

# National Pig Health Council 2014

## Testing Protocol for Semen for National Sale (both ROI & NI)

### VOLUNTARY PROTOCOLS FOR DISEASE CONTROL

Voluntary NPHC protocols have been in place for the monitoring and safe integration of imported semen, embryos and livestock on to Irish farms. No existing agreed protocol existed until now re the testing of boars producing fresh or frozen semen on the island of Ireland. This document addresses this short-coming in current protocols, specifically relating to prevention of the spread of Porcine Reproductive and Respiratory Syndrome (PRRS) through fresh or frozen semen.

New testing methods for a host of both viral and bacterial diseases have emerged over the past number of years. New testing methods will continue to emerge and it will be necessary that the protocols be revisited regularly to ensure that the most modern and reliable testing methods are applied as they become available.

### PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME (PRRS)

Two distinct strains of PRRS exist worldwide, known locally as European (Type 1) and US strains (Type 2). The US strain is usually more virulent and there is regular antigenic drift and mutation. This makes the spread of the US strain extremely difficult to curtail and control through proprietary vaccines since these vaccines need to be constantly updated and modified to address the continuous antigenic drift. The EU strain is not as virulent or as mutagenic as the US strain. The EU strain is the only one identified to date in Ireland.

Any tests available for screening for PRRS must be capable of screening for both strains of PRRS because of the possibility of the US strain entering Ireland at some future date.

### SEMEN SUPPLIERS

There are five major commercial suppliers of fresh semen on the Island of Ireland at present (2043).

Hermitage Pedigree Pigs (500 boars)	PIC (250 boars)
Glen Marshall (100 boars)	Elite Sires (100 boars)
Robert Overend (Mostly rare breeds) (40 boars)	

### REGULATIONS RELATING TO BOAR STUDS

Boars are currently required to be quarantined for 30 days prior to entry to an EU licensed boar stud. During the course of this quarantine all boars must be tested at entry and exit for the following diseases with negative results: Aujeszky's Disease, CSF and Brucella suis. The current testing protocol for boars entering a boar stud is that they produce two in-quarantine negative results for these stated diseases. There is no current legislative requirement for PRRS testing on entry or exit to

quarantine but it is prudent for all boar studs to include PRRS in it routine and quarantine testing programmes.

#### RELEVANT LEGISLATIVE REQUIREMENTS:

S.I. No. 242/1993 European Communities (trade in porcine semen – animal health) Regulations, 1993

S.I. No. 255/1965 – Livestock (Artificial Insemination) (Pigs) Regulations, 1965

Council Directive 90/429/EEC – Laying down the animal health requirements applicable to Intra-Community trade in and import of semen of domestic animals of the porcine species.

#### DISEASE TRANSMISSION THROUGH SEMEN

A range of diseases are potentially transmissible in semen. These may be either viral or bacterial in nature. Infected semen may subsequently give rise to infections in the sow that may be localised (self limiting) or can spread and pose a threat to all the stock because of lateral (pig to pig) and subsequent horizontal transmission. All extended semen contains antimicrobials to limit the risk of bacterial infections being transmitted in semen. Virucidal semen extenders are not currently available but studies suggest that extending semen may reduce the risk of transmission (of PRRS) due to reduced survivability in the diluted / extended semen.

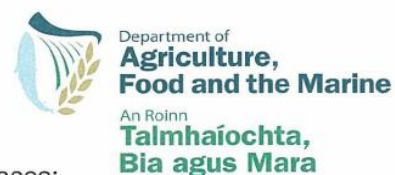
List of bacterial and viral diseases potentially transmitted in semen:

Aujeszky's Disease (Pseudorabies),	Parvovirus	Enterovirus
Adenovirus	Vesicular Disease Virus	African Swine Fever (ASF)
Classical Swine Fever (CSF)	Porcine Circo Virus Type 2 (PCV2)	Porcine Reproductive & Respiratory Syndrome (PRRS)
Torque teno virus	Mycoplasma hyopneumoniae	Foot & Mouth Disease (FMV)

#### NATIONAL DISEASE FREE STATUS

Nucleus pig units that provide boars for entry to a boar stud must have a specified high health status and operate ongoing active herd health programmes that incorporate continuous testing at farm and slaughterhouse level for a range of specific commercially important diseases. Ireland has stated national freedom from a range of viruses and bacterial infections including Classical Swine Fever (CSF) and *Brucella suis* based on ongoing surveillance carried out by the Department of Agriculture Food & the Marine (DAFM).

List of diseases for which Ireland retains a stated country freedom 2013:



To whom it may concern,

The following is the status of Ireland in relation to major pig diseases:

<b>Disease</b>	<b>Last outbreak in Ireland</b>
Foot and Mouth Disease	2001
African Swine Fever	Never recorded
Classical Swine Fever	1958
Swine Vesicular Disease	Never recorded
Vesicular Stomatitis	Never recorded
Vesicular Exanthema	Never recorded
PED( Porcine Epidemic Diarrhoea)	Never recorded
Transmissible Gastroenteritis	1984
Teschen Disease	Never recorded
Rabies	1903
Anthrax	1970
Brucella Suis	Never recorded
Aujeszky's Disease	2010

30<sup>th</sup> January 2013



John Melville MVB MAppISc MRCVS  
Superintending Veterinary Inspector

**Aujeszky's Disease:** Ireland (both ROI and NI) succeeded in achieving Annex 1 status for Aujeszky's disease in 2012. Ongoing testing and surveillance is required to maintain this status and this will be achieved through monitoring of slaughterhouse pigs plus surveillance of other blood submissions received at CVRL, Back Weston. Declared country freedom is not a sacrosanct position and can change, particularly given the threats posed by unscrupulous trade in all animals today spurred by access to the internet and facilitated by poor border controls in relation to animal imports.

## TESTING PROTOCOL:

### DETERMINATION OF APPROPRIATE SAMPLE SIZE

Viral diseases generally don't infect pigs on an individual basis. Rather, the group is generally infected. The rate of spread can vary from creeping spread (parvovirus) to explosive spread (CSF, peracute Swine Influenza). In the overall scheme of things PRRS would come under the mid-point in terms of rate of spread within a pig population. The NPHC has decided to impose a standard of 95% confidence at 10% sero-prevalence as its industry standard for determination of sample size. In relative terms, the larger stud will not have to take as many samples as the smaller stud in order to achieve the desired confidence level at the desired sero-prevalence.

		Boar Stud - Population size & Sample size			
Prevalence estimate	Confidence level	100	200	400	600
≥1%	70%	71	92	105	110
	80%	81	112	133	142
	90%	91	138	176	192
	95%	96	156	211	236
	99%	100	181	274	321
≥2%	70%	46	53	57	58
	80%	56	67	74	76
	90%	69	88	101	205
	95%	78	106	125	133
	99%	91	137	175	191
≥5%	<b>70%</b>	<b>22</b>	<b>24</b>	<b>24</b>	<b>25</b>
	80%	28	30	32	32
	90%	37	42	44	45
	95%	45	52	56	57
	99%	60	73	82	85
≥10%	<b>70%</b>	<b>12</b>	<b>13</b>	<b>13</b>	<b>13</b>
	<b>80%</b>	<b>16</b>	<b>16</b>	<b>16</b>	<b>17</b>
	<b>90%</b>	<b>21</b>	<b>22</b>	<b>23</b>	<b>23</b>
	<b>95%</b>	<b>26</b>	<b>28</b>	<b>29</b>	<b>29</b>
≥15%	99%	37	41	43	44
	<b>70%</b>	<b>9</b>	<b>9</b>	<b>9</b>	<b>9</b>
	<b>80%</b>	<b>11</b>	<b>11</b>	<b>11</b>	<b>11</b>
	<b>90%</b>	<b>15</b>	<b>15</b>	<b>15</b>	<b>16</b>
	<b>95%</b>	<b>18</b>	<b>19</b>	<b>20</b>	<b>20</b>
	<b>99%</b>	<b>26</b>	<b>28</b>	<b>29</b>	<b>29</b>

## **TESTING FREQUENCY:**

**Weekly** testing is required of the AI stations to ensure that the potential for an outbreak of disease is massively reduced. The day of testing will be determined in consultation with the testing laboratory as a commercial arrangement between the stud and the laboratory.

## **SAMPLE COLLECTION METHOD**

Sampling from the jugular vein is currently considered the best method for blood collection. Collection of very small volumes of blood onto blotting paper by pin pricking of the ear vein is carried out in some countries. New technologies may become available in time to allow for an alternative to veni-puncture for sample collection.

Sample animals should be varied over time (randomised and targeted). The random element to sampling would ensure that all animals would be tested over time and that all areas / rooms / buildings be tested each week to ensure a representative sample. The targeted nature of testing would ensure that any boar off its feed or running a temperature would be targeted on a weekly basis to ensure that any sick animal would be screened (and not collected).

## **LABORATORY TESTS**

Two major laboratory tests are currently used for detection of PRRS in pigs. The ELISA test is an antibody test, which is generally performed on blood samples. PRRS antibodies will be detectable in blood approximately 7-10 days after infection with PRRS. PRRS antibodies will persist for many months at detectable levels. Hence, the ELISA test is a very good screening test but the 7-10 day lag period between infection and a positive ELISA test being achieved provides an opportunity for infected semen to be distributed for up to 10 days before the earliest detection of infection by the ELISA test. This time period could be extended to an extent which would depend on the frequency of sample collection, the interval from sample collection to receipt of lab results and the extent of sampling.

The PCR test is now considered to be the industry standard for PRRS testing within boar studs. It is based on detection of viral or bacterial DNA and practically eliminates the lag phase between infection and antibody production associated with the ELISA test. The PCR test can be carried out on blood or semen samples, as well as other body tissues.

PRRS virus is found to circulate earlier after infection in blood than semen and also persists in blood for a longer time than it does in semen. PRRS is inconsistently shed in semen so failure to detect in the semen is not indicative of a negative result. Hence, analysis of blood, rather than semen, by PCR would be considered the gold standard for early detection of PRRS virus in a boar stud. Many PRRS outbreaks in boar studs (particularly of the European (Type1) strain) show no overt clinical signs. Therefore, monitoring of clinical signs, rectal temperatures, semen morphology and appetite as well as the aforementioned blood samples will each contribute in some part to early detection of PRRS infection in a boar stud.

**Laboratory Accreditation:**

Laboratories are required to achieve specified standardised testing protocols and procedures in order to satisfy the requirements of regulatory bodies. The gold standard for accredited laboratories is ISO 17025. It is the stated objective of the NPHC that all laboratories involved in the ongoing monitoring of PRRS status in boar studs be accredited to ISO 17025 standards.

**LABORATORY TEST TURNAROUND TIME**

The interval between sampling and receipt of test results is also critical in the entire screening process. This interval is dependent upon the efficiency of delivery of samples to the laboratory and the interval between arrival of the samples and the reporting of test results by the laboratory. The NPHC recommends that sample turnaround time be part of the laboratory accreditation protocol.

**AVAILABLE TESTING CAPACITY:**

Both private (commercial) and state laboratory capabilities should be maintained in relation to PRRS. The role of the state laboratory will be to act as a quality control agency for the commercial laboratories to ensure that they maintain test standards (both PCR and ELISA). The state laboratory will also maintain its PRRS diagnostic capability from samples submitted by PVP's

**AI STATIONS:**

Suppliers of semen for AI in Ireland will be required to sign up to these protocols and to retain records of testing for inspection and in order to continue to provide the trade guarantees to those whom they supply. Where semen crosses national boundaries (ROI to NI and NI to ROI) the AI companies will register all movements on the TRACES system to ensure traceability and quality. In the event of a product recall, in the event of a problem being detected proper records re the destination farms for AI is essential.

The ultimate purchasers / consumers of fresh semen for AI also have a role to play in this agreed protocol. The ultimate consequence of a breach in any agreed protocol could be an outbreak of disease in the farm of destination. The purchaser of semen should reserve the right to question the semen / boar testing protocol with a view to safeguarding his own stock. This could include ensuring that each semen delivery be accompanied by a certificate of testing based on the agreed protocol. If this certification is not provided based on the protocol then there is a possibility that they will refuse to purchase the semen.

**Commencement Dates:**

This voluntary arrangement will come into operation on 1/5/14. Laboratories will be given additional time until 1/7/14 to reach accreditation status for testing for PRRS by the PCR method. Weekly results for each of the stud farms participating in this scheme will be centrally maintained at a separate website operated through the IFA secretariat at this address [nphcpigs@gmail.com](mailto:nphcpigs@gmail.com)

Queries to: Executive, IFA National Pigs and Pigmear Committee, Irish Farm Centre, Naas Road, Dublin 12. Tel: 01-4260334