

**VICH**, International Co-operation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products.

**WORKING GROUP :** Biologicals Quality Monitoring.

**TOPIC:** *Test on the absence of extraneous agents*

***REVISED FORMAT***

**DRAFT TEXT**

**PRELIMINARY DRAFT**

**GUIDELINE FOR THE TESTS TO DETERMINE THE PRESENCE OF  
EXTRANEOUS AGENTS IN VETERINARY VACCINES.**

**B. Avian vaccines.**

**Date : VS 2000/01**

## **!.INTRODUCTION.**

### **1.1 Objective of the guideline.**

It is important that avian vaccines are free of contaminants, notably viral agents. Potential sources of contamination are the viral and bacterial strains used for the production of the active ingredient(s) and the starting materials of animal origin used in the production of the active ingredient and / or in the assembly of the finished product. Consequently it is necessary to demonstrate that extraneous agents are not present in biological products nor in the starting materials required for their manufacture, through the use of accepted testing procedures and sampling methods and subject to the limitations of the test.

The purpose of the guideline is to provide a description of the test methods to detect the presence of extraneous agents which shall be undertaken on all materials of animal origin used in the production of avian vaccines . It shall also provide precise information on the method and conditions of the tests to determine the absence of extraneous viruses in these substances.

### **1.2. Background.**

The materials used in the manufacture of biological products for veterinary use can be divided into two main categories:

1. Viral strains and cell substrates used in the production of the active ingredient(s).
2. Starting materials of animal origin used in the production of the active ingredients and/or in the assembly of the finished product.

Restrictions may be placed by regulatory authorities upon the use of starting materials of animal origin to minimise the risk associated with pathogens that may be potentially present in these materials e.g.: their use is not generally acceptable except when they are sterilised by a suitable, validated method.

Where the use of such substances has been shown to be essential and sterilisation not possible, it will normally be required to test and monitor the source animals for freedom from infectious agents and/or to test these substances for the absence of contaminants. [ *In the case of inactivated vaccines, the method used for inactivation of the vaccine strain may also be validated for inactivation of possible contaminants from substances of animal origin.* ]

Present methods of testing for extraneous agents of substances of animal origin are described in the European Pharmacopoeia monograph 62 (1995) and in the Code of Federal Regulations 9CFR 113 and the OIE Manual of Standards for Diagnostic Tests and Vaccines.

[ *The use of experimental animals in tests shall be minimised and if required its necessity shall be justified* ]

### **1.3. Scope of the guideline.**

The scope of the guideline is to provide guidance on the methods to determine the presence of extraneous agents in avian viral vaccines. The test methods are intended for the test on the finished product and on starting materials of animal origin used in the manufacture of the vaccine e.g. seeds , eggs and cell stocks.

## 2. COMPARATIVE DATA ON THE TEST METHODS USED IN EACH OF THE REGIONS.

### 2.1. TEST FOR THE PRESENCE OF EXTRANEIOUS VIRUSES USING EMBRYONATED EGGS.

#### GENERAL OUTLINE OF THE TEST METHODS.

##### USA :

The eggs are inoculated in the allantoic cavity and on the chorioallantoic membrane.  
After incubation for 7 days the eggs are examined.  
In case deaths have occurred, a second passage is made for verification.

##### JAPAN:

The eggs are inoculated in the allantoic cavity and on the chorioallantoic membrane.  
After incubation for **5 or 7** days the eggs are examined.

##### EUROPEAN UNION:

The eggs are inoculated in the allantoic cavity and on the chorioallantoic membrane.  
After incubation for 7 days the eggs are examined.  
A second passage is made using allantoic fluid and membrane material.  
After incubation for 7 days the eggs are examined.

	<b>USA</b> 9CFR 113.37	<b>JAPAN</b> M.R. 396-397,1993	<b>EU</b> Ph. Eur.	<b>VICH</b> PROPOSAL
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<b>INITIAL INOCULATION</b>				
<b>EGGS</b>				
Quality of the eggs.	SPF	SPF	SPF	
Age of embryo's ( in days)	9-11	9-11 ( allantois) 10-12 ( membrane)	9-11	
Number of eggs <b>(Total)</b>	20	20	20	
<b>INOCULUM.</b>				
Inoculum volume per egg ( ml)	0.1	0.1	0.2	
Number of doses in inoculum ( after neutralisation)	3	1	10	
Dilution factor due to neutralisation	10	n.a.	n.a.	
<b>TEST PROCEDURE</b>				
Inoculation into allantoic cavity ( number of eggs)	20 ( not clear)	10	10	
Inoculation on chorio-allantoic membrane ( number of eggs)	20 ( not clear)	10	10	
Incubation temperature (°C)		37		
Incubation time ( days)	7	7 for allantoic cavity 5 for membrane	7	

Daily candling	yes	n.a.	yes	
Death of embryo within 24 hours after inoculation considered as non-specific	yes	n.a.	yes	
<b>JUDGEMENT</b>				
Maximum allowed number of non-specific deaths (per group)	2	n.a.	4	
Each egg that dies during incubation period is examined	Yes	n.a.	yes	
All eggs are examined for abnormalities of membranes	Yes	Yes	Yes	
All eggs are examined for haemagglutination of allantoic fluid	Yes	Yes	Yes	
Cell sediment of allantoic fluid is examined by FA for IBV	No	No	Yes	
All embryos are examined for abnormalities	Yes	Yes	Yes	
<b>FIRST PASSAGE</b>				
<b>See Initial Inoculation</b>				
<b>INOCULUM</b>				

Pooled allantoic fluid from dead embryos in allantoic cavity			Yes	
Pooled membrane material from dead embryos on chorio allantoic membrane			Yes	
Pooled allantoic fluid from live embryos in allantoic cavity			Yes	
Pooled membrane material from dead embryos on chorioallantoic membrane			Yes	
<b>JUDGEMENT</b>				
<b>See Initial Inoculation.</b>				
<b>HAEMAGGLUTINATION TEST</b>				
Type of test ( slide or tube)		n.a.		
Source of erythrocytes		chicken		
Concentration of erythrocyte suspension (vol%)		0.5		
Ratio erythrocyte suspension / allantoic fluid		1:1		
Incubation time ( minutes)		60		
Incubation temperature (°C)		4		

**n.a. : It is not specified**



## 2.2. TEST FOR THE PRESENCE OF EXTRANEIOUS VIRUSES USING CELL CULTURES.

### GENERAL OUTLINE OF THE TEST METHODS.

#### USA :

Not known.

**JAPAN:** Minimum requirement (M.R.), 1993

Cell cultures of chicken kidney cells (CKC) and cell cultures of chicken embryonic fibroblast cells (CEFC) are inoculated.  
The CLC cultures are passaged once within a period of 14 days.  
The ECLC cultures are passaged thrice with intervals of 3-5 days.  
Every passage is examined for cytopathogenic effect.  
The final passage is examined for haemadsorbtion.

#### EUROPEAN UNION:

Cell cultures of chicken liver cells (CLC) or cell cultures of embryonic chicken liver cells (ECLC) are inoculated.  
The cell cultures are passaged at intervals of at least 5 days and observed during 20 days.  
Every passage is examined for cytopathogenic effect.  
The final passage is examined for haemadsorbtion.

	USA 9CFR 113.??	JAPAN M.R.396-397,1993	EU Ph. Eur.	VICH PROPOSAL

<b>CELLS</b>				
Quality of the eggs or chickens.		SPF	SPF	
Type of cells		primary chicken kidney cells (CKC) and primary chicken embryonic fibroblast cells(CEFC)	primary chicken liver cells <b>or</b> primary embryonic chicken liver cells	
		<b>CKC</b>	<b>CEFC</b>	
Number of monolayer cultures		4	8	5
Minimum total surface area in cm <sup>2</sup>		80	160	150
<b>INITIAL INOCULATION</b>				
<b>INOCULUM.</b>				
Inoculum volume per culture ( ml)		0.2	0.2	0.5
Number of doses in inoculum ( after neutralisation)		1	1	50
Dilution factor due to neutralisation				
<b>TEST PROCEDURE</b>				

Adsorbion time after inoculation (minutes)		60	60	60	
Temperature during adsorbion (°C)		37	37	37	
Incubation time ( days)		7	3-5	At least 5	
Incubation temperature (°C)		37	37	37	
Observation of culture every day					
<b>JUDGEMENT</b>					
Examination for cytopathogenic effect		yes	Yes	yes	
Examination for haemadsorbion		No	No	no	
<b>FIRST AND FURTHER PASSAGES</b>					
<b>INOCULUM</b>					
Passage done with pooled cell material		Yes	Yes	Yes	
Passage done with pooled medium		Yes	Yes	Yes	
Inoculation volume per culture		0.2	0.2		

<b>TEST PROCEDURE</b>					
<b>See Initial Passage</b>					
Incubation time (days)		7	3-5	5	
Observation period (days)				20	
<b>JUDGEMENT</b>					
Minimum percentage of cultures that have to be maintained until end of test				n.a.	
Examination for cytopathogenic effect		Yes		yes	
Examination for haemadsorbtion		No		no	
<b>FINAL PASSAGE</b>					
<b>INOCULUM</b>					
<b>See First Passage</b>					
<b>TEST PROCEDURE</b>					
<b>See Initial Passage</b>					
<b>JUDGEMENT</b>					
Minimum percentage of cultures that have to be maintained until end of test				80	
Examination for cytopathogenic effect		Yes	Yes	yes	
Examination for haemadsorbtion		Yes	Yes	yes	

<b>HAEMADSORPTION TEST METHOD</b>				
Origin of erythrocytes		chicken	chicken	
Concentration % vol		0.1	0,5	
Volume used for each culture				
Incubation time ( minutes)		60	20	
Incubation temperature ( °C)		4	4	
PBS buffer has pH of			7.4	

### **2.3. TEST FOR THE PRESENCE OF EXTRANEIOUS VIRUSES USING CHICKENS.**

In respect of the test for extraneous agents using chickens I like to make the following observations:

It is my understanding that the VICH process is intended to harmonise existing test methods that are presently used in Japan, USA and the EU. However, it is also my understanding that test methods that can be subject of harmonisation shall have at least already have some similarity. It is not the intention of the VICH process to impose new tests or to increase the requirements of quality control testing in one or more of the three regions.

If this reasoning is correct than the conclusion must be that the test for extraneous viruses using chickens falls outside the scope of the VICH for the following reasons:

- The test is not a regulatory requirement in Japan.
- The test used in USA and in EU are significantly different, because in the USA no serological examination is done in contrast to the situation in the EU.
- The purpose of the test in the USA is to determine safety and not to detect the presence of extraneous agents.

For the sake of completeness the information is provide in the following table.

#### **GENERAL OUTLINE OF THE TEST METHODS.**

##### **USA :**

Chickens are vaccinated with a satisfactory batch of vaccine.  
14 days later they are inoculated with 10 doses of the test vaccine using different routes of administration.  
The chickens are observed for 21 days for clinical symptoms.

##### **JAPAN:**

The test is not a regulatory requirement.

**EUROPEAN UNION:**

Susceptible chickens are inoculated with 100 doses of vaccine by the intramuscular route and with 10 doses of vaccine by eye-drop.

The chickens are observed for clinical symptoms for 14 days

The treatment is then repeated.

The chickens are observed for another 3 weeks.

Serum is collected of all chickens at moment of first treatment and at the end of the test.

The serum is examined for the presence of antibodies against relevant agents.

	<b>USA 9CFR 113.36</b>	<b>JAPAN</b>	<b>EU Ph. Eur.</b>	<b>VICH PROPOSAL</b>
<b>CHICKENS</b>				
Quality of the chickens.	susceptible		SPF	
Age of chickens ( in weeks)	young		2	
Number of chickens	20 (+ 5 controls)		20	
<b>INOCULUM.</b>				

Number of doses by intramuscular route			100	
Number of doses by subcutaneous route	10			
Number of doses by eye-drop	10		10	
Number of doses by intra tracheal route	10			
Combe scarification				
<b>TEST PROCEDURE</b>				
Number of inoculations.	1		2	
Interval between inoculations. (weeks)	n.a.		2	
Observation period ( weeks after first inoculation)	3		5	
Serum samples taken :	n.a.		At moment of first inoculation and at end of 5 week observation period	

### 3. OTHER RELEVANT INFORMATION.

#### **3.1. Specification of neutralising sera used in extraneous agent testing.**

These sera shall be shown to be free of antibodies against the following organisms.

Avian Adeno Virus  
Avian Encephalomyelitis Virus  
Avian Leucosis Virus  
Avian Nephritis Virus  
Avian Paramyxo Virus  
Avian Reo Virus  
Chick Anaemia Agent  
Egg Drop Syndrome '76 Virus  
Fowl Pox Virus  
Haemorrhagic Enteritis Virus  
Infectious Bronchitis Virus  
Infectious Bursal Disease Virus  
Infectious Laryngotracheitis Virus  
Influenza A Virus  
Marek's Disease Virus  
Newcastle Disease Virus  
Reticulo Endotheliosis Virus  
Turkey Rhinotracheitis Virus.  
Duck Viral Enteritis Virus  
Duck Viral Hepatitis Virus

### 3.2. Extraneous agents relevant for chickens and for material of chicken origin.

AGENT	SPF FLOCK		
Avian Adeno Virus	+		
Avian Encephalomyelitis Virus	+		
Avian Leucosis Virus	+		
Avian Nephritis Virus	+		
Avian Paramyxo Virus	+		
Avian Reo Virus	+		
Chick Anaemia Agent	+		
Egg Drop Syndrome '76 Virus	+		
Fowl Pox Virus	+		
Haemorrhagic Enteritis Virus	+		
Infectious Bronchitis Virus	+		
Infectious Bursal Disease Virus	+		
Infectious Laryngotracheitis Virus	+		
Influenza A Virus	+		
Marek's Disease Virus	+		
Newcastle Disease Virus	+		
Reticulo Endotheliosis Virus	+		
Turkey Rhinotracheitis Virus	+		
Duck Viral Enteritis Virus	+		
Duck Viral Hepatitis Virus	+		
Haemophilus paragallinarum	+		
Salmonella pullorum	+		
Mycoplasma gallisepticum	+		
Mycoplasma synoviae	+		
Salmonella spp	+		

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