolation from other birds & eggs until dispatch.

Signature: ..... Date :.....
Owner/Manager

Name: .....

Address: .....

.....

The contents of this declaration were explained to the Owner and his signature witnessed by:

Signature: ..... Date .....
Government Approved Veterinarian *

Name: .....

Address: .....

.....

*** A Government** Approved Veterinarian is either a Government Veterinary Officer or a specially appointed veterinarian, as authorised by the Veterinary Administration of the exporting country.

[NOTE: All pages are to be endorsed with the Official Stamp.]

FIRST VETERINARY CERTIFICATE RELATING TO EXPORT OF HATCHING EGGS OF DOMESTIC TURKEYS TO AUSTRALIA
(Source flock not vaccinated against ND).
(This certificate is to accompany the consignment of eggs)

PART A: DISEASE STATUS OF THE COUNTRY OF ORIGIN

I, ... (please print name), a Government Veterinary Officer of ..... (please print country of export) hereby certify in relation to the consignment of hatching eggs identified on Australian Import Permit Number ..... that:

[NOTE : A Government Veterinary Officer is a full-time veterinary officer of the Government of the exporting country.]

1. Disease status
   1.a. .....(country of origin) is free of highly pathogenic notifiable avian influenza

   1.b. .....(country of origin) is free of the following diseases*, in commercial poultry, game birds, and pet birds.

   Newcastle disease
   Avian paramyxovirus type 2 infection
   Avian paramyxovirus type 3 infection
   Pullorum disease (*Salmonella* Pullorum)
   Fowl typhoid (*Salmonella* Gallinarum)
   Salmonella Enteritidis infection
   Avian pneumovirus infection

   *** Delete those diseases not applicable**

2. EITHER

   * The country/zone of export is currently free of clinical Newcastle disease and clinical signs related to infection with any avian influenza virus.

   OR

   * The country/zone of export is not free of clinical Newcastle disease and clinical signs related to infection with any avian influenza virus, but stamping out and disinfection procedures were completed greater than 21 days prior to the start of collection of eggs for this consignment; and no case of clinical Newcastle disease or clinical signs related to infection with any avian influenza virus have been reported in the country or zone during the egg-collection period.

OR

* The country/zone of export is not free of clinical Newcastle disease and clinical signs related to infection with any avian influenza virus but the source flock is located on premises which are more than 40 kilometres from the nearest clinical case. Clinical Newcastle disease or clinical signs related to infection with any avian influenza virus have not occurred on any premises managed, owned or operated by the same company, group or individual as any of the source flocks, or on any premises associated with[a] the source flock within the 6 months previous to the start of collection of eggs for this consignment.

*** Delete whichever statements are inapplicable.**

[a] "associated with" includes the use of common feed trucks, dead bird pickups, servicemen etc.

3. After due enquiry I am satisfied that the source flock has not been vaccinated against Newcastle disease/avian influenza.

Signature: ..... Date: .....
Government Veterinary Officer

Name: .....

Address: .....

.....

PART B: FLOCK STATUS AND DISEASE TESTING

I, ... (please print name), a Government Approved Veterinarian of ..... (please print country of export) hereby certify in relation to the consignment of hatching eggs identified on Australian Import Permit Number ..... that:

1. The source flock, from which the eggs to be exported to Australia were derived, has been under my supervision for the previous 90 days.

2. The source flock is housed in secure rodent-proof and bird-proof buildings and is isolated by more than 400 metres from all poultry which have not been shown by testing to be of a health status equal to the source flock. Details of poultry within 400 metres of the source flock are attached.

3. The source flock has been free from the diseases listed below, during the period of 90 days prior to the collection of eggs for Australia:

   Influenza virus type A infection
   Newcastle disease
   Avian paramyxovirus type 2 infection
   Avian paramyxovirus type 3 infection
   Pullorum disease (*Salmonella* Pullorum)
   Fowl typhoid (*Salmonella* Gallinarum)
   Salmonella Enteritidis infection
   Avian pneumovirus infection

4. Pre-egg collection testing

4.a. Within 21 days before the first day of collection of eggs for export to Australia, a sample of the parent flock was tested serologically for antibody to influenza virus type A, with negative results

4.b. Within 21 days before the first day of collection of eggs for export to Australia, a sample of the parent flock was tested serologically for antibody to the following pathogens*:

   Influenza virus type A
   Newcastle disease virus
   Avian paramyxovirus types 2 and 3
   Salmonella Pullorum
   Salmonella Gallinarum
   Salmonella Enteritidis
   Avian pneumovirus

***Strike out any pathogens for which certification of country freedom has been provided under paragraph 1.b. of Part A of this certificate.**

[NOTE: In the case of testing for Influenza virus type A and Newcastle disease virus, the sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was a 5% disease prevalence in the source flock. For the other diseases listed, the sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was a 0.5% disease prevalence in the source flock (see Appendix 9). If the source flock was vaccinated against Avian pneumovirus, a random sample of 100 of these birds must be individually identified for later re-testing. The individually identified birds must be replaced randomly throughout the source flock.]

Sufficient blood was collected from each bird sampled for the performance of the required tests. Anti-coagulant was not added. The blood was allowed to clot and the serum removed.

[Note: Samples of blood may, if necessary, be incubated at 37°C for 2 hours to aid clotting and sera clarified by centrifugation. Sera may be sterilised by filtration and may be frozen. Preservatives were not added. Unless specified in a particular test, serum may not be diluted nor were samples of serum from different birds pooled.]

Where there were positive or suspicious reactors for *Salmonella* Pullorum, *Salmonella* Gallinarum or *Salmonella* Enteritidis, all of the reactors were killed and their organs cultured, and the results of the cultures are attached.

4.c. Within 21 days before the first day of collection of eggs for export to Australia, all toms used for the artificial insemination of the source flock were determined to be free of infection with *Mycoplasma iowae*. Semen from each tom used for the artificial insemination of the source flock was cultured individually and found negative for *Mycoplasma iowae*.

4.d. EITHER

4.d.1. *****Records of regular monitoring of dead-in-shell embryos, pipped eggs, etc of the source flock were examined, and all available information indicate that the flock is free from *Mycoplasma iowae*.

OR

4.d.2. *****All females in the source flock were tested by culture for Mycoplasma iowae and found to be free from the organism.

***Delete whichever is not applicable**

4.e. Within 21 days before the first day of collection of eggs for export to Australia the source flock was determined to be free of infection with *Salmonella* Arizona.

The absence of *S.* Arizona was determined by procedures to culture and isolate them from shed litter. Twenty samples were collected from each shed. Each sample was a composite sample of 3 floor and 2 nest litter samples (ie a total of 60 floor locations and 40 nest boxes per shed).

The total number of composite samples tested: .....

4.f. All tests were carried out in a government laboratory or a laboratory approved by the government of the exporting country for this specific purpose and approved by AQIS. The tests were OIE-approved tests or tests approved by AQIS - see Appendix 8. Test results are shown in the table below.

Total Number of birds in the source flock: .....

Disease agent	Test used	No of tests	No of positive results
Influenza virus type A			
Newcastle disease virus			
Avian paramyxovirus types 2 and 3			
Mycoplasma iowae			
Salmonella Pullorum			
Salmonella Gallinarum			
Salmonella Enteritidis			
Salmonella Arizona			

4.g. EITHER:

***** The source flock was not vaccinated against avian pneumovirus. .....(No of samples) were tested using the .....test, and all results were negative.

OR:

***** The source flock was vaccinated against avian pneumovirus (APV). 100 individually identified birds in the source flock were tested for APV, with individual titres recorded for each bird sampled. A list of titres is attached to this certificate. The test is scheduled to be repeated on the same sample of birds not less than 14 days after the collection of the last egg for this consignment.

*** Delete whichever is not applicable**

Signature: ..... Date: .....
Government Approved Veterinarian *****

Name: .....

Address: .....

.....

*****A **Government Approved Veterinarian** is either a Government Veterinary Officer or a specially appointed veterinarian, as authorised by the Veterinary Administration of the exporting country.

[NOTE: All pages are to be endorsed with the Official Stamp.]

FIRST VETERINARY CERTIFICATE RELATING TO EXPORT OF HATCHING EGGS OF DOMESTIC TURKEYS TO AUSTRALIA

(Source flock vaccinated against ND).

(This certificate is to accompany the consignment of eggs)

PART A: DISEASE STATUS OF THE COUNTRY OF ORIGIN

I, ... (please print name), a Government Veterinary Officer of ..... (please print country of export) hereby certify in relation to the consignment of hatching eggs identified on Australian Import Permit Number ..... that:

[NOTE : A Government Veterinary Officer is a full-time veterinary officer of the Government of the exporting country.]

1. Disease Status

1.a. .....(country of origin) is free of highly pathogenic notifiable avian influenza

1.b. .....(country of origin) is free of the following diseases*, in commercial poultry, game birds, and pet birds.

Newcastle disease
Avian paramyxovirus type 2 infection
Avian paramyxovirus type 3 infection
Pullorum disease (*Salmonella* Pullorum)
Fowl typhoid (*Salmonella* Gallinarum)
Salmonella Enteritidis infection
Avian pneumovirus infection

***Delete those diseases not applicable**

2. EITHER

* The country/zone of export is currently free of clinical Newcastle disease and clinical signs related to infection with any avian influenza virus.

OR

* The country/zone of export is not free of clinical Newcastle disease and clinical signs related to infection with any avian influenza virus, but stamping out and disinfection procedures were completed greater than 21 days prior to the start of collection of eggs for this consignment; and no case of clinical Newcastle disease or clinical signs related to infection with any avian influenza virus have been reported in the country or zone during the egg-collection period.

OR

* The country/zone of export is not free of clinical Newcastle disease and clinical signs related to infection with any avian influenza virus but the source flock is located on premises which are more than 40 kilometres from the nearest clinical case. Clinical Newcastle disease or clinical signs related to infection with any avian influenza virus have not occurred on any premises managed, owned or operated by the same company, group or individual as any of the source flocks, or on any premises associated with[a] the source flock within the 6 months previous to the start of collection of eggs for this consignment.

*** Delete whichever statements are inapplicable.**

[a] "associated with" includes the use of common feed trucks, dead bird pickups, servicemen etc.

3. After due enquiry I am satisfied that the source flock has not been vaccinated against avian influenza.

Signature: ..... Date: .....
Government Veterinary Officer

Name: .....

Address: .....

.....

PART B: FLOCK STATUS AND DISEASE TESTING

I, ... (please print name), a Government Approved Veterinarian of ..... (please print country of export) hereby certify in relation to the consignment of hatching eggs identified on Australian Import Permit Number ..... that:

1. The source flock, from which the eggs to be exported to Australia were derived, has been under my supervision for the previous 90 days.

2. The source flock is housed in secure rodent-proof and bird-proof buildings and is isolated by more than 400 metres from all poultry which have not been shown by testing to be of a health status equal to the source flock. Details of poultry within 400 metres of the source flock are attached.

3. The source flock has been free from the diseases listed below, during the period of 90 days prior to the collection of eggs for Australia:

   Influenza virus type A infection
   Newcastle disease
   Avian paramyxovirus type 2 infection
   Avian paramyxovirus type 3 infection
   Pullorum disease (*Salmonella* Pullorum)
   Fowl typhoid (*Salmonella* Gallinarum)
   Salmonella Enteritidis infection
   Avian pneumovirus infection

4. Pre-egg collection testing

   4.a. Within 21 days before the first day of collection of eggs for export to Australia, a sample of the parent flock was tested serologically for antibody to influenza virus type A with negative results.

   4.b. Within 21 days before the first day of collection of eggs for export to Australia, a sample of the parent flock was tested serologically for antibody to the following pathogens*:

   Newcastle disease virus
   Avian paramyxovirus types 2 and 3
   Salmonella Pullorum
   Salmonella Gallinarum
   Salmonella Enteritidis
   Avian pneumovirus

   ***Strike out any pathogens for which certification of country freedom has been provided under paragraph 1.b. of Part A of this certificate.**

   [Note: In the case of testing for Influenza virus type A, the sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was a 5% disease prevalence in the source flock. For the other diseases listed, the sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was a 0.5% disease prevalence in the source flock (see Appendix 9). A random sample of 100 of these birds must be individually identified for later re-testing for Newcastle disease virus and/or Avian Pneumovirus.]

Sufficient blood was collected from each bird sampled for the performance of the required tests. Anti-coagulant was not added. The blood was allowed to clot and the serum removed.

[Note: Samples of blood may, if necessary, be incubated at 37°C for 2 hours to aid clotting and sera clarified by centrifugation. Sera may be sterilised by filtration and may be frozen. Preservatives were not added. Unless specified in a particular test, serum may not be diluted nor were samples of serum from different birds pooled.]

Where there were positive or suspicious reactors for *Salmonella* Pullorum, *Salmonella* Gallinarum or *Salmonella* Enteritidis, all of the reactors were killed and their organs cultured, and the results of the tests are attached.

4.c. Within 21 days before the first day of collection of eggs for export to Australia, all toms used for the artificial insemination of the source flock were determined to be free of infection with *Mycoplasma iowae*. Semen from each tom used for the artificial insemination of the source flock was cultured individually and found negative for *Mycoplasma iowae*.

4.d. EITHER

* Records of regular monitoring of dead-in-shell embryos, pipped eggs, etc of the source flock were examined, and all available information indicate that the flock is free from *Mycoplasma iowae*.

OR

* All females in the source flock were tested by culture for *Mycoplasma iowae* and found to be free from the organism.

***Delete whichever is not applicable**

4.e. EITHER

* Within 21 days before the first day of collection of eggs for export to Australia, a random sample of 100 individually identified birds in the source flock was tested for Newcastle disease, with individual titres recorded for each bird sampled. A list of titres is attached to this certificate. After sampling, the 100 individually identified birds were replaced randomly throughout the source flock. The serological titres for Newcastle disease were not higher than $\log_2 10$, and the test is scheduled to be repeated on the same birds not less than 14 days after the collection of the last egg for this consignment.

OR

* At least 14 days prior to egg collection for this consignment, unvaccinated sentinel birds have been placed evenly throughout the source flock at a rate of 1 sentinel bird per 1000 flock birds with a minimum of 30 sentinel birds. Daily observation of these birds has been undertaken with no evidence of clinical signs of Newcastle disease. On the first day of collection of eggs for this consignment, each sentinel bird was tested for Newcastle disease with negative results. The test is scheduled to be repeated on the same sample of birds not less than 14 days after the collection of the last egg for this consignment.

***Delete the clause which is not applicable.**

Number of sentinel birds placed ..... and date placed .....

4.f. Within 21 days before the first day of collection of eggs for export to Australia the source flock was determined to be free of infection with *Salmonella* Arizona.

The absence of these bacteria was determined by procedures to culture and isolate them from shed litter. Twenty samples were collected from each shed. Each sample was a composite sample of 3 floor and 2 nest litter samples (ie a total of 60 floor locations and 40 nest boxes per shed).

The total number of composite samples tested: .....

4.g. All tests were carried out in a government laboratory or a laboratory approved by the government of the exporting country for this specific purpose and approved by AQIS. The tests were OIE-approved tests or tests approved by AQIS - see Appendix 8. Test results are shown in the table below.

Total Number of birds in the source flock: .....

Disease agent	Test used	No of tests	No of positive results
Influenza virus type A			
Avian paramyxovirus types 2 and 3			
Mycoplasma iowae			
Salmonella Pullorum			
Salmonella Gallinarum			
Salmonella Enteritidis			
Salmonella Arizona			

4.h. EITHER:

* The source flock was not vaccinated against Avian Pneumovirus. .....(No. of samples) were tested using the .....test, and all results were negative.

OR:

* The source flock was vaccinated against Avian Pneumovirus (APV). 100 individually identified birds in the source flock were tested for APV, with individual titres recorded for each bird sampled. A list of titres is attached to this certificate. The test is scheduled to be repeated on the same sample of birds not less than 14 days after the collection of the last egg for this consignment.

***Delete the clause which is not applicable.**

Signature: ..... Date: .....

Government Approved Veterinarian *

Name: .....

Address: .....

.....

* A **Government Approved Veterinarian** is either a civil service veterinarian or a specially appointed veterinarian, as authorised by the Veterinary Administration of the exporting country.

[NOTE: All pages are to be endorsed with the Official Stamp.]

SECOND VETERINARY CERTIFICATE RELATING TO THE EXPORT OF HATCHING EGGS OF DOMESTIC TURKEYS TO AUSTRALIA
(Source flock not vaccinated against ND).

This certificate relates to the post-collection observation, production records and disease status of the source flock and should be sent to the Supervising Veterinary Officer of the Torrens Island Animal Quarantine Station or the Veterinary Officer supervising the ..... Quarantine Approved Premises as soon as possible after the completion of the post-egg-collection observation period.

PART A: DISEASE STATUS OF THE COUNTRY OF ORIGIN

I, ...(please print name), a Government Veterinary Officer of ..... (please print country of export) hereby certify in relation to the consignment of hatching eggs identified on Australian Import Permit Number ..... that:

1. The country of export was free of highly pathogenic notifiable avian influenza at the time of commencement of egg collection for this consignment and has remained free of highly pathogenic avian influenza throughout the post-egg-collection observation period.

2. Clinical Newcastle disease and clinical avian influenza have not been reported during the post-egg-collection period within 40 kilometres of the location of the source flock.

3. After due enquiry, I am satisfied that the source flock has remained a closed flock and any clinical evidence of disease has been investigated and the results indicate that the following diseases have not occurred during the period since the collection of eggs for Australia:

Influenza virus type A infection
Newcastle disease
Avian paramyxovirus type 2 infection
Avian paramyxovirus type 3 infection
Pullorum disease (*Salmonella* Pullorum)
Fowl typhoid (*Salmonella* Gallinarum)
Salmonella Enteritidis infection
Avian pneumovirus infection

Signature: ..... Date: .....
Government Veterinary Officer*

Name: .....

Address: .....

.....

* A Government Veterinary Officer is a full-time veterinary officer of the Government of the exporting country.

PART B: FLOCK STATUS AND DISEASE TESTING

I, ... (please print name), a Government Approved Veterinarian of ..... (please print country of export) hereby certify in relation to the consignment of hatching eggs identified on Australian Import Permit Number ..... that:

1. Post-egg collection testing

a) The source flock, from which the eggs were derived, has been under my supervision for the 21 days since the eggs exported to Australia were collected.

b) EITHER

Between 14 and 21 days after the last day of collection of eggs for export to Australia, a sample of the parent flock was tested serologically for antibody to Influenza virus type A with negative results.

The sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was a 5% disease prevalence in the source flock (see Appendix 9).

Total number of birds in the source flock: .....
Total number of birds tested: .....

c) EITHER:

* Between 14 and 21 days after the last day of collection of eggs for export to Australia, a sample of the parent flock was tested serologically for antibody to Newcastle disease virus with negative results

The sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was a 5% disease prevalence in the source flock (see Appendix 9).

Total number of birds in the source flock: .....
Total number of birds tested: .....

OR

Post-egg-collection testing for Newcastle disease was not performed because:

* i) .....(country of origin) is officially free of Newcastle disease;

OR

* ii) Vaccination against Newcastle disease/avian influenza is totally prohibited in this country, and the source flock has not been vaccinated. No outbreak of Newcastle disease or avian influenza has been reported in the country of origin within 40 kms of the source flock, later than 21 days prior to the commencement of collection of eggs for export to Australia.

*** Delete the clauses which are not applicable.**

d) Between 14 and 21 days after the last day of collection of eggs for export to Australia, the same 100 individually identified birds that were tested previously for avian pneumovirus, were tested serologically for freedom from avian pneumovirus. Results of this testing are attached.

[Note: This testing is only required if the source flock was vaccinated against avian pneumovirus.]

e) All tests were carried out in a government laboratory or a laboratory approved by the government of the exporting country for this specific purpose and approved by AQIS. The tests were OIE-approved tests or tests approved by AQIS - see Appendix 8.

Results of post egg collection testing are shown in the table below.

Disease	Test used	No of tests	No of positive results
Influenza virus type A			
Newcastle disease virus			

f) Any clinical disease in the source flock or drop in quantity, quality, or fertility/hatchability of the eggs produced by the source flock has been investigated and the laboratory reports are attached.

Signature: ..... Date: .....
Government Approved Veterinarian *

Name: .....

Address: .....

.....

* A Government Veterinary Officer is a full-time veterinary officer of the Government of the exporting country.

[NOTE: All pages are to be endorsed with the Official Stamp.]

SECOND VETERINARY CERTIFICATE RELATING TO THE EXPORT OF HATCHING EGGS OF DOMESTIC TURKEYS TO AUSTRALIA
(Source flock vaccinated against ND).

This certificate relates to the post-collection observation, production records and disease status of the source flock and should be sent to the Supervising Veterinary Officer of the Torrens Island Animal Quarantine Station or the Veterinary Officer supervising the ..... Quarantine Approved Premises as soon as possible after the completion of the post-egg-collection observation period.

PART A: DISEASE STATUS OF THE COUNTRY OF ORIGIN

I, ...(please print name), a Government Veterinary Officer of ..... (please print country of export) hereby certify in relation to the consignment of hatching eggs identified on Australian Import Permit Number ..... that:

1. The country of export was free of highly pathogenic notifiable avian influenza at the time of commencement of egg collection for this consignment, and has remained free throughout the post-egg-collection observation period.

2. Clinical Newcastle disease and avian influenza have not been reported during the post-egg-collection period within 40 kilometres of the location of the source flock.

3. After due enquiry, I am satisfied that the source flock has remained a closed flock and any clinical evidence of disease has been investigated and the results indicate that the following diseases have not occurred during the period since the collection of eggs for Australia:

Influenza virus type A infection
Newcastle disease
Avian paramyxovirus type 2 infection
Avian paramyxovirus type 3 infection
Pullorum disease (*Salmonella* Pullorum)
Fowl typhoid (*Salmonella* Gallinarum)
Salmonella Enteritidis infection
Avian pneumovirus infection

Signature: ..... Date: .....
Government Veterinary Officer *

Name: .....

Address: .....

.....

* A Government Veterinary Officer is a full-time veterinary officer of the Government of the exporting country.

PART B: FLOCK STATUS AND DISEASE TESTING

I, ... (please print name), a Government Approved Veterinarian of ..... (please print country of export) hereby certify in relation to the consignment of hatching eggs identified on Australian Import Permit Number ..... that:

1) Post-egg collection testing

a) The source flock, from which the eggs were derived, has been under my supervision for the 21 days since the eggs exported to Australia were collected.

b) Between 14 and 21 days after the last day of collection of eggs for export to Australia, a sample of the parent flock was tested serologically for antibody to Influenza virus type A with negative results

The sample tested was of a sufficient size to give a 99% confidence of detecting the disease if there was a 5% disease prevalence in the source flock (see Appendix 9).

Total number of birds in the source flock: .....
Total number of birds tested: .....

c) EITHER

* I have examined the list of serological titres obtained from the testing of a sample of 100 individually identified birds prior to egg collection for this consignment, and am satisfied that the overall titres for antibody to Newcastle disease virus were not higher than $\log_2 10$.

Between 14 and 21 days after the last day of collection of eggs for this consignment, the same 100 birds were again blood sampled and tested for Newcastle disease, with no rise in titres suggestive of contact with Newcastle disease virus (ie 2 fold dilution increase) in any individual bird. Where suspicious rises in titre were observed, virus isolation tests were performed with negative results. Results of this testing are attached.

OR

* Not less than 14 days after the last day of collection of eggs for this consignment, the sentinel birds were tested serologically for antibody to Newcastle disease, Avian paramyxovirus type 2 infection and Avian paramyxovirus type 3 infection with negative results. Daily observation of these sentinel birds has been undertaken since the eggs were exported to Australia, and no clinical or other evidence of paramyxovirus infection has been detected. Results of this testing are attached.

* Delete the clause which is not applicable.

d) Between 14 and 21 days after the last day of collection of eggs for export to Australia, the same 100 individually identified birds that were tested previously for avian pneumovirus, were again blood sampled and tested for avian pneumovirus, with no rise in titres suggestive of contact with avian pneumovirus (ie 2 fold dilution increase) in any individual bird. Where suspicious rises in titre were observed, virus isolation tests were performed with negative results. Results of this testing are attached.

[NOTE: This testing is only required if the source flock was vaccinated against avian pneumovirus.]

e) All tests were carried out in a government laboratory or a laboratory approved by the government of the exporting country for this specific purpose and approved by AQIS. The tests were OIE-approved tests or tests approved by AQIS - see Appendix 8. Results of post egg collection testing are shown in the table below.

Disease	Test used	No of tests	No of positive results
Influenza virus type A			

f) Any clinical disease in the source flock or drop in quantity, quality, or fertility/hatchability of the eggs produced by the source flock has been investigated and the laboratory reports are attached.

Signature: ..... Date: .....
Government Approved Veterinarian *

Name: .....

Address: .....

.....

* A **Government Approved Veterinarian** is either a Government Veterinary Officer or a specially appointed veterinarian, as authorised by the Veterinary Administration of the exporting country.

[NOTE: All pages are to be endorsed with the Official Stamp.]

CERTIFICATE FROM THE SUPERVISING VETERINARY OFFICER, TORRENS ISLAND ANIMAL QUARANTINE STATION,
OR THE AUSTRALIAN GOVERNMENT VETERINARY OFFICER SUPERVISING THE QUARANTINE APPROVED PREMISES

Details of imported Consignment:

a. Consignor: .....

b. Date of Arrival: .....

c. Identification of consignment: .....

d. Description of Contents: .....

*****I, ... (please print name), Supervising Veterinary Officer of the Torrens Island Animal Quarantine Station (TIAQS) certify that:

OR

*I, ... (please print name), the Supervising Veterinary Officer at the ..... Quarantine Approved Premises (QAP) certify that:

*** Delete whichever is not applicable**

1. The consignment of eggs described above was carried directly from the aircraft to the egg hatchery. After arrival, the eggs were stacked on plastic egg flats and were either:

a) fumigated with formaldehyde gas (generated by the addition of 35 cc of commercial formalin [40% solution] to 17.5 grams of potassium permanganate for each 2.38 cubic metres of fumigation space) in a suitable room or cabinet with forced ventilation at a temperature of at least 20 ^{o}C and a relative humidity of between 80% and 90% for 20 minutes with no free water; or

b) disinfected by submerging in a 0.5% (1:200) solution of VirkonS and allowed to dry with the disinfectant on them.

The eggs were then incubated to hatch the quarantine flock.

A quantity of eggs from Australian SPF flocks, which met the "Specifications of Tests which are to be applied to Specific Pathogen Free Chicken Flocks used in the Production and Testing of Avian Viral Vaccines" (Australian Government Department of Health and Ageing), were treated similarly and incubated to hatch the sentinel flock. The ratio of Australian SPF chicks to imported poults at the day-old stage was 1:50. In the case where the 1:50 ratio resulted in the number of sentinel chickens being less than 100, a minimum of 100 sentinel chickens were reared concurrently with the quarantine flock.

2. All packing materials consigned with the imported eggs were destroyed.

3. EITHER

* All eggs and imported and sentinel birds in the TIAQS were observed daily for evidence of disease and, where abnormalities were observed, a full investigation was carried out and a report is attached.

OR

* To the best of my knowledge, all eggs and imported and sentinel birds in the ..... QAP were observed daily for evidence of disease. Where abnormalities were observed and reported to me, a full investigation was carried out and a report is attached.

*** Delete whichever is not applicable**

4. Cloacal swabs were collected from a sample of birds in the quarantine flock at 6 weeks of age. The sample tested was of a sufficient size to give a 99% confidence of detecting the diseases tested if there was a 5% disease prevalence in the source flock. Each sample and each bird was so identified that a second sample could have been collected later from any specified bird. Cloacal swabs from groups of no more than 5 birds were pooled and tested for freedom from the following pathogens with negative results in each case:

a. haemagglutinating agents by direct inoculation of the allantoic cavity of 9-11 day-old chick embryos with cloacal swabs. Any agents isolated were specifically identified and influenza virus type A, Newcastle disease virus, avian paramyxovirus type 2 and type 3 were absent.

b. infectious bursal disease virus

5. All sentinel birds were bled at 6 weeks of age. Sufficient blood was collected from each bird sampled for the performance of the required tests. Each sample and each bird was so identified that a second sample could have been collected later from any specified bird. Anti-coagulant was not added. The blood was allowed to clot and the serum removed.

[Note: Samples of blood may, if necessary, be incubated at 37°C for 2 hours to aid clotting and sera clarified by centrifugation. Sera may be sterilised by filtration and may be frozen. Preservatives were not added. Unless specified in a particular test, serum may not be diluted nor were samples of serum from different birds pooled.]

Each serum sample collected was tested for antibodies to the following pathogens:

Influenza virus type A
Newcastle disease virus
Salmonella Pullorum
Salmonella Gallinarum
Salmonella Enteritidis
Infectious bursal disease virus
Avian Pneumovirus

Those birds with positive serology were necropsied and the samples sent to AAHL or ..... (an Approved Government Laboratory). Results of any necropsies undertaken are attached.

Where there were positive or suspicious reactors for *Salmonella* Pullorum, *Salmonella* Gallinarum, *Salmonella* Enteritidis, all of the reactors were killed and their organs cultured, and the results of the tests are attached.

6. The absence of Salmonella Enteritidis, Salmonella Pullorum, Salmonella Gallinarum and Salmonella Arizona was determined by early microbial monitoring techniques - see Appendix 8.

7. Specifications for the tests above are detailed in Appendix 8.

8. The quarantine flock (at day-old) was vaccinated/not vaccinated [delete one] with HVT virus for Marek's disease by the importer.

9. All birds in the TIAQS were only allowed feed which had been irradiated to 2.5 mega rads and only allowed drinking water which had been sterilised either by physical means or by acidification to pH 2.

[NOTE: This condition is OPTIONAL for birds in an QAP. However, the manager/director should ensure that the health status of the quarantine flock is not compromised by the provision of any contaminated feed/water.]

10. EITHER

*****During the quarantine period in the TIAQS, all appropriate security measures with respect to the egg hatchery, to staff associated with the hatchery and to all materials entering or leaving the hatchery were taken. To my knowledge at no stage during the quarantine period was there a breakdown in security.

OR

*I have audited/supervised the audit of the program according to the requirements set out in the Approved Quarantine Manual. As far as could be ascertained, during the quarantine period, all appropriate security measures with respect to the egg hatchery, to staff associated with the hatchery and to all materials entering or leaving the hatchery were taken. To the best of my knowledge, at no stage during the quarantine period was there a breakdown in security at the ..... Quarantine Approved Premises.

*** Delete whichever is not applicable**

11. I attach the reports from AAHL/..... (Approved Government Laboratory) which identify the samples tested with the birds in the quarantine and sentinel flock, establish the validity of all tests and state the results of all tests.

12. I attach the declaration by the owner/manager of the source flock and the two certificates (with enclosures) which I have received from the Government Veterinary Officer of the Country of Export, which refer to this consignment.

13. After consideration of all relevant information, I certify that the progeny of this consignment *ARE or *ARE NOT qualified to be released from quarantine.

Signature: ..... Date: .....

Name: .....

*****Supervising Veterinary Officer
Torrens Island Animal Quarantine Station

OR

* Supervising Veterinary Officer
..... Quarantine Approved Premises

Address: .....

.....

*** Delete whichever is not applicable**

[NOTE: All pages are to be endorsed with the Official Stamp.]

APPENDIX 8

(A) Approved Tests For The Importation Of Fertile Eggs

PATHOGEN	TESTS	OIE/AAHL/SCAHLS
Infectious bursal disease virus	AGID, VN	OIE
	ELISA	AAHL, OIE
Influenza virus type A	ELISA	AAHL
	AGID	OIE, SCAHLS
Newcastle disease virus	HIT	AAHL, OIE, SCAHLS
Avian paramyxovirus types 2 and 3	HIT	OIE
Salmonella Pullorum, *Salmonella* Gallinarum, *Salmonella* Enteritidis	RSAT	AAHL, OIE
	WBTAT	SCAHLS
Avian pneumovirus	ELISA	AAHL

HIT haemagglutination inhibition test
ELISA enzyme-linked immunosorbent assay
RSAT rapid serum agglutination test
AGID agar gel immunodiffusion test
VN virus neutralisation test
WBTAT whole blood tube agglutination test

OIE Office International Des Epizooties
AAHL Australian Animal Health Laboratory
SCAHLS Subcommittee on Animal Health Laboratory Standards, Australia

(B) Comparison of AAHL, SCAHLS and OIE Protocols and Test Interpretations

Avian Influenza

Isolation	OIE	at least 5 eggs used, allantoic fluid checked for HA by AGID
	AAHL	at least 3 eggs used, allantoic fluid checked for HA by ELISA
Serology	OIE	AGID
	SCAHLS	AGID
	AAHL	ELISA (more specific and more sensitive) >60% inhibition is positive, 40 - 60% inhibition is inconclusive.

Newcastle Disease

Isolation	OIE	at least 5 eggs used
	AAHL	at least 3 eggs used

Serology OIE, SCAHLS and AAHL classify sera with antibody titres equal to or greater than 1/8 as positive.

***Salmonella* Pullorum**

Serology SCAHLS use whole blood tube agglutination test.
OIE and AAHL use rapid serum agglutination test.
any clumping is considered positive.

IBD

Serology	OIE	AGID
	AAHL	ELISA

APV

Serology AAHL uses commercial ELISA kit; S/P ratio >0.3 is positive.

(C) Examination for *Salmonella* spp in eggs/birds at Torrens Island Quarantine Station/ Quarantine Approved Premises

a) Serological Testing

Testing for *S.* Pullorum, *S.* Enteritidis and *S.* Gallinarum requires serology using the group specific test. Serum is to be collected from all SPF in-contact sentinel birds at 6 weeks of age.

Testing for *Salmonella* Arizona relies on cultures from a range of environmental and post-mortem samples.

b) Hatchery Sampling for Bacteriological Culture of *Salmonella* spp.

The testing is designed to identify *S.* Enteritidis, *S.* Arizona, *S.* Pullorum and *S.* Gallinarum.

Hatchery Waste:
Samples of shell debris and membranes are to be collected from each hatching tray and pooled. Specimens from each incubator are to be kept separate. Emphasis will be on the collection of the most moist egg shells and debris. Approximately 200 ml of material may be collected from each tray, 2200 ml per incubator (a subsample of up to 200g per incubator should be cultured).

Pipped embryos:
All pipped embryos are to be sampled. The livers and alimentary tracts are to be removed aseptically and placed in separate containers, either individually or in pools of up to 20. Containers must be labelled so that sample pairs can be identified.

Mortalities:
All birds which die within 10 days after hatching are to be sampled. The liver and caecum are to be removed aseptically from each bird, keeping the two separate. Specimens are preferably submitted individually or if large numbers are involved may be pooled into groups of up to 5. Containers must be labelled to allow sample pairs to be identified. Mortality rates of <5% can be expected (<50 pools of 5).

Culls:
5% of healthy culls from 1 to 10 days of age are to be randomly selected for sampling, with a minimum of 10 and a maximum of 45 culls being submitted. These may be pooled into groups of up to 5. Contaminated tissues such as caecum should be submitted separately from uncontaminated tissues, for example liver. Healthy culls older than 10 days need not be sampled.

Faeces:
A representative sample from all imported chickens is to be submitted by collection of faeces which accumulates on the floor of each holding carton during sorting (up to 100g may be cultured).

c) Culture

Hatchery waste:
Pooled samples of hatchery waste from each incubator are to be cultured separately in Shott bottles of selenite broth at the rate of 10g of debris per 90 ml broth.

Pipped embryos:
Pooled samples of liver and alimentary tract are to be cultured in separate selenite broths at a rate of no more than 10g of tissue per 90 ml of broth.

Mortalities and Unhealthy Culls (and any suspicious or positive reactors to *S*. Pullorum or *S*. Gallinarum):
Individual samples - samples of liver and alimentary tract are to be cultured separately. The tissues should be cultured directly onto SBA and BGA plates and in selenite broth.
Pooled samples - Pools of liver and alimentary tract are to be cultured separately in selenite broth, no more than 10g of tissue per 90 ml of broth.

Healthy culls:
Cultured as for pooled mortality samples.

Faeces:
Samples from each sorting unit should be kept separate. Faeces and faeces-contaminated material are to be cultured in selenite broth at a rate of no more than 10g of material per 90 ml selenite broth.

<u>Individual Mortalities eg liver, heart, blood, yolk sac</u>

Sample	1. BA, XLD, BGA for 37°C, 24hrs*	Select colonies
	AND	
	2. selenite broth for 37°C, 24hrs and then MAC, XLD, BGA for 37°C, 35hrs*	Select colonies

Hatchery Waste, Pipped Embryos, Faeces, Culls, Pooled Mortalities

Sample selenite broth for 37°C, 24hrs and then
MAC, XLD, BGA for 37°C, 24hrs* Select colonies

Dead in Shell Embryos

Wash eggs, then
Flame in 70% alcohol and open each egg aseptically, then
Homogenise to paste in pools of 1-5

1. BA (loopful) for 37°C, 24 hrs Select colonies

AND

2. 10ml Paste to 900 ml selenite broth for 37°C, 24hrs and then
   MAC, XLD, BGA for 37°C, 24hrs* Select colonies

Suspect *Salmonella* spp. colonies

Single colony
Urea broth for 4-16 hrs, 37°C, then

1. Urea - ve TSI, LIA, API20E for 37°C, 24hrs If *Salmonella* sp
   MAT with grouping sera
2. Urea +ve Discard

Serogrouping of isolates can be carried out using reference antisera and standard procedures.

Serotyping of isolates requires dispatch to a Salmonella reference laboratory eg IMVS Adelaide.

APPENDIX 9

SAMPLE SIZE FOR 99% CONFIDENCE OF DETECTING 0.5% AND 5% PREVALENCE OF DISEASE

Population Size	Sample Size to detect 0.5% prevalence	Sample Size to detect 5% prevalence
10	10	10
20	20	20
30	30	30
40	40	36
50	50	42
60	60	47
70	70	51
80	80	54
90	90	57
100	100	59
120	120	63
140	140	67
160	160	69
180	179	71
200	198	73
250	244	76
300	286	78
350	325	80
400	360	81
450	392	82
500	421	83
600	470	84
700	512	85
800	546	85
900	576	86
1000	601	86
1200	642	87
1400	674	87
1600	699	88
1800	720	88
2000	737	88
3000	792	89
4000	821	89
5000	840	89
6000	852	90
7000	861	90
8000	868	90
9000	874	90
10000	878	90
a	919	90